Silvia Hoirisch-Clapauch

A coagulação e os transtornos mentais: Foco na cognição.

Tese submetida ao Programa de Pós-graduação de Psiquiatria e Saúde Mental (PROPSAM), da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do grau de doutor.

Orientador: Prof. Antonio Egidio Nardi

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Aluna de Doutorado de Psiquiatria e Saúde Mental na Universidade Federal do Rio de Janeiro, com início em agosto de 2013. Bolsista da Capes. As pesquisas foram custeadas com o auxílio da Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, bolsa ADT1 E-26/190.050/ 2011 e projeto contemplado no Edital Pensa Rio E-26/110.643/2012) e do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 456615/2013-0).

Esta tese é dedicada aos pacientes com esquizofrenia que moram no Instituto de Psiquiatria. Vocês me assustavam com o olhar transtornado, quando eu tinha cinco anos. Na faculdade, eu me assustava com a agitação psicomotora, as alucinações e a imprevisibilidade com que vocês eram capazes de acabar com as vidas de vocês e de pessoas próximas. O que me assusta hoje, não é nem a aparência, nem os sintomas de vocês, mas o fato de alguns psiquiatras resistirem a aceitar ideias consolidadas no campo da neurociência.

"Schizophrenia is in need of a treatment as miraculous as clot buster is for acute ischemic stroke."

Thomas Insel, Diretor do Instituto Nacional de Saúde Mental dos Estados Unidos, no congresso da Associação Americana de Psiquiatria, em 2014, referindo-se ao ativador do plasminogênio tissular recombinante.

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SUMÁRIO

LISTA DE ABREVIATURAS

ADAMTS13: desintegrina e metaloproteinase com domínio de trombospondina 13

ATP: adenosina trifosfato

BDNF: fator neurotrófico derivado do cérebro

C4b-BP: proteína ligadora da fração do complemento C4b

HFSE: Hospital Federal dos Servidores do Estado, Ministério da Saúde

HIV: vírus da imunodeficiência adquirida

IgG e IgM: Imunoglobulinas G e M

IPUB: Instituto de Psiquiatria da Universidade Federal do Rio de Janeiro

ISRS: inibidores seletivos da recaptação da serotonina

MMP: metaloproteinases da matriz

NMDA: N-metil-D-aspartato

PAI-1: inibidor do ativador do plasminogênio

PGRMC1: receptor de membrana, resultante da fusão do receptor da progesterona com o do PAI-1

PROS1: gene da proteína S

PTT: púrpura trombocitopênica trombótica

TAFI: inibidor da fibrinólise ativado pela trombina

TAM: receptores tirosina quinase Tyro3, Axl e Mer

TGF-β: fator de crescimento transformador β

tPA: ativador do plasminogênio tissular

VEGF: fator de crescimento do endotélio vascular

VLDL: lipoproteína de muito baixa densidade

RESUMO

Introdução: Cinco pacientes com esquizofrenia ou transtorno esquizoafetivo, usando warfarin para profilaxia de trombose venosa profunda, evoluíram com remissão mantida dos sintomas psicóticos. Nenhum tinha lesão isquêmica cerebral, corroborando o que é relatado em exames de neuroimagem de pacientes com esquizofrenia, que mostram atrofia cerebral, em especial do hipocampo e córtex pré-frontal. O ativador do plasminogênio tissular (tPA) regula a coagulação e também participa de processos que previnem a atrofia cerebral.

Objetivo: Buscar evidências de que proteínas que regulam a coagulação influenciam transtornos acompanhados por disfunção cognitiva, como a esquizofrenia.

Métodos: Primeiro, avaliamos se as hipóteses formuladas para a esquizofrenia envolveriam baixa atividade do tPA. A seguir, buscamos marcadores de baixa atividade do tPA em 70 indivíduos com esquizofrenia ou transtorno esquizoafetivo e 98 sem transtorno mental. Depois, estudamos o papel da baixa atividade do tPA na patogênese de comorbidades não iatrogênicas da esquizofrenia. Também avaliamos se a eficácia dos tratamentos antipsicóticos depende da normalização da atividade do tPA. Por fim, analisamos como a baixa atividade do tPA influencia a fisiopatologia de várias condições acompanhadas por disfunção cognitiva.

Resultados: A ativação de neurotrofinas, da relina e do receptor N-metil-D-aspartato são alguns dos processos influenciados pelo tPA, defeituosos na esquizofrenia. Identificamos uma alta prevalência de condições que resultam em baixa atividade do tPA em pacientes com esquizofrenia ou transtorno esquizoafetivo, incluindo altos níveis de insulina, triglicerídeos, homocisteína, anticorpos antifosfolipídio e deficiência da proteína S livre. A proteína S tem propriedades tróficas, antiapoptóticas e mitogênicas, independentes do tPA. Reforça a hipótese de que a disfunção do tPA e da proteína S participe da fisiopatologia da esquizofrenia e do transtorno esquizoafetivo, o fato de várias intervenções eficazes no tratamento da psicose corrigirem a atividade destas serino proteases. Tudo indica que a disfunção do tPA ou de seu produto final, a plasmina, também integre a fisiopatologia de várias outras condições acompanhadas de déficit cognitivo. Os resultados foram publicados em onze artigos, anexados a esta tese.

Conclusão: As evidências sugerem que a atividade defeituosa do tPA, da plasmina e da proteína S tenha papel importante na patogênese de transtornos acompanhados de disfunção cognitiva, como a esquizofrenia.

Palavras chave: ativador do plasminogênio, plasmina, proteína S, esquizofrenia.

ABSTRACT

Background: Five patients with schizophrenia spectrum disorders on chronic warfarin therapy for deep-vein thrombosis showed sustained psychosis remission. None of them had ischemic brain lesions, corroborating other author's findings. Schizophrenia is not accompanied by ischemic brain lesions, but by atrophy of the hippocampus and prefrontal cortex. Tissue plasminogen activator (tPA) regulates coagulation and catalyzes processes that prevent brain atrophy.

Objective: To search for evidence that proteins that regulate coagulation influence disorders accompanied by cognitive abnormalities, such as schizophrenia.

Methods: First, we analyzed whether low tPA activity could link the many hypotheses formulated for schizophrenia pathogenesis. Then, we searched for markers of low tPA activity in a group of 70 patients with schizophrenia spectrum disorders and 98 controls without mental disorders. Next, we reviewed the influence of low tPA activity on schizophrenia comorbidities unrelated to medications, and how tPA performs in interventions effective in treating psychotic symptoms, such as warfarin. We also analyzed the role of tPA in conditions accompanied by cognitive impairment.

Results: The activation of neurotrophins, reelin and the N-methyl-D-aspartate receptor are some of the neuroprotective and neuroregenerative mechanisms mediated by tPA, reported as defective in disorders accompanied by cognitive abnormalities, such as schizophrenia. Patients diagnosed with schizophrenia spectrum disorders had a high prevalence of conditions affecting tPA activity, including hyperinsulinemia, hypertriglyceridemia, hyperhomocysteinemia, antiphospholipid antibodies and free-protein S deficiency. Free-protein S has trophic, antiapoptotic and mitogenic properties that do not depend on tPA. The hypothesis that low activity of tPA and/or protein S contributes to the pathophysiology of schizophrenia spectrum disorders is further supported by the ability of many interventions effective in treating psychosis to restore tPA and protein S activity. Last, not least, tPA dysfunction seems to have an important role in the pathophysiology of different conditions accompanied by cognitive dysfunction. Our results were published in 11 papers attached to this thesis.

Conclusion: Evidence suggests that dysfunctional activity of tPA, plasmin and protein S, has a pivotal role in the pathophysiology of disorders accompanied by cognitive dysfunction, such as schizophrenia.

Key words: plasminogen activator, plasmin, protein S, schizophrenia.

INTRODUÇÃO

A hemostasia é um mecanismo dinâmico, que mantém o sangue líquido dentro do vaso íntegro, transformando-o em uma rolha gelatinosa, o coágulo, quando o vaso é lesado.[1] O coágulo é composto por plaquetas, presas por uma rede de fibrina, que também aprisiona os outros glóbulos sanguíneos.

Como vários dos fatores que formam a fibrina são ativados de forma sequencial,[2] o mecanismo ficou conhecido como cascata da coagulação. Tal termo caiu em desuso, quando se descobriu que a coagulação depende da ativação concomitante de várias vias, que se retroalimentam de modo explosivo. Hoje, o conjunto de reações que culmina na formação da rede de fibrina é denominado via da coagulação.[1]

As células endoteliais produzem e ativam fatores anticoagulantes e substâncias fibrinolíticas, ou seja, capazes de dissolver a rede de fibrina. Isto dificulta a formação do coágulo no vaso íntegro, favorecendo sua formação aos locais onde o endotélio está lesado ou inexiste, como em ferimentos, placas ateroscleróticas fragmentadas ou válvulas mecânicas intracardíacas.

Condições que contribuem para que coágulos sejam formados no vaso íntegro compreendem a imobilização, como em cirurgias, trauma, confinamento ou doenças neurológicas que cursam com paresia de extremidades, presença de cateter venoso central ou marca-passo, neoplasias, varizes e doenças mieloproliferativas, como a policitemia vera e a trombocitemia essencial.[3,4] Também aumentam o risco de tromboembolismo a hiperviscosidade e trombofilias.[5,6]

As trombofilias representam ganho de função dos fatores da coagulação, ou deficiência das substâncias anticoagulantes ou pró-fibrinolíticas. São classificadas como hereditárias ou adquiridas e os pacientes com trombofilia, como trombofílicos fracos ou fortes. O risco é grande de que trombofílicos fortes tenham tromboses sem causa definida, recorrentes ou que coloquem a vida em risco.[7]

São exemplos de trombofílicos fortes aqueles com deficiência de antitrombina III e os com persistência de anticorpos antifosfolipídio.[8] Recomenda-se que todo trombofílico forte, que já tenha tido uma trombose sistêmica, seja anticoagulado

a vida toda.[8] Isso porque, após a reabsorção do coágulo, há lesão endotelial residual, que prejudica os mecanismos anticoagulante e fibrinolítico, aumentando a chance de novas tromboses. Indivíduos com eventos tromboembólicos potencialmente fatais – especialmente se estes não tiverem sido provocados por hormônios, imobilização prolongada ou outra situação claramente trombogênica – têm indicação de serem anticoagulados de forma perene, mesmo que não sejam classificados como trombofílicos fortes.

Em 1999, o Hospital Federal dos Servidores do Estado, um hospital terciário filiado ao Ministério da Saúde, criou um Ambulatório de Trombofilia, que hoje é referência no Rio de Janeiro. Nesse ambulatório, o tratamento não se limita à anticoagulação. Inclui, também, correção de problemas metabólicos, além de orientação para que os pacientes evitem o sedentarismo, a desidratação, o consumo exagerado de carboidrato, o uso de estrogênios e progesterona de terceira geração e outras situações que aumentam o risco trombótico.

Quando pensamos na relação entre a coagulação e os transtornos mentais, a primeira associação costuma ser com acidentes vasculares cerebrais, perturbando o afeto e os processos cognitivos. O fato é que alguns elementos que modulam a coagulação também participam de importantes reações neuroquímicas. Como tal, a atividade reduzida desses elementos pode comprometer o afeto e os processos cognitivos, independente de problemas circulatórios.

O objetivo deste doutorado foi criar modelos para explicar a fisiopatologia de doenças acompanhadas de alterações cognitivas, incorporando elementos que regulam a coagulação.

Inicialmente, discutiremos aspectos inerentes à coagulação, explicando o motivo pelo qual decidimos desenvolver modelos na interface hematologia-psiquiatria. A seguir, analisaremos como substâncias que regulam a coagulação influenciam a fisiopatologia, as comorbidades e o tratamento de uma doença que pode evoluir com grave déficit cognitivo: a esquizofrenia. Faremos, também, considerações sobre a fisiopatologia de outras doenças caracterizadas por alterações cognitivas: a púrpura trombocitopênica trombótica, as dificuldades no aprendizado e os transtornos do espectro autista.

Os assuntos foram divididos em itens, a maioria dos quais se refere a artigos publicados no decorrer do doutorado. As referências bibliográficas estão limitadas àquelas não mencionadas nos artigos.

REMISSÃO DA PSICOSE COM WARFARIN

Em 2003, recebemos no Ambulatório de Trombofilia do HFSE um paciente de 19 anos, encaminhado para elucidar a etiologia e tratar uma trombose venosa profunda proximal. Apesar da trombose não ter sido provocada, esse jovem tinha várias condições que aumentam o risco trombótico: era sedentário, tinha compulsão alimentar por doces, sobrepeso, hiperhomocisteinemia, hipertrigliceridemia e mutação do gene da protrombina G20210A.

O tratamento proposto incluiu anticoagulação com o warfarin, ácido fólico para reduzir os níveis séricos de homocisteína e mudança no estilo de vida. Após pouco mais de seis meses, tanto o peso, quanto as alterações metabólicas haviam normalizado. Neste ponto, decidimos suspender a medicação anticoagulante, mas, para nossa surpresa, o paciente se recusou a fazê-lo, alegando que o warfarin tinha sido responsável pelo desaparecimento das alucinações auditivas que lhe tiravam a tranquilidade há muito tempo.

Nos anos subsequentes, mais quatro pacientes, com diversas entidades mórbidas, relataram acentuada melhora nos sintomas psicóticos alguns meses após o início do warfarin, a ponto de prescindirem de medicações antipsicóticas. Três tinham síndrome do anticorpo antifosfolipídio e uma tinha deficiência hereditária da proteína C funcional, aliada à deficiência da proteína S, esta relacionada à doença inflamatória intestinal em atividade.

Como todos os cinco estavam usando anticoagulante para prevenir recorrência de trombose venosa profunda, a primeira suspeita foi de que os sintomas psicóticos tivessem sido causados por lesões isquêmicas cerebrais. Contrariando as expectativas, mas corroborando o que é classicamente descrito na literatura, nenhuma tomografia cerebral mostrou isquemia. O que, em geral, se observa nos exames de imagem dos pacientes psicóticos, particularmente naqueles com esquizofrenia, não são lesões isquêmicas, mas atrofia cerebral, em especial no hipocampo e no córtex pré-frontal. A redução do volume cerebral já pode ser observada em exames de imagem no primeiro episódio psicótico e se agrava com a progressão da doença.

Nossa hipótese, então, foi que o warfarin, alterando algum componente da coagulação, da anticoagulação ou da fibrinólise, pudesse reverter os danos causados pela atrofia cerebral. Havia duas possibilidades não excludentes: o warfarin promoveria plasticidade neuronal ou estimularia a neurogênese, que no adulto existe especificamente nas áreas envolvidas em processos cognitivos, como o hipocampo e o córtex pré-frontal.

Ao realizarmos um levantamento da literatura, cruzando no site de busca PubMed (www.ncbi.nlm.nih.gov/pubmed/) cada elemento que participa da formação da rede de fibrina ou que regula a coagulação com os termos *neurogenesis* e *hippocampus,* encontramos um único resultado: o ativador do plasminogênio tissular (tPA). O warfarin aumenta os níveis do tPA no sangue e o tPA atravessa a barreira hematoencefálica.[9,10]

Para entender melhor os achados em apreço, procuramos o Prof. Antonio Egidio Nardi, que nos propôs desenvolver uma linha de pesquisa para testar a hipótese de que o aumento dos níveis do tPA teria sido responsável pela melhora dos sintomas psicóticos. Nosso primeiro artigo foi publicado como relato de casos:

Hoirisch-Clapauch S, Nardi AE. Psychiatric remission with warfarin: Should psychosis be addressed as plasminogen activator imbalance? Med Hypotheses 2013; 80: 137-41.

- ✓ **O que o artigo tem de inovador**: Foi o primeiro trabalho a aventar a possibilidade de que a baixa atividade do tPA possa ser um elemento importantíssimo na fisiopatologia da esquizofrenia. Também foi o primeiro a postular que a correção da atividade do tPA possa ter sido o responsável pelo alívio dos sintomas psicóticos.

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Psychiatric remission with warfarin: Should psychosis be addressed as plasminogen activator imbalance?

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ABSTRACT

Background: Psychotic patients are at increased risk of thromboembolism that cannot be ascribed to physical restraint or medication. Patients with chronic schizophrenia or long-term depressive illness do not display ischemic brain injuries on magnetic resonance imaging, as expected in patients with thrombotic tendency, but atrophy of specific brain regions, which indicates abnormal neuronal plasticity.
Hypotheses: We postulate that a relationship between psychosis pathophysiology and thrombotic tendency may comprise proteins that participate not only in the anticoagulation-fibrinolysis mechanism, but also in neuronal plasticity.
Case description: Five psychotic patients with thrombotic episodes on chronic warfarin therapy attained remission of psychotic symptoms and are free of psychotropic medication from 2 to 11 years. All patients have at least one thrombophilia related to inhibition of plasminogen activators, including prothrombin G20.210A polymorphism, hyperhomocysteinemia, antiphospholipid antibody syndrome and protein C deficiency.
Discussion: Plasminogen activators participate in blood clot dissolution and tissue repair, such as remodeling of hippocampus after stress, trauma, stroke or seizures. A significant prevalence of both thromboembolism and psychotic events can be seen in circumstances characterized by physiological or pathological inhibition of plasminogen activators, such as puerperium, confinement, polycystic ovary syndrome, antiphospholipid antibody syndrome and chronic inflammatory disorders.
Conclusion: Our findings suggest that normalization of plasminogen activator levels in the brain may induce long-term remission of psychotic symptoms. Randomized controlled studies may help clarify the role of anticoagulation in the treatment of psychosis.

Introduction

Several observational studies using different methodologies have shown an increased risk of venous thromboembolism in psychiatric patients. Some authors ascribe the elevated risk to physical restraint, as seen in catatonia and neuroleptic malignant syndrome or to the use of antipsychotic medication, in particular clozapine, chlorpromazine and thioridazine [1–3].

Nonetheless, both d-dimers and soluble P-selectin were found to be significantly increased in an antipsychotic-naïve group of patients with acute psychosis, compared with healthy controls matched for age, gender and body mass index. A positive d-dimer result indicates the presence of an abnormally high level of fibrin fragments, which are produced when a blood clot is degraded, while soluble P-selectin is released when platelets are activated. Since d-dimers and soluble P-selectin are considered markers for thrombotic phenomena, it seems that at least part of venous thromboembolic events seen in patients with acute psychosis might be induced by pathogenic mechanisms related to psychosis itself rather than to antipsychotic treatment [3].

We report five patients with psychotic symptoms, who entered psychotic symptoms remission months to years after beginning warfarin therapy and remain free of psychotropic medication from 2 to 11 years. Based on this finding, we discuss the mechanisms potentially involved in an increased prevalence of psychosis in subjects with conditions characterized by plasminogen activator inhibition and an increased prevalence of thrombotic events in psychotic patients.

Psychosis and brain imaging

If one assumes that thrombotic tendency could play a significant role in the etiology of psychosis, ischemic brain injuries would have been expected in neuroimaging studies, but in fact this has not been observed. Instead, magnetic resonance imaging has shown decreased cortical thickness in lateral prefrontal, right mid-

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dle and superior temporal lobe cortex when compared to healthy controls [4].

Furthermore, on high-resolution magnetic resonance images, patients with chronic schizophrenia display bilateral hippocampal volume reduction, while patients with first-episode schizophrenia display left hippocampal volume reduction and patients at high risk for psychosis have normal baseline hippocampal volume whether or not they subsequently develop a psychotic illness [5]. It has been well established that in long-term depressive illness the hippocampus and prefrontal cortex also undergo atrophy, but remodeling of the hippocampus can be reversible [6].

Therefore, a relationship between the pathophysiology of psychosis and thrombotic tendency is likely to occur at the biochemical level, comprising proteins that play a role both in the coagulation-anticoagulation-fibrinolytic process and the restoration of hippocampus after injury.

Neuronal plasticity and the fibrinolytic mechanism

Searching in PubMed on November 4, 2012 the Medical Subject Headings: ''neuronal plasticity'' AND hippocampus AND proteins that participate in the anticoagulation mechanism, such as ''tissue factor pathway inhibitor'', ''antithrombin III'', ''functional protein C'' or ''free protein S'', no items were found. The same occurred with ''neuronal plasticity'' AND hippocampus AND proteins that participate in the hemostatic mechanism, such as ''factor V'', ''factor VII'', ''factor VIII'', ''factor X'', ''factor XI'', ''factor XII'', ''factor XIII'' or prothrombin. Searching ''neuronal plasticity'' AND hippocampus AND thrombin resulted in three papers. When ''neuronal plasticity'' AND hippocampus'' were entered with ''plasminogen activator'', the result was 26 medical papers. Based on evidences showing that neuronal plasticity in the hippocampus depends on plasminogen activators, we hypothesize that warfarin could induce remission of psychotic symptoms through normalization of plasminogen activators in specific regions of the brain, as the hippocampus.

Plasminogen activators and the hippocampus

The urokinase-type plasminogen activator (uPA) and tissue plasminogen activator (tPA) are inducible serine proteases that participate in blood clot dissolution, embryonic development, cell adhesion, proliferation, differentiation and migration, as well as in matrix degradation, apoptosis, and angiogenesis [7,8].

Of note, plasminogen activators play an important role in hippocampal remodeling after stress, trauma, stroke or seizures [7,9]. In mice, for example, the expression of uPA receptor (uPAR) in the normal hippocampus is low and confined to a small population of astrocytes and interneurons. In animals undergoing status epilepticus, uPAR expression increases dramatically, supporting the idea that uPA contributes to brain remodeling [7].

Different findings support the notion that uPA and tPA are involved in emotional behavior. First, hippocampal neurons are among the few brain neurons that express uPA gene, suggesting that uPA is involved in the specific hippocampal functions, i.e., learning, memory, emotional processing, and vulnerability to stress [7]. Although tPA is more abundant in the hippocampus than uPA, its production is not restricted to the hippocampus. Actually, tPA can also be produced by the amygdala, the hypothalamus and cerebellar Purkinje cells, where it modulates responses that include fear, anxiety, autonomic and endocrine functions, and motor learning [8]. Also, neuronal overexpression of tPA in transgenic mice improves learning, while tPA inhibition disturb emotional behavior [9]. Furthermore, both uPA and tPA have an important a role in the limbic system, especially in depolarization-evoked dopamine release in the nucleus accumbens [10]. Disruption of the mesolimbic dopaminergic pathway is a common feature of schizophrenia and drug addiction [11,12].

Patients and methods

Detailed clinical data were extracted from medical records of 5 patients with thrombotic tendency and psychotic features treated at the Anticoagulation Clinic of a tertiary Brazilian public hospital by one of the authors (SHC). All patients were followed by a well-trained psychiatric team supervised by the other author (AEN). In all cases, the psychiatric diagnosis was based on Structured Clinical Interview Diagnostic [13].

Thrombophilia screening was performed according to the following guidelines: unprovoked thrombosis, recurrent deep venous thrombosis episodes, three or more unexplained embryonic losses or one fetal loss related to placental thrombosis and infarcts.

Thrombophilia panel comprised a complete blood cell count, fibrinogen, 12-h fast lipid profile, anticardiolipin antibodies IgM and IgG, lupus anticoagulant, antithrombin III, free protein S, functional protein C and factor V Leiden, prothrombin G20.210A and MTHFR C677T/A1298C polymorphisms. Antithrombin III, free protein S, functional protein C, anticardiolipin antibodies and lupus anticoagulant were screened more than 1 month after the thrombotic event. Free protein S was performed at least 1 month after discontinuation of oral contraceptives or hormonal therapy, and at least 6 weeks after pregnancy, puerperium, infectious or inflammatory episodes. Functional protein C and free protein S were dosed at least 3 weeks after discontinuation of warfarin. Antithrombin III was dosed at least 1 week after discontinuation of heparin. Due to budget restrictions, patients were not screened for anti-β2 glycoprotein 1 IgM or IgG.

Whenever positive, anticardiolipin antibodies or lupus anticoagulant were reassessed at least 12 weeks apart, according to the International Society on Thrombosis and Haemostasis guidelines.

Once deep venous thrombosis was identified, initial management consisted of sequential heparin and warfarin anticoagulation. Patients were advised about warfarin interactions, including other drugs, herbs and carotene. According to our routine, to increase the chances that the test used to monitor the effect of warfarin, International Normalized Ratio or INR, could remain within the therapeutic range, caffeine, [36] dark green vegetables, vegetable fat, as in cookies or crackers, and soy, olive or canola oil were restricted. Regular INR assessment showed that all five patients remained $\geqslant 2$ for over 85% of the time, which means that during most of the time adequate anticoagulation and/or fibrinolysis was achieved.

Case reports

Patient 1

An 18-year-old boy was admitted with an unprovoked episode of proximal deep venous thrombosis in the leg. He was overweight, reporting to be sedentary and to have a carbohydrate binge eating disorder. He complained of chronic hallucinatory voices but had no regular psychiatric treatment and no other psychopathological symptom or diagnosis. Thrombophilia screening disclosed three distinct conditions that impair plasminogen activation: heterozygous prothrombin G20.210A, hypertriglyceridemia (282 mg/DL) and hyperhomocysteinemia (18 μM) [14–16]. A brain CT scan was normal.

The patient was started on warfarin and folic acid 10 mg/day. Besides, he was recommended to start exercises and avoid carbohydrate overeating. After 6 months he was told to withhold oral

anticoagulation, but he refused to do so, alleging that the chronic hallucinatory voices he experienced had disappeared. After eleven years on oral anticoagulation, he remains asymptomatic.

Discussion

Since genes that codify plasminogen activator inhibitor 1 (PAI-1) synthesis have insulin response and considering that correlation between triglyceridemia and insulin levels is almost direct, either hypertriglyceridemia or hyperinsulinemia correlate with PAI-1 levels [15,17]. Noteworthy, antipsychotic-naïve schizophrenic patients have a significantly higher mean plasma insulin, proinsulin, 32, 33-split proinsulin, and C-peptide level, as opposed to healthy control subjects [16], which means that in these patients hyperinsulinemia results from excessive insulin synthesis.

Patient 1 was homozygous for methylene tetrahydrofolate reductase C677T, a polymorphism that impairs homocysteine metabolism. In this setting, homocysteine levels are usually normalized with folic acid supplementation. Homocysteine levels are also modulated by insulin, so excessive carbohydrate intake and resultant hyperinsulinemia may further increase homocysteine levels [15,18].

Patient 2

A 50-year-old woman searched for psychiatric care due to delusional and hallucinatory paranoid episodes. She had agitation and mood incongruent psychotic symptoms and was diagnosed as bipolar I disorder, mixed episode. From 23 to 43 years of age she experienced seven first-trimester miscarriages and a premature birth. An episode of left popliteal venous thrombosis related to oral contraceptives occurred at the age of 32.

A brain CT scan was normal. Anticardiolipin antibodies, assessed twice with an interval of 12 weeks, showed negative IgG antibodies and positive IgM antibodies (55 and 43 MPL) and she was diagnosed with antiphospholipid antibody syndrome (APS) and was placed on warfarin. After 13 months she became asymptomatic for her psychotic and mood disorder. She remained free from psychotropic treatment for the last 4 years.

Discussion

Patients with APS are at increased risk for thromboembolic events as a result of down regulation of proteins that inhibit coagulation and fibrinolysis, such as protein C, and plasmin [19]. As the risk of recurrent thrombosis in APS is high, patients with a thrombotic event are placed on lifelong warfarin.

A high prevalence of antiphospholipid antibodies, such as anticardiolipin antibodies and lupus anticoagulant, can be found in psychotropic-naïve, acutely psychotic patients, as compared to healthy controls [20]. Similarly, patients with APS have a significant prevalence of psychiatric manifestations, such as psychosis, delirium, depression, anxiety, aggressive behavior, mania, depression, and bipolar disorders [20,21]. Since placental angiogenesis depends on metalloproteinase activation by plasmin, plasminogen activator inhibition increases the risk of miscarriages and preterm deliveries [22,23]. Adverse obstetric events, such as recurrent miscarriages, are important clues to APS diagnosis.

Patient 3

A 38-year-old obese woman was diagnosed as bipolar I disorder, mixed episode with delusions, and was placed on lithium, sodium divalproate, and phenobarbital. When she was 35 years old, she gave birth to a premature infant in a pregnancy complicated by pre-eclampsia and a deep venous thrombosis episode in the 3rd trimester. Five years later, a preoperative evaluation disclosed a prolonged activated partial thromboplastin time (aPTT) that proved to be a persistent strong lupus anticoagulant. A brain CT scan, performed to rule out ischemic lesions, came out normal.

The diagnosis of APS was made and the patient was placed on long-term warfarin. At that time, she was oriented to engage in regular physical activity, such as daily walking. After 2 years, psychotropic medication was tapered out. She lost 22 kg (48.5 lb) and remains asymptomatic for her bipolar disorder for the last 2 years without any psychotropic medication.

Discussion

In antiphospholipid antibody syndrome, sometimes psychiatric symptoms present many years before the onset of somatic symptoms [22].

Patient 4

A 29-year-old woman was diagnosed as puerperal psychosis with mood-incongruent features and catatonic symptoms after a preterm delivery. Psychotic symptoms did not respond to medication and response to electroconvulsive therapy was transient. She was unable to take care of her child and remained in bed most of the time. An iliofemoral venous thrombosis episode occurred when her daughter was one-year-old, and she was diagnosed with antiphospholipid antibody syndrome based on a persistent positive lupus anticoagulant. Repeated brain CT scans were normal. After 8 months on warfarin she became asymptomatic for the mood disorder with psychotic symptoms and remains asymptomatic for the last 2 years.

Discussion

Most women experience some kind of mood disorder during puerperium, but symptoms are transient and mild. About 10–15% of women have a more disabling and persistent form of depression and approximately 1–2 per 1000 women experience postpartum psychosis, usually within the first 2 postpartum weeks. Mood-incongruent symptoms can be seen in 65% of patients [24]. Other symptoms include mania or mixed episode, delusional beliefs related to the infant or auditory hallucinations [25]. It seems that Patient 4 had a baseline condition that impaired plasminogen activation – APS – antiphospholipid antibodies - and a trigger that intensified the problem and favored the emergence of the acute psychotic episode – the puerperium [26].

Patient 5

A 37-year-old female was admitted with acute deep-venous thrombosis. She had an eleven-year history of ulcerative rectocolitis treated with sulfasalazine, a ten-year history of bipolar I disorder with depressive and maniac episodes treated with sodium valproate, quetiapine and clonazepam, a recent history of polycystic ovaries and four episodes of deep venous thrombosis in the leg since she was 30 years old, the first related to oral contraceptives, followed by three unprovoked thrombotic events. She had been medicated with anticoagulants for 3–6 months after each thrombotic episode and reported a sedentary lifestyle.

Repeated testing showed low levels of functional protein C. All brain CT scans were normal. She was placed on lifelong warfarin. Psychotropic medication was tapered out progressively, and she remains asymptomatic for the last 5 years.

Discussion

Patient 5 had a baseline condition and a trigger that inhibit plasminogen activator: hereditary deficiency of protein C [27] and a chronic inflammatory disease. In chronic inflammatory diseases, tumor necrosis alpha and interleukin-1 induce PAI-1 synthesis by endothelial cells [28]. Ensuing tPA inhibition by PAI-1 might explain the high prevalence of psychosis in autoimmune diseases, such as Guillain–Barré syndrome, ulcerative colitis, Crohn's disease, Sjögren's syndrome, autoimmune hepatitis and systemic lupus erythematous [29,30]. High levels of PAI-1 can be detected in cerebrospinal fluid of patients with neuropsychiatric lupus erythematous reinforcing the concept that plasminogen activator imbalance might play a role in psychiatric conditions [31].

As plasmin is required to activate metalloproteinases involved in ovary remodeling, low plasminogen activator levels are implicated in the pathophysiology of polycystic ovaries [32]. It is possible that low plasminogen levels could also explain the high percentage of psychiatric disorders found in women with polycystic ovary syndrome (PCOS): it has been estimated that 26% of these women have major depression and 11% of them have bipolar disorder [33].

Conclusions

A number of conditions at increased risk for acute psychotic events, including antiphospholipid antibody syndrome [21], chronic inflammatory diseases [29], polycystic ovary syndrome [33] and confinement [34,35], may have as a common denominator the pathological inhibition of plasminogen activators. We believe that a baseline condition that promotes imbalance of plasminogen activators combined to a trigger that potentiates the problem could play a crucial role in the pathophysiology of psychotic events.

Randomized controlled studies might help clarify if medications able to restore the levels of plasminogen activators in the brain could increase the chances of psychotic patients to achieve long-term psychiatric remission. Potential candidates include heparin and direct inhibitors of factor Xa in therapeutic (not prophylactic) doses and warfarin. Considering that excessive insulin production related to either sedentary lifestyle or excessive carbohydrate consumption is a prevalent condition that inhibits plasminogen activators, it would be wise to assess the impact of a carbohydrate-balanced diet combined with structured exercise in psychotic patients.

Conflict of interest statement

The authors disclose no conflict of interest.

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O ATIVADOR DO PLASMINOGÊNIO TISSULAR

O tPA é uma serino protease, que converte o plasminogênio em plasmina. A plasmina dissolve a rede de fibrina, restaurando, assim, a patência vascular (Figura 1). Em virtude de suas propriedades fibrinolíticas, o tPA recombinante é usado no tratamento do acidente vascular cerebral isquêmico, do infarto do miocárdio e da embolia pulmonar.[10-12]

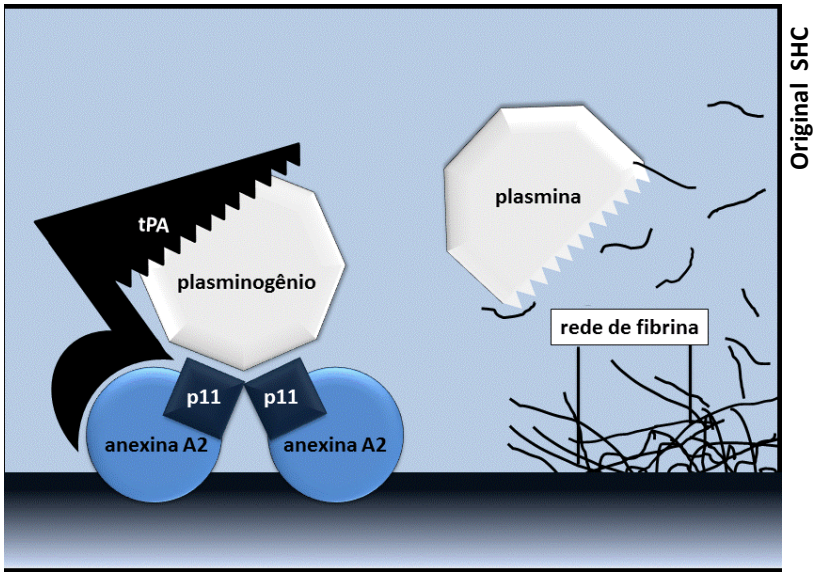

Figura 1. Esquema de ativação do plasminogênio pelo ativador do plasminogênio tissular (tPA) no endotélio, com resultante geração de plasmina. A reação é acelerada na presença de um receptor composto por duas moléculas de anexina A2 e duas da proteína p11.

A geração de plasmina é acelerada quando o tPA e o plasminogênio se ligam a um heterotetrâmero composto por duas moléculas de anexina A2 e duas da proteína p11, também conhecida como S100A10.[13,14] A anexina A2, uma proteína que se liga ao cálcio e a fosfolipídios, participa da regulação da proteólise extracelular e de interações celulares.[14] Já a p11, por facilitar o acúmulo de diversos receptores da serotonina e do receptor metabotrópico do glutamato 5 na superfície celular, modula funções neuronais, que incluem a regulação do humor.[15]

Tanto o tPA, quanto a uroquinase, um ativador do plasminogênio produzido no urotélio, mediam proteólise do tecido extracelular. Como resultado, ambos participam de processos dependentes da remodelação tissular fisiológica ou patológica, como ovulação, consolidação óssea pós-fratura, funções cognitivas, respostas emocionais e angiogênese placentária ou tumoral.[16-18]

Para que a estrutura tissular sofra modificações, é preciso que proteínas da matriz extracelular sejam degradadas por metaloproteinases da matriz. A primeira metaloproteinase da matriz identificada foi a colagenase. Gross e Lapiere estudavam a metamorfose, em 1962, e descobriram uma enzima, na cauda dos girinos, que degradava o colágeno fibrilar.[19] As 23 metaloproteinases da matriz do ser humano contêm um domínio catalítico e um pró-domínio inibitório.[20] A remoção do pró-domínio depende de uma sequência de reações envolvendo outras metaloproteinases da matriz e o sistema tPA-plasmina.[21]

Sabe-se hoje que a função das metaloproteinases vai muito além da modificação da arquitetura tissular. Essas enzimas também convertem proteínas estruturais da matriz em moléculas capazes de promover quimioatração, proliferação, sobrevida celular, sinalização e diferenciação.[22]

O tPA E A FISIOPATOLOGIA DA ESQUIZOFRENIA

No cérebro, o tPA catalisa vários processos subjacentes à neurogênese, à modificação de circuitos neuronais e ao sistema de recompensa. Alguns destes processos independem da ativação de metaloproteinases. São exemplos: a clivagem do precursor do fator neurotrófico derivado do cérebro (BDNF) em um fator maduro, com propriedades antiapoptóticas e a liberação de dopamina no núcleo *accumbens.* O tPA também é responsável pela ativação do fator de crescimento endotelial vascular (VEGF), da relina e de receptores N-metil-D-aspartato (NMDA). A ativação dos receptores NMDA ativa a proteína quinase Akt, que participa do metabolismo da glicose, da proliferação e da migração celular (Figura 2).

Como várias destas reações neuroquímicas são essenciais e indispensáveis para o processamento cognitivo, adequadas reações afetivas e recuperação do equilíbrio mental após eventos traumáticos, é provável que a redução da resiliência e o impacto negativo sobre o bem-estar psíquico, observado após eventos traumáticos, decorram da baixa atividade do tPA. Partindo-se do pressuposto que indivíduos com esquizofrenia podem cursar com perturbações cognitivas e afetivas e não recuperar o equilíbrio mental após eventos traumáticos, postulamos que a atividade do tPA estaria gravemente comprometida na esquizofrenia.

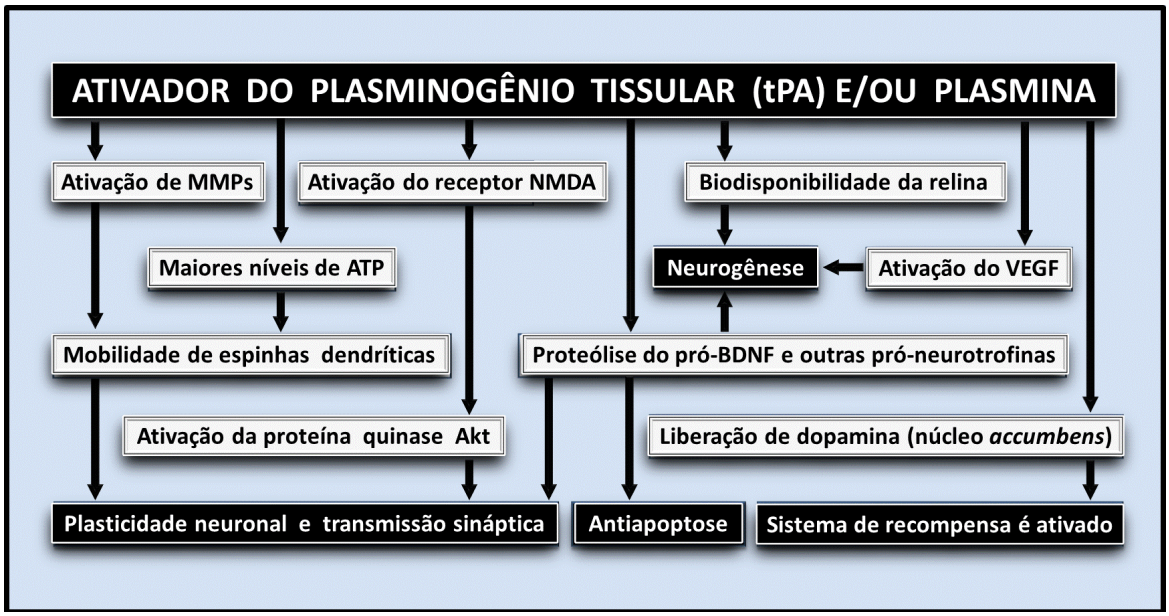

Figura 2. A influência do tPA e da plasmina em processos neuroquímicos. MMPs: metaloproteinases da matriz; NMDA: N-metil-D-aspartato; ATP: adenosina trifosfato; VEGF: fator de crescimento do endotélio vascular; pró-BDNF: precursor do fator neurotrófico derivado do cérebro.

Reforça essa hipótese o fato de condições caracterizadas por hipercortisolismo, como a síndrome de Cushing ou vivências traumáticas, poderem cursar com sintomas psicóticos. O cortisol estimula o promotor do gene que codifica o inibidor do ativador do plasminogênio (PAI)-1, um potente inibidor do tPA (Figura 3).

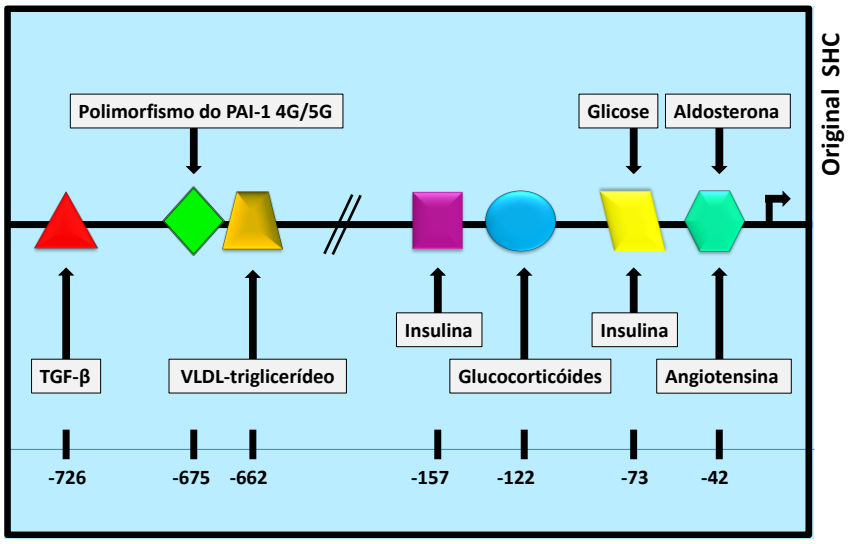

Figura 3: O promotor do gene do inibidor do ativador do plasminogênio (PAI)-1 responde ao fator de crescimento transformador (TGF)-β, à lipoproteína de muito baixa densidade (VLDL, carreador de triglicerídeo), à insulina, ao cortisol, à angiotensina e à aldosterona. A deleção de uma guanina no promotor do PAI-1 (PAI-1 4G/5G ou 4G/4G) aumenta a transcrição do PAI-1. Modificado de Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost 2005; 3: 1879-83.

No sistema nervoso central, o tPA também é inibido pela neuroserpina. Mutações no gene da neuroserpina podem resultar em um quadro de demência familiar, que também se acompanha de atrofia cerebral.

Neste modelo proposto para a esquizofrenia, a atividade aberrante do tPA e da plasmina não só tornaria os neurônios mais vulneráveis, como também inibiria a reconfiguração sináptica e a neurogênese. Como resultado, haveria perda da substância cinzenta e branca.

A constatação de que a baixa atividade do tPA era um elemento comum a várias alterações químicas identificadas na esquizofrenia, serviu de base para a elaboração de um artigo, descrevendo um novo modelo para esse transtorno mental:

Hoirisch-Clapauch S, Nardi AE. Multiple roles of tissue plasminogen activator in schizophrenia pathophysiology. Semin Thromb Hemost 2013; 39: 950-4.

- ✓ **O que o artigo tem de inovador**: Esse foi o primeiro trabalho a identificar um denominador comum a diversas alterações químicas prevalentes na esquizofrenia: a atividade defeituosa do tPA.

Multiple Roles of Tissue Plasminogen Activator in Schizophrenia Pathophysiology

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Abstract

Schizophrenia, a disabling mental disorder, is characterized by brain atrophy, especially in the superior temporal gyrus and the medial temporal lobe, which includes the hippocampus and the amygdala. The model that better explains brain atrophy includes a trigger and a predisposing condition. The trigger is exemplified by illicit drugs or environmental stressors that promote release of substances harmful to the neurons, such as glucocorticoids or noradrenalin. Predisposed patients would have one or more conditions that impair neuronal plasticity and neurogenesis. Evidence indicates that abnormal tissue plasminogen activator (tPA) activity is an important predisposing condition. tPA plays an important role in synaptic regulation and plasticity, and in neurogenesis, being crucial to the biology of memory, learning, and emotions. Several biochemical abnormalities seen in schizophrenics are related to decreased levels or impaired activity of tPA, including deficient dopamine transmission at D1 receptors in the prefrontal cortex, impaired cleavage of a precursor of brain-derived neurotrophic factor into its mature form (mature brain-derived neurotrophic factor), abnormal *N*-methyl-D-aspartate receptor-mediated signaling, reduced Akt phosphorylation, and abnormal activation of reelin. Clinical conditions related to schizophrenia, such as hyperhomocysteinemia, insulin resistance, and type 2 diabetes mellitus are characterized by a loss of tPA function or decreased tPA levels. This article reviews how low levels or abnormal function of tPA are related to the pathogenesis of schizophrenia.

Keywords
- tissue plasminogen activator
- neurogenesis
- schizophrenia
- mechanism of action
- pathophysiology

Schizophrenia is a chronic, severe, and disabling mental disorder. The disease is characterized by positive symptoms (paranoid or bizarre delusions, auditory hallucinations), negative symptoms (social withdrawal, flat affect, reduced capacity to experience pleasure), and cognitive deficits (impairment of memory and attention, disorganized speech and thinking).

Magnetic resonance imaging studies and postmortem findings of schizophrenic patients provide compelling evidence for brain atrophy. Affected brain regions comprise enlarged lateral ventricles and specific gray matter volume reductions, especially prominent in the superior temporal gyrus and medial temporal lobe brain regions, which includes the hippocampus and the amygdala.[1]

To explain brain atrophy, it would be reasonable to assume a predisposing condition and a trigger. The trigger would be an illicit drug or an environmental stressor, which promotes synthesis and release of substances capable of damaging neurons, such as glucocorticoids or noradrenalin.[2] Predisposed patients would have one or more neurochemical abnormalities that would impair neuronal protection and/or prevent both neuronal plasticity and neurogenesis.[3]

Since there are isolated cases of schizophrenia in some families and clusters in others, it seems that incapability to

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promote neurogenesis might result from DNA mutations, acquired factors, or both.

Evidence indicates that abnormal function or low levels of tissue plasminogen activator (tPA) should be regarded as an important parameter when it comes to decipher the pathophysiology of schizophrenia. For decades, the main role of tPA was considered to be the regulation of intravascular thrombolysis. Apart from its fibrinolytic action, tPA is widely distributed in the central nervous system, where it actively participates in mechanisms of synaptic regulation, synaptic plasticity, integrity of the blood–brain barrier, and neurogenesis, being crucial in the biology of memory, learning, and emotions.[4,5]

Neurogenesis, for instance, requires activation of matrix metalloproteinases (MMP),[6] brain-derived neurotrophic factor (BDNF),[7] vascular endothelial growth factor,[8] and *N*-methyl-D-aspartate (NMDA) receptors.[9] Of note, tPA itself, or through activation of plasmin, participates in all these processes.[4,10–12]

This article reviews how low levels or the loss of function of tPA are related to the pathogenetic mechanisms of schizophrenia.

Schizophrenia Pathophysiology

Dopaminergic Hypothesis

Dopamine is a major neurotransmitter, involved in behavior, thoughts, and interest in new experiences and reward. Feelings of pleasure and well-being derived from alcohol, cocaine, nicotine, and methamphetamine, for example, are mediated by dopamine function in terminals of mesolimbic neurons in the nucleus accumbens.[13]

The classical hyperdopaminergic hypothesis of schizophrenia derived from the capability of dopaminergic-enhancing drugs to induce positive symptoms of schizophrenia. Nonetheless, neither negative symptoms nor cognitive impairment is affected by drugs that inhibit dopamine receptor. The dopaminergic hypothesis currently accepted is based on imaging and postmortem studies. It states that a deficit in dopamine transmission at D1 receptors in the prefrontal cortex is related to negative symptoms and cognitive impairment, whereas the excess of subcortical dopamine transmission at D2 receptors is related to positive symptoms.[14,15]

How dopamine is related to tPA. In $tPA^{-/-}$ mice, dopamine release is markedly diminished, and injection of either tPA or plasmin into the nucleus accumbens corrects the problem.[13] Also, activation of dopamine receptors, especially D1, promotes tPA release from postsynaptic neurons into the extracellular space. tPA converts plasminogen to plasmin, and then plasmin acts on presynaptic dopaminergic neurons via plasminogen activator receptor-1 to potentiate the activity-dependent release of dopamine in the nucleus accumbens.[16]

Brain-Derived Neurotrophic Factor Hypothesis

BDNF is a neurotrophin synthesized by dopamine cells. In immature neurons, BDNF is involved in growth, differentiation, maturation, and survival, whereas in mature neurons, BDNF plays an important role in synaptic plasticity, augmentation of neurotransmission, and regulation of receptor sensitivity. BDNF imbalance is associated with disruptions in brain circuitry, neurodevelopmental impairment, and abnormal cognitive function.[17]

The majority of studies in drug-naive patients with schizophrenia show a reduction of BDNF protein expression, as detected through enzyme-linked immunosorbent assay and Western blotting.[18]

How BDNF is related to tPA. BDNF is released as a precursor. In response to neuronal activity, secreted pro-BDNF is proteolytically cleaved into mature BDNF (mBDNF) by tPA. It must be remembered that pro-BDNF and mBDNF, by interacting with their respective receptors, work in opposite directions: pro-BDNF facilitates long-term depression and mBDNF participates in long-term potentiation. Long-term potentiation and long-term depression are two common forms of synaptic plasticity; while the first strengths neuronal synapse, the second weakens it. Cleavage of BDNF proneurotrophin is therefore an important regulatory mechanism.[19]

N-Methyl-D-Aspartate Receptor Hypothesis

Activation of NMDA receptors requires membrane depolarization plus stimulation by glutamate and the coagonist glycine. Excitatory neurotransmitter glutamate is critically important for most aspects of normal brain function, including cognition, memory, and learning. When excess glutamate accumulates outside the cells, hyperactivation of postsynaptic glutamate receptors causes massive calcium influx that damages the mitochondria and activates proapoptotic genes.[20]

Activated NMDA receptor opens an ion channel that allows flow of sodium and small amounts of calcium ions into the cell and potassium out of the cell. Inhibition of NMDA receptors depends on several extracellular ions, including zinc. One mechanism of action of NMDA antagonists is to reduce the excitation of parvalbumin-expressing fast-spiking interneurons, resulting in disinhibition of pyramidal cells. Overactive pyramidal cells, particularly those in the hippocampus, can drive a hyperdopaminergic state, thus producing the positive symptoms of psychosis.[20]

Ketamine, an NMDA receptor antagonist, is able to pathologically increase resting brain functional connectivity and to reproduce both negative and positive symptoms, as well as many of the cognitive deficits associated with schizophrenia in normal volunteers.[21] NMDA receptor antagonists can also reduce the expression of GAD67, an isoform of glutamate decarboxylase that catalyzes the synthesis of γ-aminobutyric acid (GABA) from glutamate. GABA is a major inhibitory neurotransmitter, and impaired GABA synthesis due to downregulation of GAD67 has been demonstrated in the cortex and hippocampus of schizophrenic patients.[22]

How the NMDA receptor is related to tPA. Plasmin and tPA potentiate signaling mediated by glutamatergic receptors by modifying the properties of the NMDA receptor. NDMA receptor has two extracellular domains: the subunits NR1 and NR2. Calcium influx through NMDA receptor subunit NR2 requires cleavage of NMDA receptor by plasmin, a process that removes zinc.[23] Cleavage results in transient phosphorylation

of the extracellular signal-regulated kinases 1 and 2 and activation of cyclic adenosine monophosphate response element-binding protein (CREB). CREB turns on a cell signaling pathway that protects neurons from the deleterious effects of excitotoxicity.[24]

tPA released by neurons is constitutively endocytosed by astrocytes via the low-density lipoprotein-related protein receptor and is then exocytosed in a regulated manner. The exocytotic recycling of tPA by astrocytes is inhibited in the presence of extracellular glutamate.[25]

Akt Hypothesis

Akt, also known as protein kinase B, shows a virtually ubiquitous distribution and plays important roles in nervous system. Two Akt isoforms, Akt2 and Akt3, specifically regulate some aspects of apoptosis and cell growth in cultured neurons through phosphorylation of serine and threonine amino acid residues of several enzymes. These enzymes include glycogen synthase kinase 3 and hexokinases, which are regulators of mitochondrial cytochrome C. Disruption of Akt2 and Akt3 induces a small but significant reduction of cell viability in cortical neurons that correlates with the activation of caspase 3.[26]

How Akt is related to tPA. Subtle changes in the microenvironment induce the release of neuronal tPA into the synaptic space, which then interacts with plasminogen on the cell surface, leading to plasmin-induced, NMDA receptor-mediated Akt phosphorylation.[27]

Hyperhomocysteinemia Hypothesis

Different studies have reported elevated homocysteine levels in a significant percentage of schizophrenic patients.[28,29] The correlation is high: A 5-μmol/L increase in homocysteine levels is associated with a 70% increased risk for schizophrenia.[29] Elevated levels of homocysteine result from incapability of recycling homocysteine into methionine. Chronic hyperhomocysteinemia leads to increased levels of intracellular S-adenosyl homocysteine, a potent inhibitor of methyltransferases, such as DNA methyltransferase. As DNA methylation promotes gene silencing, the consequences of reduced methylation include altered gene expression and reduced neurotransmitter synthesis, which contribute to neuropsychiatric disease.[30]

However, some genes implicated in the pathophysiology of schizophrenia, such as reelin promoter, are hypermethylated, not hypomethylated,[31] which suggests another explanation for the increased risk for schizophrenia seen in patients with hyperhomocysteinemia.

How homocysteine is related to tPA. Homocysteine prevents binding of plasminogen and tPA to annexin, a phospholipid-binding protein that increases the catalytic efficiency of tPA-dependent plasminogen.[32]

Reelin Hypothesis

Reelin is an extracellular matrix protein synthesized and secreted from GABAergic neurons in the adult brain. This signaling protein mediates neuronal migration, orientating them in a ventricular-to-pial disposition and also modulates synaptic function and plasticity in adult synapses. In addition, reelin participates actively in glutamate receptor homeostasis. Indeed, blocking this protein secretion in the hippocampus rapidly and reversibly changes the subunit composition of NMDA glutamate receptors, which means that continuous reelin secretion is a strict requirement to maintain the composition of NMDA receptors.[33,34] Reelin mRNA and protein levels are reduced by approximately 50% in postmortem brain from schizophrenic patients.[31]

How reelin is related to tPA. In physiological conditions, reelin is anchored to the extracellular matrix and its active segment is released by tPA.[35] In fact, neurons from mice lacking the tPA gene ($tPA^{-/-}$) migrate 51% as fast as neurons from wild-type mice.[36]

Metabolic Hypothesis

A significant difference in baseline 2-hour postprandial blood sugar can be seen in medication-naive patients with schizophrenia, compared with matched healthy controls.[37] Actually, schizophrenia has been related to insulin resistance, diabetes mellitus, and other metabolic abnormalities through several susceptibility genes, which include gamma-secretase-mediated ErbB4 signaling, adipocytokine signaling, and TCF7L2.[38]

TCF7L2, for example, is a transcription factor involved in the Wnt/β-catenin signaling, a pathway that represses proglucagon synthesis in enteroendocrine cells. Different point mutations of the *TCF7L2* gene have been associated with increased risk for type 2 diabetes and schizophrenia.[39,40]

How metabolism is related to tPA. Both insulin and its precursor, proinsulin, stimulate the synthesis of plasminogen activator inhibitor-1 (PAI-1). PAI-1 is the most potent inhibitor of tPA.[41]

Nicotine—The Self-Medication Hypothesis

The smoking rate among patients with schizophrenia is 59%, with a sixfold chance of being a smoker at first-episode psychosis compared with healthy controls.[42] Cessation rates are lower in schizophrenics than in general population.[43]

How nicotine is related to tPA. Nicotine increases tPA plasmatic levels and promotes the release of tPA into the extracellular space in the nucleus accumbens.[44] The heavy use of nicotine has been viewed as an attempt at self-medication.[45] Actually, tPA release provides a reasonable explanation for the finding that nicotine enhances cognitive functions in schizophrenics.[46]

Low Prevalence of Neoplasia

Since tobacco smoking rates in individuals with schizophrenia are typically higher than in the general population, one would expect a high prevalence of lung cancer in schizophrenics. Nonetheless, different epidemiological studies in schizophrenics quote a lower incidence of cancer in general, and lung cancer in particular, which suggests that schizophrenia may afford some protection.[47–49] Siblings and parents of schizophrenic patients also show a significant reduction in cancer incidence as compared with the general population.[50]

How the prevalence of neoplasia is related to tPA. Migration of endothelial cells to provide tumor nutrition requires significant upregulation of proteolysis. Conversely, chemical inhibition of the system reduces angiogenesis in vitro. The plasminogen system plays an essential role in tumor angiogenesis: tPA and plasmin can act either directly or indirectly, by activating MMP or by liberating growth factors and cytokines sequestered within the extracellular matrix.[51]

Conclusion

We suggest that inadequate synthesis and release of tPA or low tPA activity be considered a critical component of schizophrenia pathophysiology. Low levels of tPA or decreased tPA activity are common denominators of several biochemical abnormalities seen in schizophrenia. In addition, tPA actively participates in the mechanisms of neurogenesis and angiogenesis, which might justify not only the impaired neurogenesis but also the low prevalence of neoplastic diseases among schizophrenics. Translational trials are needed to support our hypothesis.

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PREVALÊNCIA DE MARCADORES DE BAIXA ATIVIDADE DO tPA EM PACIENTES COM ESQUIZOFRENIA OU TRANSTORNO ESQUIZOAFETIVO

Assumindo que a remissão dos sintomas psicóticos tenha resultado da normalização da atividade do tPA e/ou da plasmina pelo warfarin, postulamos que pacientes com esquizofrenia, não medicados com warfarin, teriam baixa atividade dessas serino proteases. Para provar esta hipótese, avaliamos a prevalência de marcadores de baixa atividade do tPA ou da plasmina em um grupo de 70 pacientes com esquizofrenia, diagnosticados por critérios estabelecidos no Manual de Diagnóstico e Estatística dos Transtornos Mentais (DSM)-IV. Os resultados foram comparados com os de 98 controles, sem transtorno mental.

O painel incluiu marcadores de baixa atividade do tPA metabólicos (aumento dos triglicerídeos, da insulina e homocisteína) e não metabólicos (deficiência das proteínas C funcional e S livre, o polimorfismo 4G/5G no gene do PAI-1, a mutação 20210 no gene da protrombina e anticorpos antifosfolipídio, como anticorpos anticardiolipina e anti-β2 glicoproteína I isotipos IgG e IgM e anticoagulante lúpico).

A hipertrigliceridemia é um potente estímulo para a síntese hepática de lipoproteínas de muito baixa densidade (VLDL), que carreiam triglicerídeos. O VLDL e a insulina, induzindo transcrição do PAI-1, reduzem a atividade do tPA (Figura 3).

Duas condições podem comprometer a função do tPA ou da plasmina, sem alterar os níveis: a hiperhomocisteinemia e anticorpos antifosfolipídio (Figura 4).

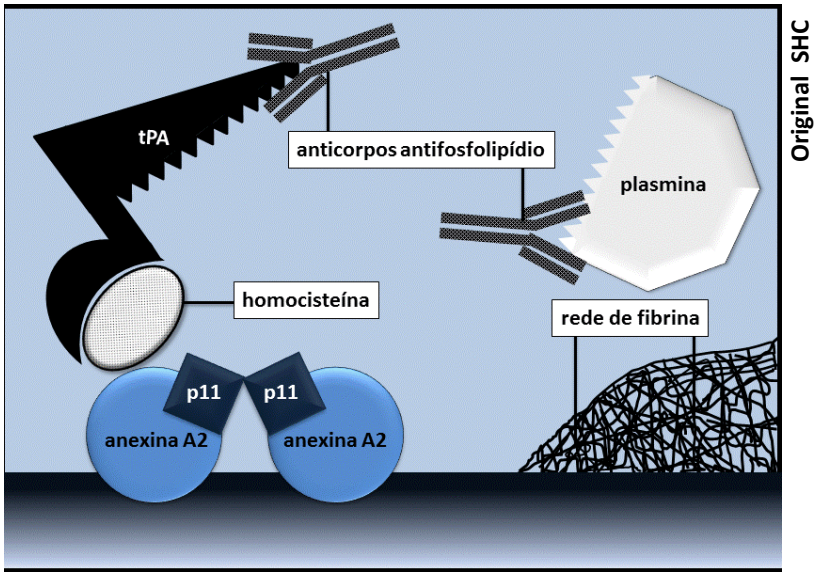

Figura 4. A homocisteína e os anticorpos antifosfolipídio podem afetar a atividade do tPA.

A carência de folato, das vitaminas B12 e B6, o consumo excessivo de café e a hiperinsulinemia podem contribuir para elevar os níveis de homocisteína.

A proteína S é um anticoagulante natural, que também tem propriedades antiapoptóticas, mitogênicas e tróficas. O aumento da atividade do tPA pela proteína S envolve a sua ligação à proteína C funcional, com resultante neutralização do PAI-1. A atividade da proteína S está reduzida na inflamação. Isto porque a C4b-BP uma grande glicoproteína que inibe a via do complemento, se liga à proteína S, inibindo-a. Ou seja, só a proteína S livre é ativa (Figura 5). Também está reduzida em pacientes que usam estrogênio, na gestação e puerpério.

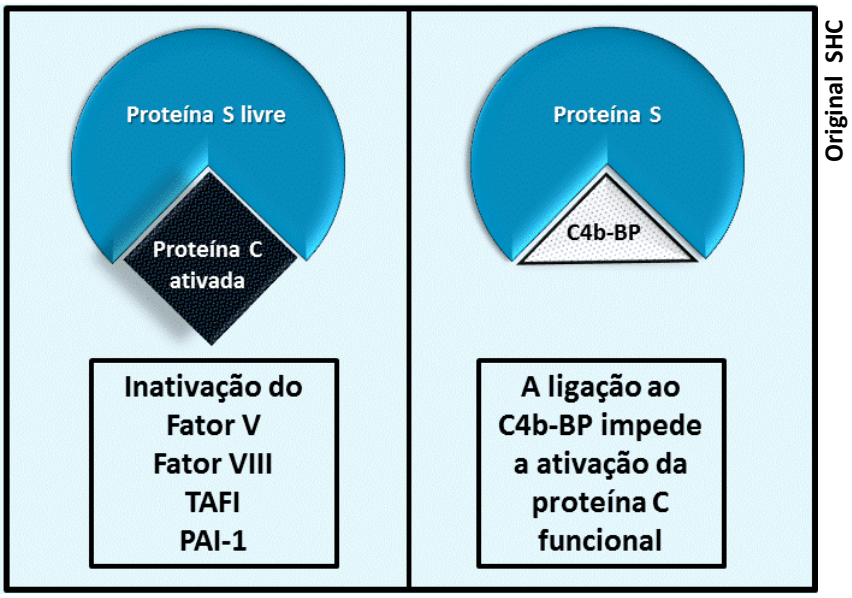

Figura 5. Proteína S livre, ativa e ligada ao C4b-BP (proteína ligadora à fração do complemento C4b). PAI-1, inibidor do ativador do plasminogênio; TAFI: inibidor da fibrinólise ativado pela trombina.

As propriedades antiapoptóticas, mitogênicas e tróficas da proteína S decorrem da sua interação e ativação dos receptores tirosina quinase TAM (Tyro3, Axl e Mer).[23] No sistema nervoso central, estes receptores que transferem fosfato promovem proliferação e diferenciação das células tronco neurais, além de influenciar a sobrevida das mesmas, em um mecanismo que envolve a regulação de neurotrofinas.[23]

Se a diminuição dos níveis da proteína S livre decorrente de processos inflamatórios é comum, a deficiência hereditária acomete apenas 0,03 a 0,1% da população geral. De 1510 pacientes acompanhados no Ambulatório de Trombofi-

lia do Hospital Federal dos Servidores do Estado, por exemplo, só cinco (0,3%) têm deficiência hereditária da proteína S. Mais de 200 mutações diferentes já foram descritas no gene que codifica a produção da proteína S, PROS1. Isto inviabiliza que se identifique uma prevalência aumentada de mutações que resultam em transcrição diminuída da proteína S, em grandes estudos genômicos de pacientes com esquizofrenia. A deficiência é transmitida por herança autossômica dominante.

A atividade do tPA também depende do polimorfismo 4G/5G no gene do PAI-1, de alta prevalência na população em geral. Comparado com cinco guaninas em série (5G), a sequência de quatro guaninas (4G) aumenta a transcrição do PAI-1, que é maior em indivíduos 4G/4G, menor nos 5G/5G e intermediária nos 4G/5G.

À exceção de dois pacientes, que eram usuários de cocaína no momento da pesquisa, todos os outros 68 tinham marcadores positivos (1–6 marcadores, média 2,1). Problemas metabólicos como hiperinsulinemia (44% vs. 11%), hipertrigliceridemia (29% vs. 11%) e hiperhomocisteinemia (27% vs. 6%) foram mais prevalentes em pacientes do que nos controles (Tabela 1).

Tabela 1. Marcadores metabólicos de baixa atividade do tPA e/ou da plasmina (modificado de Schizophr Res 2014; 159: 118-23.)

	Pacientes (n = 70)	**Controles (n = 98)**	**Taxa de risco (IC 95%)**	**P**
Hiperinsulinemia de jejum, com glicemia normal	41%	11%	5,6 (2,5-12,2)	<0,001
Insulina >50 µU/mL	10%	0	23,2 (1,3-414)	0,03
Limite máximo e mínimo, participantes com hiperinsulinemia (µU/mL)	13,8–119	14–28,1		<0,001
Hipertrigliceridemia de jejum	29%	11%	3,2 (1,4-7,1)	0,005
Triglicerídeos >300 mg/dL	7%	0	16,5 (0,9-304)	0,05
Limite máximo e mínimo, participantes com hipertrigliceridemia (mg/mL)	154–650	152–252		<0,001
Hipertrigliceridemia mais hiperinsulinemia	26%	7%	4,5 (1,8-11,4)	0,001
Hiperhomocisteinemia	27%	6%	5,7 (12,1-15,2)	<0,001
Homocisteína >30 µmol/L	3 (4%)	0	10,2 (0,5-200)	0,1
Limite máximo e mínimo, participantes com hiperhomocisteinemia (µmol/L)	13,6–59	13,7–20,1		<0,001
Hiperhomocisteinemia mais hiperinsulinemia	17%	0	42,0 (2,4-724)	0,01
2–4 alelos do polimorfismo da metilenotetrahidrofolato redutase C677T ou A1298C, participantes com hiperhomocisteinemia	42%	100%	0,05 (0,002-1,1)	0,06

Baixos níveis de proteína S livre foram encontrados em 21% dos pacientes e em nenhum controle. Só um dos pacientes com redução de proteína S livre (2%) tinha evidência de infecção e nenhuma participante estava no período gravídico-puerperal ou usava estrogênio, sugerindo deficiência hereditária em 20% dos pacientes. Um quinto dos pacientes com baixos níveis de proteína S livre (ponto de corte <67% para homens, <54% para mulheres) tinham tido um ou mais episódios de trombose venosa profunda. A deficiência de proteína S livre aumentou as chances de ter um parente de primeiro grau com esquizofrenia em 145 vezes (IC 95%, 15,7–1345). Nenhum paciente ou controle tinha deficiência da proteína C funcional.

A persistência de anticorpos antifosfolipídio em título alto ou moderado foi vista em 30% dos pacientes e em nenhum controle.

A prevalência dos polimorfismos do PAI-1 4G/5G e 4G/4G foi maior entre os pacientes do que nos controles, mas a diferença não teve significado estatístico, possivelmente por conta do pequeno número de participantes. No nosso estudo, a hiperinsulinemia e a hiperhomocisteinemia foram altamente sinérgicas com o polimorfismo PAI-1 4G/5G ou 4G/4G para o diagnóstico de esquizofrenia.

Tabela 2. Marcadores não metabólicos de baixa atividade do tPA e/ou da plasmina (modificado de Schizophr Res 2014; 159: 118-23.)

	Pacientes (n = 70)	Controles (n = 98)	Taxa de risco (IC 95%)	P
Persistência de ≥1 anticorpo antifosfolipídio em título médio ou alto	30%	0	85,5 (5,07-1442)	0,002
Anticoagulante lúpico forte positivo ou anticorpo anticardiolipina ≥80 MPL	9%			
Síndrome do anticorpo antifosfolipídio	8%			
Baixos níveis da proteína S livre	21%	0	55,0 (3,2-937)	0,005
Baixos níveis da proteína S livre mais hiperhomocisteinemia	6%			
Baixos níveis da proteína S livre mais hiperinsulinemia	10%			
Baixos níveis da proteína S livre mais anticorpos antifosfolipídio	6%			
Polimorfismo do PAI-1 4G/5G ou 4G/4G	60%	48%	1,6 (0,8-2,9)	0,1
Polimorfismo do PAI-1 4G/5G ou 4G/4G mais hiperinsulinemia	20%	2%	12,0 (2,6-54,7)	0,001
Polimorfismo do PAI-1 4G/5G ou 4G/4G mais hipertrigliceridemia	17%	5%	3,8 (1,3-11,4)	0,01
Polimorfismo do PAI-1 4G/5G ou 4G/4G mais hiperhomocisteinemia	24%	1%	31,1 (4,0-240)	0,001

É possível que nossos resultados tenham sido influenciados pela medicação psicotrópica. No entanto, já foi demonstrado que pacientes com esquizofrenia, virgens de tratamento, têm maior nível sérico dos precursores de insulina, de hipertrigliceridemia, de hiperhomocisteinemia e de anticorpos antifosfolipídio do que a população em geral.

Cabe ressaltar que anticorpos antifosfolipídio e baixos níveis de proteína S livre, apesar de altamente prevalentes no nosso grupo de pacientes, não foram preditores independentes da esquizofrenia em uma análise multivariada. Isto sugere que várias condições que comprometam a atividade do tPA ou da plasmina precisem existir de forma concomitante, para impedir o reparo neuronal.

Os achados, reforçando a hipótese de que indivíduos com esquizofrenia ou transtorno esquizoafetivo teriam baixa atividade do tPA, foram publicados como:

Hoirisch-Clapauch S, Nardi AE. Markers of low activity of tissue plasminogen activator/plasmin are prevalent in schizophrenia patients. Schizophr Res 2014; 159: 118-23.

- ✓ **O que o artigo tem de inovador**: Esse é o primeiro estudo da prevalência de trombofilias em pacientes com esquizofrenia.
- ✓ Nosso estudo também permite que se relacione a deficiência de proteína S a várias situações caracterizadas por alterações cognitivas ou episódios psicóticos, como o puerpério, o lupus eritematoso sistêmico e a síndrome de Sjögren. Além disso, oferece a primeira evidência – mesmo que indireta – de que a deficiência hereditária de proteína S possa ser um fator de risco para esquizofrenia.

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Markers of low activity of tissue plasminogen activator/plasmin are prevalent in schizophrenia patients

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ABSTRACT

Introduction: Clot buster tissue plasminogen activator (tPA) and its end-product plasmin play a well-defined role in neurochemistry. They mediate a number of events that culminate in tolerance against excitotoxicity, hippocampal neurogenesis, synaptic remodeling, neuronal plasticity, cognitive and emotional processing. Abnormalities in these processes have been implicated in schizophrenia pathogenesis.
Methods: Laboratory markers of low activity of tPA/plasmin were analyzed in 70 schizophrenia adults (DSM-IV), and 98 age-matched controls, consecutively selected at university hospitals.
Results: All but two patients had positive markers (1–6, mean 2.1). Twenty-nine patients and 11 controls had hyperinsulinemia (44% vs. 11%) and 20 patients and 11 controls had hypertriglyceridemia (29% vs. 11%). Both insulin and triglycerides stimulate production of plasminogen activator inhibitor (PAI)-1, a major tPA inhibitor. Nineteen patients and six controls had hyperhomocysteinemia (27% vs. 6%), a condition that impairs tPA catalytic activity. Fifteen patients (22%) but no controls had free-protein S deficiency, a condition that reduces PAI-1 inhibition. Twenty-one patients (30%) but no controls had 1–3 antiphospholipid antibodies in medium or/high levels. Such antibodies are able to inhibit tPA/plasmin activity. Both PAI-1 polymorphism 4G/5G and heterozygous prothrombin G20210A were more prevalent in patients (60% vs. 48% and 2% vs. 1%, respectively), but difference lacked significance. PAI-1 polymorphism was synergistic with hyperinsulinemia. Protein C deficiency was not detected in patients or controls.
Conclusion: We have found a high prevalence of markers of low tPA/plasmin activity in a sample of schizophrenia patients. Our findings should be validated in large studies, preferably in medication-naïve patients.

1. Introduction

We have previously reported that five patients with schizophrenia or schizoaffective disorders on warfarin therapy for long-term prevention of recurrent venous thromboembolism attained psychotic symptom remission and remain free of psychotropic medication for 2–11 years. On neuroimaging studies, none of these patients had brain ischemia (Hoirisch-Clapauch and Nardi, 2013a).

The only elements of the coagulation pathway that play a major role in the neurochemistry are clot buster tissue-plasminogen activator (tPA) and its end-product plasmin. tPA catalyzes important neuronal processes, such as cleavage of brain-derived neurotrophic factor precursor to anti-apoptotic mBDNF, proteolysis of vascular endothelial growth factor, N-methyl-D-aspartate (NMDA) receptor activation and regulation of dopamine release. As a result, tPA is involved in synaptic remodeling, neuronal plasticity, cognitive and emotional processing, in tolerance to excitotoxicity and hippocampal neurogenesis (reviewed in Hoirisch-Clapauch and Nardi, 2013b).

Assuming that psychotic symptom remission could have resulted from warfarin-induced normalization of tPA/plasmin activity, we decided to screen a group of schizophrenia patients for markers of low activity of these serine proteases, including metabolic markers (hyperhomocysteinemia, hyperinsulinemia and hypertriglyceridemia) and non-metabolic markers (lupus anticoagulant, anticardiolipin antibodies, anti-β2 glycoprotein 1 antibodies, low levels of functional protein C, low levels of free-protein S, 4G/5G polymorphism of the PAI-1 gene and prothrombin G20210A mutation). Two thrombophilias that do not affect the activity of tPA/plasmin, antithrombin III deficiency and factor V Leiden were also assessed. Results were compared to matched controls without any psychiatric disorder.

2. Methods

2.1. Study design

From January to December 2013, unrelated adults diagnosed with schizophrenia according to DSM-IV (American Psychiatric Association,

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1994), and age-matched controls without a psychiatric diagnosis were consecutively recruited for the study. Patients and controls were respectively selected among in- and outpatients regularly seen at a psychiatric clinic and from the staff of a general hospital, in Rio de Janeiro, Brazil. Exclusion criteria consisted of pregnancy or the puerperium, use of estrogen, insulin, metformin, selective serotonin reuptake inhibitors or anticoagulant therapy within the month before blood sampling.

Study protocol included a detailed chart review of the patients and a brief interview, aiming at assessing demographic information and medication, illicit drug use, cigarette smoking, and caffeine consumption, a physical examination and blood tests of all participants.

The study was approved by the Institutional Review Board as 0040.0.249.000-11. Informed written consent was obtained from every participant or his legal representative before enrollment in accordance with the Declaration of Helsinki.

2.2. Study population

The sample comprised 70 patients: 46 (66%) reported being Caucasians, 23 (33%) reported being Afro-Brazilians or Indigenous-Brazilians, and one (1%) reported being Asian. Of the 98 controls, 46 controls (47%) reported being Caucasians and 52 (53%) reported being Afro-Brazilians or Indigenous-Brazilians. Only three of the patients were medication-naïve. Table 1 summarizes the baseline characteristics of the study population.

2.3. Screening for thrombophilia disorders and metabolic abnormalities

Fasting blood samples for determination of glucose, insulin, C-peptide, creatinine, homocysteine, complete blood-cell count, erythrocyte sedimentation rate, free-protein S, functional-protein C, antithrombin III, antinuclear antibody, antiphospholipid antibodies and polymorphisms were drawn in the morning. When levels of free-protein S, functional-protein C, antithrombin III or lupus anticoagulant were not determined immediately, the plasma was frozen within 30 min of collection and stored at −80 °C until analysis.

Blood for homocysteine analysis was collected in tubes containing ethylenediaminetetraacetic acid. Homocysteine was determined by chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Germany). Free-protein S was quantified by immunoturbidimetric assay (Diagnostica Stago, France). Protein C and antithrombin III activity was measured by chromogenic assay (Siemens Healthcare Diagnostics, Germany). Deficiency of a natural anticoagulant had to be confirmed on a repeat sample after 4–12 weeks.

The presence of factor V Leiden G1691A, prothrombin G20210A, the methylenetetrahydrofolate reductase gene polymorphisms C677T and A1298C and the 4G/5G polymorphism in the plasminogen activator inhibitor (PAI)-1 promoter was determined by polymerase chain reaction (PCR) with sequence specific primers (Roche Diagnostics GmbH, Germany).

Table 1
Sample characteristics.

	Patients ($n = 70$)	Controls ($n = 98$)
Males	38 (54%)	49 (50%)
Age range (years)	18–72	18–71
Age (mean ± standard deviation)	42 ± 11	43 ± 14
Caucasians	46 (66%)	46 (47%)
Drug abusers	13 (19%)	3 (3%)
Cigarette smokers (one or more packs per day)	33 (47%)	6 (6%)
Treatment-refractory schizophrenia	41 (59%)	0
Schizophrenia patients refractory to clozapine	17 (24%)	0
Use of atypical antipsychotic within 30 days before sampling	21 (30%)	0
Blood sampling during an acute episode	29 (41%)	0
Past electroconvulsive therapy	33 (47%)	0

Anticardiolipin and anti-β2 glycoprotein I antibodies were measured with enzyme-linked immunosorbent assay (Diagnostica Stago, France). Lupus anticoagulant was screened using dilute Russell's viper venom time reagent and LA2 confirmation test (Siemens Healthcare Diagnostics, Germany), according to the International Society on Thrombosis and Haemostasis guidelines (Pengo et al, 2009). When antiphospholipid antibodies were detected, the test was repeated after >12 weeks to confirm persistence and sera were tested for antinuclear antibodies by indirect immunofluorescence assay on HEp-2 cells (Bio-Rad, USA).

2.4. Definitions

Heavy smoking was defined as self-reported consumption of ≥20 cigarettes per day. High caffeine consumption was defined as ≥750 mg of caffeine per day as filtered coffee. Hyperhomocysteinemia was defined as homocysteine levels ≥13.2 μmol/L. Hyperinsulinemia was defined as ≥20% increase in insulin values according to quartiles of body mass index, with normal fasting glucose levels. Hypertriglyceridemia was defined as fasting triglycerides ≥150 mg/dL. Cut-off value for anticardiolipin antibody or anti-β2 glycoprotein I antibody was: medium levels ≥40 GPL- or MPL-U/mL and high levels ≥80 GPL- or MPL-U/mL. Cut-off value for positive lupus anticoagulant was ≥1.3 and for strong lupus anticoagulant, ≥2. Cut-off value for low free-protein S was <67% for men and <54% for women.

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviation and compared by two-tailed Student's *t* test. Discrete variables are presented as percentage and compared using chi-square analysis or Fisher's exact test, as appropriate. Alpha significance was set at 0.05. Softwares Epi Info version 7 and "R" were used for data analysis.

3. Results

Seventy-six patients were invited. Two declined and 74 were enrolled, but four failed to complete all aspects of the protocol. One hundred controls were invited and enrolled, but only 98 completed the study. Of the 70 patients studied, 68 had 1–6 markers of low tPA/plasmin activity (mean 2.1). Chronic patients and those studied during acute episodes exhibited the highest number of markers (3–6 markers per patient, mean 3.1). The two patients without markers were cocaine abusers at the time of the survey. Missing data comprised <2% of all results.

3.1. Laboratory data: metabolic markers

Table 2 shows the prevalence of metabolic markers among patients and controls. Four patients had fasting hyperglycemia (126–197 mg/mL) and therefore their insulin levels were not measured. All patients and controls with hyperinsulinemia had also elevated C-peptide levels, indicating increased insulin production. Elevated insulin levels were found in nine lean patients who were not on atypical antipsychotics or chlorpromazine and did not have any infectious or inflammatory disorder.

All 12 patients who displayed both hyperhomocysteinemia and hyperinsulinemia were heavy smokers and high caffeine consumers, with coffee sweetened with sugar. Four controls with hyperhomocysteinemia were heavy smokers and high caffeine consumers, but coffee was sweetened with non-caloric sweeteners. One patient with hyperhomocysteinemia had pernicious anemia. All participants with hyperhomocysteinemia had normal creatinine levels.

Table 2
Metabolic markers of low tPA/plasmin activity.

	Patients ($n = 70$)	Controls ($n = 98$)	Odds ratio (95% CI)	*P*
Fasting hyperinsulinemia with normal glucose levels	29 (41%)	11 (11%)	5.59 (2.54–12.2)	<0.001
Insulin levels ≥50 μU/mL	7 (10%)	0	23.2 (1.30–414)	0.03
Mean insulinemia (participants with hyperinsulinemia, μU/mL)	41 ± 27	19 ± 6		<0.001
Insulin range (participants with hyperinsulinemia, μU/mL)	13.8–119	14–28.1		<0.001
Hyperinsulinemia with grade III obesity (body mass index ≥40 kg/m^2)	3 (4%)	0	10.2 (0.51–200)	0.1
Elevated C-peptide	31 (44%)	11 (11%)	6.28 (2.86–13.7)	<0.001
Fasting hypertriglyceridemia	20 (29%)	11 (11%)	3.16 (1.40–7.13)	0.005
Triglycerides >300 mg/dL	5 (7%)	0	16.5 (0.89–304)	0.05
Mean triglyceridemia (participants with hypertriglyceridemia, mg/mL)	276 ± 120	167 ± 29		<0.001
Triglyceride range (participants with hypertriglyceridemia, mg/mL)	154–650	152–252		<0.001
Hypertriglyceridemia plus hyperinsulinemia	18 (26%)	7 (7%)	4.50 (1.76–11.4)	0.001
Hyperhomocysteinemia	19 (27%)	6 (6%)	5.71 (12.1–15.2)	<0.001
Homocysteine levels >30 μmol/L	3 (4%)	0	10.2 (0.51–200)	0.1
Mean homocysteinemia (participants with hyperhomocysteinemia, μmol/L)	21.1 ± 11	14.4 ± 0.7		<0.001
Homocysteine range (participants with hyperhomocysteinemia μmol/L)	13.6–59	13.7–20.1		<0.001
Hyperhomocysteinemia plus hyperinsulinemia	12 (17%)	0	42.0 (2.44–724)	0.01
2–4 alleles of the methylenetetrahydrofolate reductase C677T or A1298C polymorphism (participants with hyperhomocysteinemia)	8 (42%)	6 (100%)	0.05 (0.002–1.15)	0.06
2–4 alleles of the methylenetetrahydrofolate reductase C677T or A1298C polymorphism (participants with hyperhomocysteinemia)	8 (42%)	6 (100%)	0.05 (0.002–1.15)	0.06
2–4 alleles of the methylenetetrahydrofolate reductase C677T or A1298C polymorphism (all participants)	16 (23%)	14 (14%)	1.77 (0.8–3.93)	0.1

3.2. Laboratory data: non-metabolic markers

Table 3 shows the prevalence of non-metabolic markers among patients and controls. One patient had three antiphospholipid antibodies, three patients had two, and 18 had one antibody. All patients with positive anti-β2 glycoprotein I antibody also had anticardiolipin antibodies in medium or high titer, but the reverse was not true. Although four patients with one or more positive antiphospholipid antibodies had had recurrent deep-vein thrombosis, none of them was treated with anticoagulants for more than six months.

Of the 15 patients with low free-protein S levels, three were women (range: 38–50%) and 12 were men (range: 32–66%). Three of the patients with low levels of free-protein S had had one or more episodes of deep-vein thrombosis, and six had at least one first-degree relative with a history of thromboembolism. All patients with reduced levels of free-protein S had normal erythrocyte sedimentation rate and normal leukocyte count, without left shift or toxic granulation, except for a patient with periodontitis, whose free-protein S levels were 32–40%. Another patient with severe periodontitis had normal levels of free-protein S.

Nine of the 15 patients with free-protein S deficiency and one control reported one or more first-degree relatives with schizophrenia. Compared to controls, low levels of free-protein S increased the chances of having a first-degree relative with schizophrenia by 145 times (95% confidence interval, 15.7–1345.6).

Thrombophilias that may affect tPA/plasmin activity, which were not significantly different in patients and controls, comprised PAI-1 polymorphism 4G/5G or 4G/4G (60% vs. 48%, $P = 0.1$), heterozygous prothrombin G20210A (2% vs. 1%, $P = 0.4$), and functional protein C deficiency (not detected in both groups). Prevalence of thrombophilias that do not affect tPA/plasmin activity, such as heterozygous factor V Leiden (4% vs. 2%, $P = 0.2$) and antithrombin III deficiency (not detected in both groups), was not significantly different.

3.3. Multivariate analysis

In a multivariate analysis, only hyperinsulinemia, hyperhomocysteinemia and hypertriglyceridemia remained as independent predictors of schizophrenia.

Table 3
Non-metabolic markers of low tPA/plasmin activity.

	Patients ($n = 70$)	Controls ($n = 98$)	Odds ratio (95% CI)	*P*
Any persistent antiphospholipid antibody in medium or high titer	21 (30%)	0	85.5 (5.07–1442)	0.002
Positive lupus anticoagulant	16			
Strong positive lupus anticoagulant	7			
IgM anticardiolipin antibody	6			
High titer IgM anticardiolipin antibody	2			
Primary antiphospholipid antibody syndrome	8			
Low free-protein S levels	15 (22%)	0	55.0 (3.22–937)	0.005
Free-protein S range (women with low free-protein S levels, %)	38-50			
Free-protein S range (men with low free-protein S levels, %)	32-66			
Low-protein S levels plus obesity (BMI = 30 and 33 kg/m^2)	2			
Low-protein S levels plus invasive infection	1			
Low protein S levels plus hyperhomocysteinemia	4 (6%)			
Low-protein S levels plus hyperinsulinemia	7 (10%)			
Low-protein S levels plus antiphospholipid antibodies	4 (6%)			
PAI-1 4G/5G or 4G/4G polymorphism	42 (60%)	48 (48%)	1.56 (0.83–2.9)	0.1
PAI-1 4G/5G or 4G/4G polymorphism plus hyperinsulinemia	14 (20%)	2 (2%)	12.0 (2.63–54.7)	0.001
PAI-1 4G/5G or 4G/4G polymorphism plus hypertriglyceridemia	12 (17%)	5 (5%)	3.84 (1.28–11.4)	0.01
PAI-1 4G/5G or 4G/4G polymorphism plus hyperhomocysteinemia	17 (24%)	1 (1%)	31.1 (4.02–240)	0.001
PAI-1 4G/5G or 4G/4G polymorphism plus low protein S levels	6 (9%)	0	19.8 (1.09–358)	0.04
PAI-1 4G/5G or 4G/4G polymorphism plus antiphospholipid antibodies	11 (16%)	0	38.0 (2.20–658)	0.01

4. Discussion

Considering that most patients studied were not drug-naïve, it is possible that some of our findings were influenced by antipsychotic medication. Nonetheless, some authors have reported a high prevalence of markers of low tPA activity, such as antiphospholipid antibodies, hyperinsulinemia or hyperhomocysteinemia in medication-naïve schizophrenia patients (Delluc et al., 2014; Kale et al, 2010; Ryan et al., 2003). Additionally, it has been demonstrated that schizophrenia patients exhibit high levels of PAI-1, independent of antipsychotics and that PAI-1 levels are also elevated in first-degree relatives of schizophrenia patients (Carrizo et al., 2008).

Conditions such as hyperhomocysteinemia or antiphospholipid antibodies may affect the catalytic properties of tPA or plasmin (Hajjar et al., 1998; Krone et al., 2010) rather than tPA or plasmin levels. Due to lack of availability of tests that could assess tPA activity in our laboratories, we decided to search for markers of low activity of tPA or plasmin.

4.1. Metabolic markers

Although polymorphisms may contribute to an increased risk for hyperhomocysteinemia, metabolic markers of low tPA/plasmin activity are usually acquired conditions.

Increased insulin levels may impair activity of plasminogen activator because PAI-1 promoter has receptors for insulin and insulin precursors (Fig. 1). Increased insulin synthesis may result from excessive consumption of carbohydrates coupled with lack of physical activity or from peripheral resistance to insulin action. Insulin resistance, defined as abnormal response of skeletal muscles to the hormone, is usually mediated by tumor necrosis factor (TNF)-α, a cytokine produced during inflammatory processes, such as infection and obesity (Carvalho-Filho et al., 2005). Insulin resistance can also derive from continuous skeletal muscle exposure to insulin, which causes degradation of occupied receptors, reducing the number of receptors on the myocyte surface (Gavin et al., 1974).

Hyperinsulinemia due to insulin resistance is a common side-effect of both conventional and atypical antipsychotics (Teff et al., 2013). However, different authors have shown that treatment-naïve schizophrenia patients display higher serum levels of insulin precursors than controls, which attests increased production of insulin (Guest et al., 2010; Ryan et al., 2003). It seems that high carbohydrate consumption coupled with a sedentary lifestyle may contribute to the pathophysiology of hyperinsulinemia in schizophrenia patients, medication-naïve or not.

Hyperinsulinemia is a risk factor for two prevalent comorbidities in unmedicated schizophrenia patients: high body mass index and hypertension (Mitchell et al., 2013). The hormone promotes fat accumulation, which may lead to obesity (Shanik et al., 2008) and aldosterone produced by perivascular fat increases tensional levels (Briones et al., 2012).

PAI-1 promoter responds to very-low density lipoprotein (VLDL), the most important carrier of triglyceride in plasma (Fig. 1). Hypertriglyceridemia provides a robust stimulus for hepatic VLDL synthesis and VLDL-triglyceride induces PAI-1 transcription in endothelial cells, which is greater in individuals with PAI-1 polymorphism 4G/5G or 4G/4G (Eriksson et al., 1998).

Hyperhomocysteinemia is a classic risk factor for venous and arterial occlusions. Normal homocysteine levels depend on the intake and absorption of folate, cobalamin and pyridoxine, and on folate recycling. Homozygous or double-heterozygous individuals with methylenetetrahydrofolate reductase polymorphism C677T or A1298C have a decreased ability to recycle folate (Weisberg et al., 1998). In these patients, low folate intake contributes to increase homocysteine levels and folic acid supplementation is usually effective in correcting the problem.

Plasma homocysteine levels are inversely related to glomerular filtration rate and directly related to serum insulin levels, cigarette smoking, and coffee drinking (Björck et al, 2006; Nurk et al., 2004; Van Guldener et al., 2001; Verhoef et al., 2002). The percentage of heavy smokers among schizophrenia patients is high. Cigarette smoking causes bronchoconstriction and caffeine bronchodilation. Heavy coffee drinking is highly prevalent among heavy smokers and if coffee is consumed with sugar, heavy coffee drinking can contribute to hyperinsulinemia.

Different studies have shown a high prevalence of hyperhomocysteinemia in schizophrenia patients (Levine et al., 2002). The correlation is high: an increase of 5 μmol/L increases the risk of schizophrenia by 1.7 (Muntjewerff et al., 2006).

4.2. Non-metabolic markers

Non-metabolic abnormalities that impair tPA/plasmin activity include hereditary, acquired and mixed hereditary and acquired disorders. Since

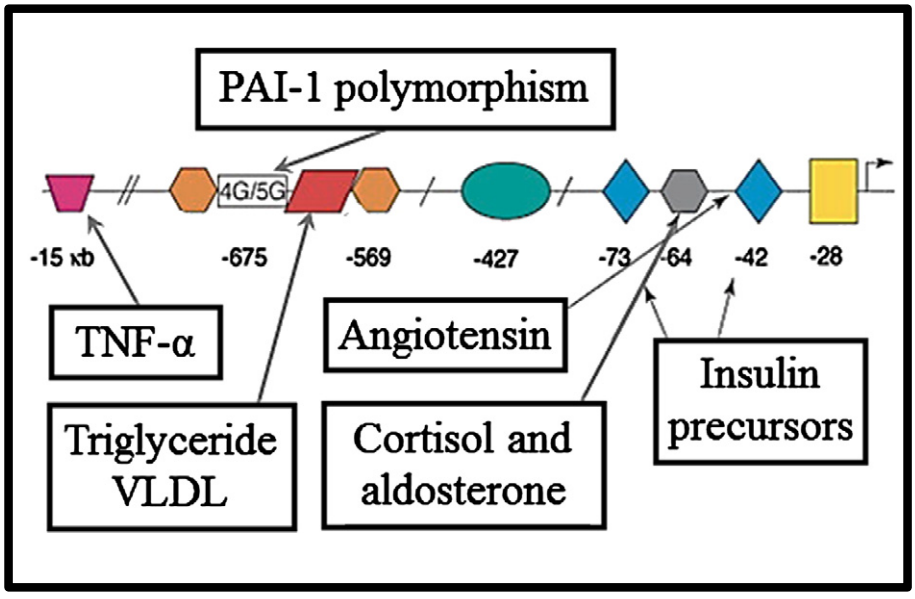

Fig. 1. Plasminogen activator inhibitor (PAI)-1 promoter responds to tumor necrosis factor (TNF)-α, to triglycerides and very-low density lipoproteins (VLDL), to angiotensin, to cortisol and aldosterone and to insulin precursors. A common guanosine deletion in the promoter region of the PAI-1 gene, known as PAI-1 polymorphism 4G/5G, is associated with increased PAI-1 transcription. Modified from Vaughan, 2005. PAI-1 and atherothrombosis. J. Thromb. Haemost 3 (8) 1879-83.

hereditability of schizophrenia is high, a number of studies have been trying to identify chromosomal regions that could harbor schizophrenia genes (Allen et al., 2008).

A non-metabolic condition prevalent in our sample of schizophrenia patients was protein S deficiency. Protein S, which has been shown to have mitogenic properties, accelerates neutralization of PAI-1, therefore increasing tPA activity (de Fouw et al., 1986; Rezende et al., 2004). Protein S deficiency is an autosomal-dominant coagulation abnormality that affects only 0.03–0.1% of the general population (Middeldorp and van Hylckama, 2008). Several mutations resulting in protein S deficiency have been described (Lind-Hallden et al., 2012), which explains why genoma studies have been failed to identify correlation. Indeed, functional assays are recommended to identify the deficiency.

Inflammatory disorders, activating complement, may reduce protein S activity. Protein S circulates as free active form or bound to a component of the complement pathway. There are several reports of cognitive abnormalities or psychotic episodes in situations characterized by low protein S levels, such as the puerperium, systemic lupus erythematosus, Sjögren syndrome or AIDS (Bissuel et al., 1992; Blanc et al., 2013; Busby et al., 2013; Faught et al., 1995; Jennekens and Kater, 2002; Spinelli, 2009; Zadura et al., 2009) We cannot rule out the possibility that some patients with low free-protein S levels had inflammatory disorders. However, since schizophrenia was highly prevalent in first-degree relatives of these patients, we suspect that at least in some cases the condition was inherited.

Stress may increase protein S levels (Hannan et al, 2000) and since nine patients in this study with normal protein S levels reported fear of needles, it is possible that the prevalence of reduced free-protein S levels among patients was underestimated.

Antiphospholipid antibodies were highly prevalent in our sample of patients, but no control exhibited any of the five antibodies. A high incidence of acute psychotic episodes has been reported in patients with antiphospholipid antibodies in medium or high levels (Kurtz and Muller, 1994; Schwartz et al, 1998). In some of them, thrombotic events or obstetric complications that characterize antiphospholipid antibody syndrome may be preceded by psychiatric symptoms for years (Eaton et al., 2010). Positive lupus anticoagulant and anticardiolipin antibodies are present in 0.2% and 2% of the general population, respectively (Lockwood et al., 1989). However, a strong lupus anticoagulant or high levels of IgM anticardiolipin antibodies, detected respectively in seven (10%) and two (3%) patients, are extremely rare.

4.3. Non-significant variables

Prevalence of a common polymorphism in the PAI-1 promoter, known as PAI-1 4G/5G, was higher among patients than controls, but because of the small number of participants difference did not achieve statistical significance. PAI-1 activity is highest in 4G/4G homozygotes, lowest in 5G/5G homozygotes, and intermediate in 4G/5G heterozygotes (Fig. 1). PAI-1 promoter is up-regulated by inflammatory cytokines, such as interleukin-6, TNF-α and tumor growth factor-β, and by renin, angiotensin and aldosterone (reviewed in Brown, 2010). We postulate that inflammatory conditions could increase the risk of schizophrenia through mechanisms involving increased PAI-1 levels and/or decreased free-protein S levels. As seen above, PAI-1 levels are influenced by insulin and VLDL-triglyceride levels. In this study, hyperinsulinemia, but not hypertriglyceridemia was synergistic with PAI-1 polymorphism 4G/5G or 4G/4G for schizophrenia diagnosis.

Neither factor V Leiden nor deficiency of antithrombin III is associated with reduced activity of tPA/plasmin and according to our hypothesis we were not expecting to find an increased prevalence of these thrombophilias in individuals with schizophrenia. Nonetheless, we were expecting an increased prevalence of both protein C deficiency and prothrombin G20210A mutation – a single base mutation that causes prothrombin levels to be elevated – in patients than in controls. One possible explanation is that both thrombophilias are infrequent among Brazilians. The other explanation is that both protein C deficiency and prothrombin G20210A impair fibrinolysis through an effect mostly mediated by activatable fibrinolysis inhibitor (TAFI), rather than by tPA/plasmin (Colucci et al., 2004; Esmon, 2003).

4.4. A proposed model for schizophrenia involving low tPA/plasmin activity

Psychotic episodes can be triggered by traumatic events. During highly stressful situations the hippocampus is bombarded by substances, such as cortisol, adrenaline and noradrenaline (Brown et al, 1999; Jensen et al, 2011), which stimulate the synthesis of PAI-1, a potent inhibitor of tPA. PAI-1 promoter has glucocorticoid response (Van Zonneveld et al., 1988). Although epinephrine per se inhibits PAI-1 synthesis, it stimulates the synthesis of angiotensin II, which potentiates the stimulus for synthesis of PAI-1 by cortisol (Brown et al, 2000). Adrenaline causes hyperglycemia through activation of glycogen synthase and dephosphorylation of serine 641, while cortisol promotes a state of insulin resistance (Jensen et al, 2011; Lundberg, 2005). In these circumstances, there is compensatory insulin production, which further stimulates the synthesis of PAI-1 (Festa et al., 1999). It has been proposed that PAI-1 has a negative impact on psychological well-being, as occurs after traumatic events (McEwen and Gianaros, 2010).

Healthy individuals exposed to traumatic events are expected to recover. In our proposed model of schizophrenia, aberrant tPA/plasmin functioning would not only render neurons vulnerable to excitotoxicity, but would also prevent restoring neurogenesis, thus decreasing gray matter volume (Asami et al., 2012). The finding that markers highly prevalent in patients, such as antiphospholipid antibodies and low free-protein S levels, were not significant in a multivariate analysis somehow reinforces the hypothesis that several conditions impairing tPA/plasmin activity must be met in order to increase neuronal susceptibility and prevent repair processes.

5. Limitations

One limitation of our study is that we did not take into account confounders such as high blood pressure, body-mass index, smoking, coffee-drinking, alcohol intake and drug abuse, recent acute psychotic episode, or schizophrenia duration. Additionally, it is imperative that our findings be validated in medication-naïve, first-episode patients.

6. Conclusions

We have found a high prevalence of markers of reduced tPA/plasmin activity in a sample of schizophrenia patients, compared to controls. Our findings should be validated in large studies, preferably in medication-naïve patients. Randomized controlled interventions are required to examine if interventions aimed at correcting tPA/plasmin activity may improve the course of schizophrenia.

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Contributors

Both authors have equally contributed to the manuscript.

Conflict of interest

Dr. Hoirisch-Clapauch and Dr. Nardi report no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2014.08.011.

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A INFLUÊNCIA DA BAIXA ATIVIDADE DO tPA NAS COMORBIDADES DA ESQUIZOFRENIA

Supondo-se que a atividade do tPA esteja seriamente comprometida na esquizofrenia, era de se esperar que problemas relacionados à incapacidade de degradar a fibrina do coágulo ou de modificar a estrutura da matriz extracelular fossem comuns nestes pacientes. De fato, pacientes com esquizofrenia têm elevado risco de doenças cardiovasculares e tromboembólicas, obesidade, disfunções sexuais e reprodutivas, doenças inflamatórias, o tabagismo e o abuso de substâncias psicoativas.

A dificuldade de dissolver a fibrina do coágulo é uma das explicações para a alta prevalência de doenças cardiovasculares e tromboembólicas, que contribuem para que a expectativa de vida nesses pacientes seja cerca de 15 anos menor que a da população em geral. De 253 pacientes com esquizofrenia ou transtorno esquizoafetivo, avaliados por nós no Instituto de Psiquiatria da UFRJ, 23% já tiveram trombose venosa profunda ou acidente vascular cerebral, 74% dos quais são classificados como trombofílicos fortes (dados não publicados).

A relação entre a obesidade e os níveis de PAI-1 é complexa. Adipócitos produzem PAI-1 e também produzem citocinas que estimulam a produção de PAI-1. Além disso, a insulina e a leptina estimulam o promotor do PAI-1. Mais de 20% dos pacientes com esquizofrenia ou transtorno esquizoafetivo são obesos ou têm excesso ponderal.

A proteólise extracelular alterada prejudica a ovulação e a angiogênese placentária, o que dificulta a reprodução nesse grupo de doentes. A evidência de que a patogênese da psicose tenha aspectos em comum com a patogênese de disfunções reprodutivas é enfatizada pelo risco de natimortos ou recém-natos de baixo peso, que é duas vezes maior em gestações que cursam com um episódio psicótico do que população em geral, mesmo quando se controla para a taxa de tabagismo. Mães com psicose afetiva também têm maior risco de partos prematuros, bebês de baixo peso ou com baixo peso para a idade gestacional, do que as mães da população em geral.

A repercussão da baixa atividade do tPA não se restringe à angiogênese fisiológica. Tudo leva a crer que a inibição da angiogênese tumoral contribua

para que a prevalência de tumores malignos nos pacientes com esquizofrenia seja menor que a esperada.

Doenças inflamatórias também aumentam o risco para esquizofrenia. A inflamação se acompanha de resistência insulínica e aumento nas citocinas. O promotor do PAI-1 responde à insulina e a citocinas, ou seja, a atividade do tPA é menor nas doenças inflamatórias. Por conta da ativação do sistema do complemento, a atividade da proteína S também está reduzida nas doenças inflamatórias (Figuras 3 e 5).

Se considerarmos que a deficiência de tPA é um elemento de suma importância na fisiopatologia da esquizofrenia, o abuso de drogas e o tabagismo pesado poderiam ser considerados uma "tentativa de automedicação".[24] Isto porque a morfina, a cocaína, a anfetamina e a nicotina elevam os níveis de tPA no núcleo *accumbens*.

A hipótese defendendo que a hipofibrinólise e as alterações da proteólise participem da constelação etiológica de diversas comorbidades não iatrogênicas da esquizofrenia foi publicada em dois artigos:

Hoirisch-Clapauch S, Nardi AE. Low activity of plasminogen activator: a common feature of non-iatrogenic comorbidities of schizophrenia. CNS Neurol Disord Drug Targets 2015; 14: 325-30.

Hoirisch-Clapauch S, Brenner B, Nardi AE. Adverse obstetric and neonatal outcomes in women with mental disorders. Thromb Res 2015; 135: S60-3.

- ✓ **O que os artigos têm de inovador**: Fornecem uma explicação para o alto risco de doenças cardiovasculares e tromboembolismo venoso, observado de forma consistente nos pacientes com esquizofrenia, inclusive nos virgens de tratamento especializado.
- ✓ Também ajudam a compreender outras comorbidades da psicose em apreço, como o alto risco para complicações obstétricas e perinatais, e a alta prevalência de usuários de substâncias psicoativas e de tabagistas nesse grupo.

Low Activity of Plasminogen Activator: A Common Feature of Non-Iatrogenic Comorbidities of Schizophrenia

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Abstract: Understanding the pathogenesis of non-iatrogenic comorbidities of schizophrenia may provide insights into the pathogenesis of schizophrenia itself. First-episode, drug-naïve schizophrenia patients are at high risk of thromboembolic events, diseases related to substance abuse, sexual dysfunction, reproductive disorders, inflammatory and autoimmune diseases, as well as complications of hyperinsulinemia or hyperhomocysteinemia. This review focuses on the role of reduced plasminogen activator activity in non-iatrogenic comorbidity of schizophrenia. By preventing thrombus dissolution, low tissue plasminogen activator activity increases the risk of thrombotic events. Components of the plasminogen activator system also play a key role in reproduction. Both illicit drugs and tobacco increase plasminogen activator levels in the central nervous system, which seems to relieve symptoms of the mental disorder. Chronic alcoholism, sexual dysfunction, inflammatory and autoimmune disorders, and complications of hyperinsulinemia or hyperhomocysteinemia are somehow related to low plasminogen activator activity. Plasminogen activator mediates several neurochemical processes that seem to prevent or reverse gray-matter atrophy seen in first-episode schizophrenia patients. Such processes include cleavage of brain-derived neurotrophic factor precursor to an anti-apoptotic neurotrophin and activation of N-methyl-D-aspartate receptor. Controlled, randomized studies are needed to determine if measures aimed at correcting plasminogen activator activity can improve the quality of life, reduce morbidity and mortality rates, and particularly improve the course of schizophrenia.

Keywords: Comorbidity, drug naïve, medication naïve, metabolism, plasminogen activator, schizophrenia.

INTRODUCTION

The observation that five patients with schizophrenia spectrum disorders on chronic warfarin therapy for recurrent deep vein thrombosis entered psychiatric remission, and were able to withhold antipsychotic agents, led us to postulate that tissue plasminogen activator (tPA), an element of the coagulation pathway, could play an important role in schizophrenia pathogenesis [1] (Fig. **1**).

Clot-buster tPA mediates important neurochemical reactions that prevent or reverse gray-matter atrophy [2-4], a condition already seen in first-episode patients [5]. Such reactions include cleavage of brain-derived neurotrophic factor precursor (proBDNF) to an anti-apoptotic neurotrophin [6] and activation of N-methyl-D-aspartate (NMDA) receptor. Activated NMDA receptor upregulates cyclin-dependent kinase inhibitor p21, which inhibits apoptosis and is involved in neuronal regeneration in models of cerebral hypoxia and ischemia *in* and *ex vivo* [7].

To endorse our hypothesis, we have searched for markers of low tPA activity in a group of 70 schizophrenia patients, and 98 controls. Many markers of low tPA activity, including hyperinsulinemia, hyperhomocysteinemia, low levels of free-protein S and antiphospholipid antibodies were highly prevalent in schizophrenia patients [8].

Given that most schizophrenia patients studied were not drug naïve, we wished to investigate whether tPA plays a role in the pathogenesis of non-iatrogenic disorders that accompany schizophrenia.

THROMBOSIS AND CARDIOVASCULAR DISORDER

Thrombotic events and cardiovascular disorder account for the 2-3 times higher mortality rate in schizophrenia patients than in the general population [9-11]. The increased risk of thromboembolic events [12, 13] is usually associated with psychotropic medication and with immobility, as in restraint or catatonia. However, based on evidence that first-episode patients display higher levels of markers of thrombogenesis than controls, it has been postulated that mechanisms involved in the pathogenesis of psychosis might also contribute to the thrombotic tendency [13]. There is no evidence of increased risk of myocardial infarction or arterial stroke in drug-naïve schizophrenia patients [14, 15], possibly because the mental disorder is typically diagnosed before the age of 30.

By preventing intravascular thrombus degradation, low tPA activity increases the chances of both venous and arterial occlusions.

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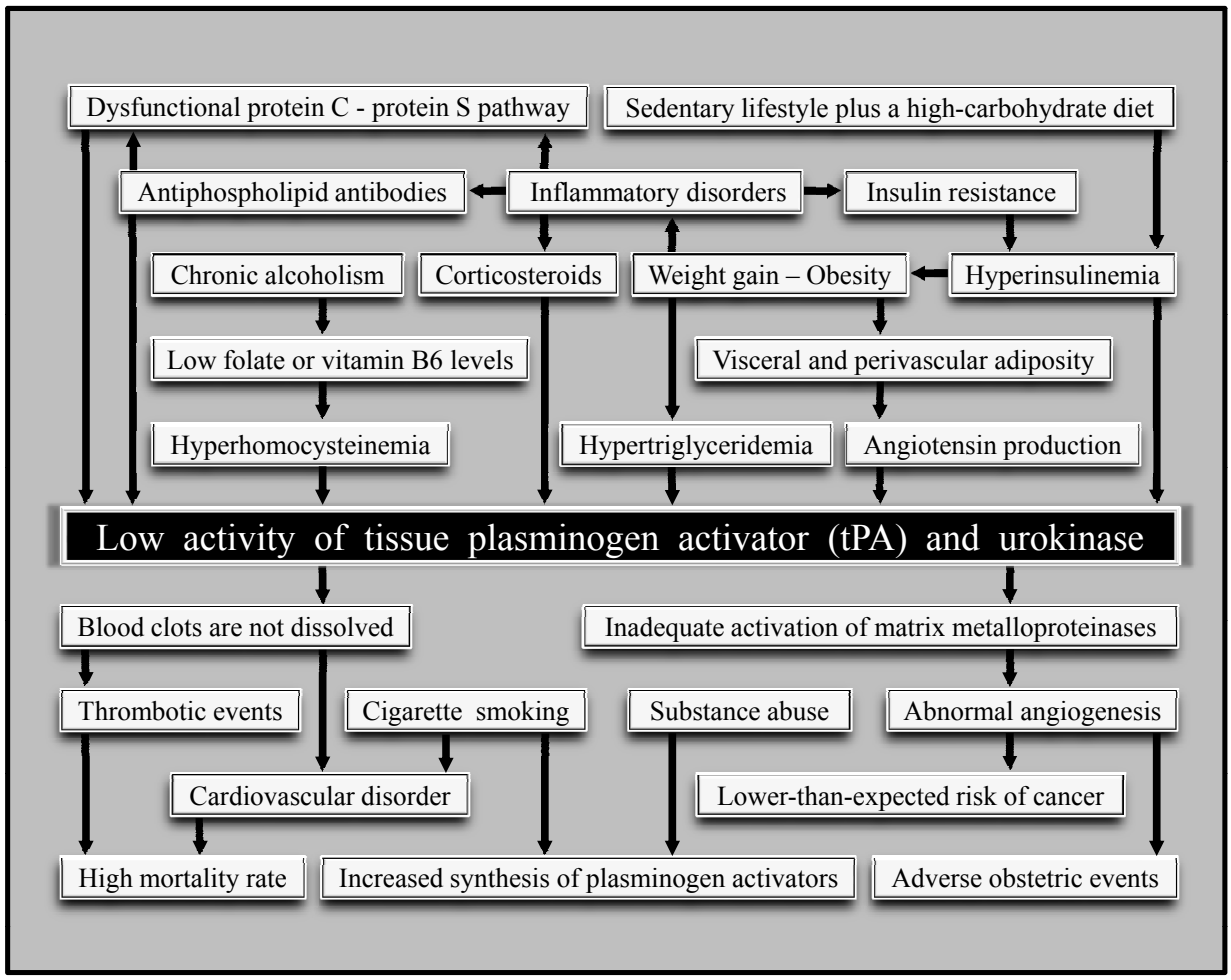

Fig. (1). How plasminogen activator influences non-iatrogenic comorbidity of schizophrenia.

SUBSTANCE ABUSE

The most common comorbidity of schizophrenia seems to be substance abuse [16]. Illicit drug use and cigarette smoking have been considered an attempt at self-medication by schizophrenia patients [17, 18].

Drug addiction is highly prevalent in first-episode patients, with hallucinogens and stimulants being the most commonly used [19]. Cannabis decreases the levels of plasminogen activator inhibitor (PAI)-1, a major inhibitor of plasminogen activators urokinase and tPA [20, 21].

Chronic cocaine use induces both tPA and urokinase mRNA in the mesolimbic dopaminergic pathway, including the ventral tegmental area, the nucleus accumbens and the hippocampus, while acute drug use induces preferably urokinase, with slight but significant tPA changes in the nucleus accumbens and the striatum [22]. Repeated but not-single dose methamphetamine induces the expression of tPA mRNA in the frontal cortex, nucleus accumbens, striatum, and hippocampus [23]. tPA and urokinase play important roles in regulating the rewarding and reinforcing effects of cocaine and methamphetamine [24].

If prevalence of drug-naïve schizophrenia patients who abuse cocaine or hallucinogens is high, prevalence of heavy cigarette smokers is huge. Even though tobacco smoking increases tPA levels in the central nervous system [25], it may increase cardiovascular risk by causing endothelial dysfunction that reduces the capacity of the endothelium to release tPA [26]. There are few chances that schizophrenia patients will make any attempt to quit smoking and if so, will be successful at quitting [18], probably because correction of tPA deficiency seems to relieve symptoms of the mental disorder.

The availability and legality of alcohol contribute to its widespread use by people with schizophrenia [27]. Although heavy alcohol drinkers have higher tPA antigen than moderate drinkers or abstainers [28], heavy alcohol intake stimulates insulin production. Since PAI-1 promoter has insulin response [29], the net result is low plasminogen activator activity.

SEXUAL DISFUNCTION AND REPRODUCTIVE DISORDERS

Components of the plasminogen activator system play a key role in the reproductive system. Urokinase influences follicular development, ovulation, menstruation and embryo implantation [30], while tPA has an important role in placental angiogenesis and placental vessel remodeling that

supports fetal growth and development [31]. Based on the hypothesis that patients with schizophrenia might have reduced activity of plasminogen activators, an increased prevalence of reproductive disorders would be expected in this population, which really occurs.

Schizophrenia mothers are more likely than controls to have placental abruption and give birth to low-birth-weight and/or small-for-gestational-age babies, not only in pregnancies that take place before schizophrenia is diagnosed, but also in pregnancies occurring after the onset of the disease, in which psychotropic medications are not used [32-34]. Even after adjustment for primiparity, age, education, hypertension and the high incidence of smoking during pregnancy, women with at least one psychotic episode during pregnancy have more than twice the risk of having a preterm, a low-birth-weight neonate or a stillbirth than controls [35]. Also, compared to controls, fetuses of women with chronic schizophrenia are at higher risk of having oxygen deprivation due to placental insufficiency, a condition that reduces the chances of survival of the fetus and the neonate [34, 36]. Some authors have found that schizophrenia pregnancies are at high risk for preeclampsia, whereas others have demonstrated that schizophrenia reduces the risk of preeclampsia [37, 38]. One possible explanation for the discrepancy is that many schizophrenia mothers continue to smoke during pregnancy, and cigarette smoking decreases the risk of preeclampsia and eclampsia [39].

Plasminogen activator plays an important role in ovulation, which may contribute to the increased prevalence of polycystic ovaries in medicated schizophrenia patients [40]. Prevalence of polycystic ovaries in drug-naïve women with schizophrenia has not been estimated yet.

Erectile and ejaculatory dysfunction is a common complain of treatment-naïve schizophrenia males, usually attributed to negative symptoms [41]. Low activity of plasminogen activator may be relevant to the pathogenesis of erectile dysfunction. Penile erection depends on the release of nitric oxide in the corpus cavernosum. Plasminogen activator-mediated vascular endothelial growth factor (VEGF) activation induces endothelial production of nitric oxide [42].

LOW RISK OF CANCER

First-episode patients are at lower-than-expected risk of neoplasia [43, 44]. The low prevalence of lung cancer in a population with a high prevalence of cigarette smoking may seem paradoxical at first glance, but it makes sense if we consider that low tPA activity impairs angiogenesis that supports solid tumor growth. The incidence of malignant tumors in parents and siblings of schizophrenia patients also seems to be lower than in the general population [45], leading to the hypothesis that low tPA activity in schizophrenia might have a hereditary component.

INFLAMMATORY AND AUTOIMMUNE DISEASES

The risk of having schizophrenia increases by 29% with a prior autoimmune disease, by 60% with any hospitalization with infection, and by more than twofold with both risk factors [46]. It is possible that chronic bacterial infections could also influence the course of the mental disorder. Periodontitis, for example, is highly prevalent among drug-naïve schizophrenia patients [47]. Smoking and bad hygiene habits are partially responsible for the problem. Nicotine affects gingival blood flow and stimulates cytokine production that affects connective tissue turnover, which may increase pocket depth, affect periodontal attachment, and eventually lead to tooth loss [48].

Some autoimmune diseases are prevalent in schizophrenia patients, their parents and siblings. Sjögren's syndrome, autoimmune hepatitis, Crohn's disease and Guillain-Barré syndrome, for instance, may increase the risk of schizophrenia [49]. Also, persistent antiphospholipid antibodies, such as lupus anticoagulant or IgM anticardiolipin antibodies are detected in about 30% of the patients with schizophrenia, independent of psychotropic drug use [8, 50]. In these patients, psychosis may occur before the thrombotic events or obstetric complications that characterize the antiphospholipid antibody syndrome [50, 51]. Antiphospholipid antibodies can interfere with multiple steps in the mechanism of action of plasminogen activator [52].

Low activity of plasminogen activator in autoimmune disorders may also result from abnormalities in the protein C – protein S pathway, from corticosteroid therapy or from insulin resistance. Protein S circulates in two forms: the functionally active free-protein S or bound to a protein that belongs to the complement system, C4b binding protein. Free-protein S is a cofactor of activated protein C, which has the ability to inhibit PAI-1 [53]. C4b binding protein levels are significantly elevated in inflammatory disorders, which partially accounts for the low activity of tPA in inflammation. Importantly, some antiphospholipid antibodies interfere with protein C – protein S pathway. Corticosteroid therapy may reduce activity of plasminogen activator, because PAI-1 gene has a glucocorticoid-response element [54]. Insulin resistance related to inflammatory disorders will be discussed in the following section.

METABOLIC PROBLEMS RELATED TO HYPERINSULINEMIA

Assuming that reduced activity of tPA might be involved in the pathogenesis of schizophrenia, and knowing that insulin decreases tPA activity, one would expect a high prevalence of hyperinsulinemia in treatment-naïve schizophrenia patients. Indeed, medication-naïve patients display increased plasma levels of insulin and insulin precursors pro-insulin and C-peptide [55, 56]. In line with the hypothesis that hyperinsulinemia might contribute to the pathogenesis of schizophrenia, patients with type 1 diabetes, a condition characterized by decreased insulin production, have a lower prevalence of schizophrenia than the general population [57].

Most metabolic disturbances prevalent in first-episode patients, including increased body mass index, hypertension, hypertriglyceridemia and the metabolic syndrome [58, 59] are somehow related to hyperinsulinemia. Although insulin stimulates nitric oxide production in the endothelium, which decreases peripheral vascular resistance [60], in smokers and

in individuals with hyperlipidemia, nitric oxide combines with superoxide to form free radicals that abolish vasodilation [61]. Long-term hyperinsulinemia leads to obesity. Adipose tissue, especially mesenteric fat tissue synthesizes angiotensin [62]. PAI-1 promoter responds to angiotensin [63].

Triglycerides are hydrolyzed from fat and released into the circulation as fatty acids. In obese individuals, the liver is flooded with fatty acids and uses them as substrate to synthesize triglycerides that are exported to adipocytes. Hypertriglyceridemia, a bidirectional energy transfer between adipocytes and hepatocytes [64], is a potent stimulus for synthesis of a triglyceride carrier, very-low-density lipoprotein (VLDL). VLDL induces transcription of PAI-1 in endothelial cells [65].

The diagnosis of metabolic syndrome, which has a tremendous impact on morbidity and mortality by cardiovascular disease, is based on the combination of three or more risk factors as follows: central obesity, hypertension, glucose intolerance, hypertriglyceridemia, and low levels of high-density cholesterol [66]. Increased PAI-1 level has been considered the true villain of metabolic syndrome [63].

Increased insulin production may result from a high-carbohydrate diet combined with a sedentary lifestyle or from insulin resistance. Both physical inactivity and unhealthy dietary behaviors are prevalent among schizophrenia patients [58].

Insulin resistance may result from inflammatory conditions, such as obesity or chronic bacterial infection. Inflammation is accompanied by tumor necrosis factor (TNF)-α production, which induces synthesis of nitric oxide. Nitric oxide modifies proteins involved in insulin signaling [67], reducing the response of myocytes to insulin. Impaired uptake of glucose by skeletal muscles is usually accompanied by compensatory hyperinsulinemia [68].

Arranz *et al*. [69] have not found an increased prevalence of insulin resistance in drug-naïve schizophrenia patients. Considering that physical activity reverses insulin resistance, it is possible that lifestyle characteristics of the study population could have influenced the results. Other metabolic abnormalities, besides hyperinsulinemia, may account for the reduced activity of plasminogen activator seen in medication-naïve schizophrenia patients. One of them is hyperhomocysteinemia.

METABOLIC PROBLEMS RELATED TO HYPERHOMOCYSTEINEMIA

Hyperhomocysteinemia is highly prevalent in never-medicated schizophrenia patients [70]. The correlation between homocysteine levels and the mental disorder is significant: an increase of 5 mmol/L increases the risk of schizophrenia by 1.7 [71].

Homocysteine is not a dietary constituent. The amino acid, formed exclusively upon demethylation of methionine, is metabolized through folate- and vitamin B6-dependent pathways and eliminated by the kidney [72]. Interestingly, it has been reported that severity of negative symptoms is inversely correlated with serum folate levels [73]. Chronic alcoholism, which may be associated with psychotic symptoms, is an important cause of hyperhomocysteinemia [74], probably because chronic alcohol intake interferes with folate and vitamin B6 metabolism. Although there are no studies assessing the status of vitamin B6 in treatment-naïve schizophrenia patients, decreased vitamin B6 levels have been demonstrated in a high percentage of treatment-resistant schizophrenia patients, such as those who remain hospitalized for long periods or who are taking large doses of psychotropic drugs [75].

Homocysteine levels are directly correlated with cigarette smoking, caffeine consumption or insulin levels [76]. Heavy smokers are likely to be heavy coffee drinkers possibly because cigarette smoking causes bronchoconstriction and caffeine is a bronchodilator [77]. If coffee is consumed with sugar, heavy coffee drinking may induce hyperinsulinemia. Homocysteine inhibits an important step in tPA mechanism of action: its binding to annexin [72].

CONCLUSION

Decreased activity of plasminogen activator seems to play an important role in the pathogenesis of conditions prevalent in drug-naïve schizophrenia patients. Interventions aiming at correcting plasminogen activator activity include vitamin supplementation, regular physical activity, a balanced diet, and anticoagulant therapy. Controlled randomized studies are needed to determine if these measures can reduce comorbidity, improve quality of life, reduce mortality rates, and improve the course of schizophrenia.

CONFLICT OF INTEREST

The authors have declared that there are no conflict of interest in relation to the subject of this study.

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Adverse obstetric and neonatal outcomes in women with mental disorders

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ABSTRACT

The brain and the placenta synthesize identical peptides and proteins, such as brain-derived neurotrophic factor, oxytocin, vascular endothelial growth factor, cortisol, and matrix metalloproteinases. Given the promiscuity between neurochemistry and the mechanism of placentation, it would be expected that mental disorders occurring during pregnancy would increase the risk of adverse obstetric and neonatal outcomes. Indeed, expectant mothers with anxiety disorders, post-traumatic stress disorder, schizophrenia, or depressive disorders are at higher risk of preterm birth, low-birth-weight and small-for-gestational-age infants than controls. These mental illnesses are accompanied by a procoagulant phenotype and low activity of tissue plasminogen activator, which may contribute to placental insufficiency. Another risk factor for pregnancy complications is hyperemesis gravidarum, more common among women with eating disorders or anxiety disorders than in controls. Severe hyperemesis gravidarum is associated with dehydration, electrolyte imbalance and malnutrition, all of which may increase the risk of miscarriages, of low-birth-weight babies and preterm birth. This paper reviews some aspects of mental disorders that may influence pregnancy and neonatal outcomes.

Introduction

A number of peptides and proteins are produced by both the brain and the placenta. Oxytocin, for example, is synthesized in amnion, chorion, decidua, and neurons. In the peripartum period, oxytocin not only controls labor and milk ejection, but also regulates anxiety, mood symptoms and other complex mechanisms of social cognition and behavior, including neonate recognition and bonding between mother and baby [1]. Brain-derived neurotrophic factor, a well-known neurotrophin, and matrix metalloproteinases (MMPs) have a major role in adult neurogenesis and neuroprotection against excitotoxicity, and also participate in the processes of embryo implantation, trophoblast invasion, placental angiogenesis and vascular remodeling [2–4].

Among other substances that play a dual role in the central nervous system and the placenta are somatostatin, neurotensin, enkephalin, cortisol, insulin-like growth factor 1, vascular endothelial growth factor and the transcription factor cyclic AMP-responsive element-binding protein (CREB).

Given the promiscuity between neurochemistry and the mechanism of placentation, it would be expected that women with mental disorders would be at increased risk for obstetric complications. Unsurprisingly, depressive disorders, anxiety disorders, post-traumatic stress disorder, schizophrenia, and eating disorders occurring during pregnancy are independent risk factors for adverse obstetric and neonatal outcomes [5] (Fig. 1).

Depressive disorders

Periods of sadness are inherent aspects of human experience. While grief is a normal response to pregnancy complications, major depressive disorder is a disabling illness that increases the risk for adverse obstetric outcomes. If untreated, antenatal depression is associated with worsening of the psychiatric condition that may lead to suicide attempts, and with increased risk of a postpartum episode.

According to the American Psychiatric Association [6], the diagnosis of a major depressive disorder requires at least five of the following features to be present during the same 2-week period, one of them being depressed mood or loss of interest. With the exception of weight change and suicidal ideation, each criterion must be present nearly every day: (i) depressed mood most of the day; (ii) marked diminished interest or pleasure in all or almost all activities most of the day; (iii) insomnia or hypersomnia; (iv) psychomotor agitation or retardation; (v) fatigue or loss of energy; (vi) feelings of worthlessness, or excessive/ inappropriate guilt; (vii) diminished ability to think or concentrate, or indecisiveness; (viii) recurrent thoughts of death, suicide ideation or suicide attempt; (ix) weight loss when not dieting, or weight gain. The diagnosis also requires that symptoms cause significant distress or impairment in important areas of functioning, such as social or professional activities. Besides, the episode should not be attributable to any

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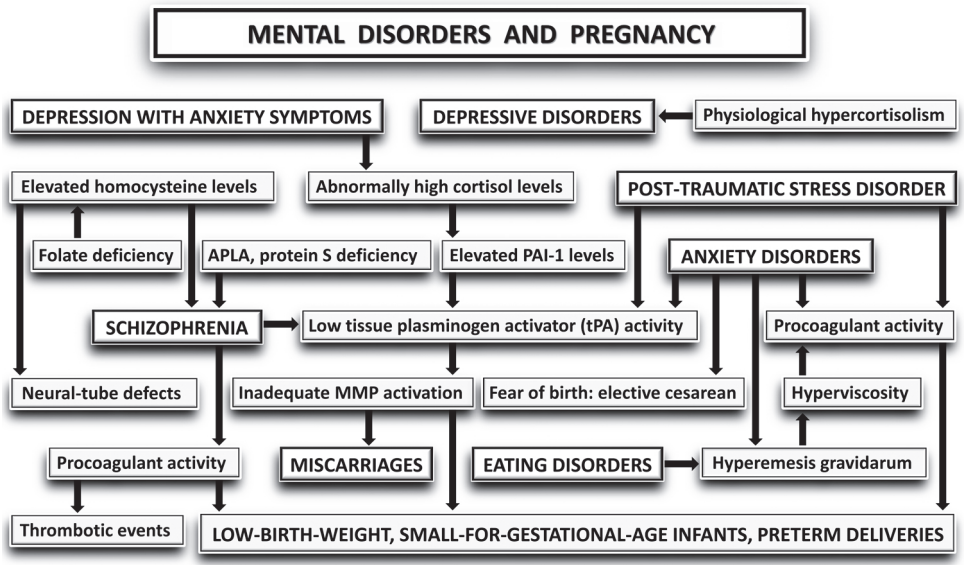

Fig. 1. Legend: PAI-1: plasminogen activator inhibitor-1; MMP: matrix metalloproteinase; APLA: antiphospholipid antibodies.

substance effect, and the symptoms must not be related to another medical condition. Importantly, depressive disorders are often accompanied by anxiety.

Elevated estrogen levels in pregnancy doubles the levels of corticosteroid binding globulin, resulting in low catabolism of cortisol by the liver, and a two-fold half-life of cortisol in plasma [7]. As a result, a steady rise in cortisol during normal pregnancy is noted, peaking during the third trimester at about two and three times non-pregnant values. Mothers with comorbid depression and anxiety synthesize an excessive amount of cortisol that further increases the already high levels of cortisol seen in normal pregnancies [8].

Chronic hypercortisolism may hinder fertility. In addition, a high percentage of pregnancies with Cushing's syndrome are complicated by fetal wastage, intrauterine growth restriction, preterm deliveries and neonatal death [9]. Elevated levels of cortisol that accompany severe depressive disorders may also increase the risk of pregnancy complications, such as recurrent unexplained miscarriages [10]. Although it has been reported that more women who use antidepressants miscarry than unexposed women [11], it is possible that women who require antidepressants have a more severe clinical course that could influence the obstetric outcome.

In 1992, Steer et al. [12] alerted to the fact that mothers with depressive symptoms had more than three times the odds of preterm birth, of low-birth-weight or small-for-gestational-age infants than mothers without depressive symptoms. These results were not confirmed in meta-analyses, possibly because treatment became more effective over the years. Nonetheless, it is indisputable that antenatal depression poses a modest but statistically significant risk of preterm birth and low-birth weight [13].

One possible explanation for the adverse obstetric events found in mothers with depressive disorders involves elevated levels of plasminogen activator inhibitor (PAI)-1, a finding consistently reported in depressive disorders. Both placental angiogenesis and vascular remodeling – required to sustain fetal growth – depend on extracellular matrix degradation by MMPs, such as MMP-2 and MMP-9 [4]. MMPs are secreted as latent enzymes, whose activation depends on stromelysin-1, also known as MMP-3, and on plasminogen [4]. MMP-3 is activated by plasmin and both plasminogen and plasmin are inhibited by PAI-1.

If a depressive episode increases the risk of adverse obstetric events, pregnancy itself increases the risk of having a depressive episode. It seems that high cortisol levels contribute to depressive episodes that affect 12% of pregnant women [14]. Cortisol levels are inversely related to dopamine levels, which plays a pivotal role in reward-motivated behavior, and to serotonin levels, which regulates mood, appetite and sleep.

Anxiety disorders and post-traumatic stress disorder

Fear is an adaptive reaction to real threat or perceived imminent threat, while anxiety corresponds to anticipation of future threat. The two responses overlap, but fear is usually linked to the fight-or-flight reaction, whereas anxiety is often associated with apprehension and physical tension. Anxiety disorders differ from transient fear or anxiety by being persistent and/or by an out-of-proportion response [6]. Although some level of anxiety may be experienced by most pregnant women without affecting pregnancy results, anxiety disorders – which include generalized anxiety disorder, social anxiety disorder, specific phobia, panic disorder, and agoraphobia – may increase the likelihood of adverse obstetric and neonatal outcomes.

Acute and chronic stress are characterized by platelet activation and increased levels of factors VII, VIII, XII, fibrinogen and von Willebrand factor antigen. While in acute stress tissue plasminogen activator (tPA) levels increase and fibrinolysis is activated, in chronic stress tPA levels decrease and PAI-1 levels increase, and therefore fibrinolysis is inhibited [15]. It is possible that increased procoagulant activity and decreased fibrinolytic activity, inherent to chronic anxiety symptoms, could increase the risk of adverse obstetric and neonatal outcomes seen in women with anxiety disorders, by causing placental vessel thrombosis. Of note, several studies indicate that acute cortisol reactivity to laboratory stressors may be blunted during pregnancy [8].

Panic attacks are highly stressful situations. As such, they may hasten the clotting time and induce a hypercoagulable state, similar to what is observed in procedures likely to provoke acute anxiety, such when the specific phobia patient fears needles [16]. Mothers with panic disorder have a higher risk of preterm deliveries and small-for-gestational-age infants than controls, especially if a panic attack is experienced during gestation [17].

Another problem significantly associated with anxiety disorders is excessive vomiting [18]. Severe hyperemesis gravidarum is associated with dehydration, electrolyte imbalance and malnutrition, all of which may increase the risk of miscarriages, of low-birth-weight babies and preterm birth. The risk is even higher if hyperemesis is associated with low pregnancy weight gain [19]. Also, compared

to healthy women, highly anxious mothers have an increased chance to deliver through elective cesarean section [20], which may artificially shorten pregnancy length.

Post-traumatic stress disorder (PTSD), which is not currently classified under anxiety disorders [6], is highly prevalent in the pregnancy following stillbirth, especially if pregnancy occurs with an interval of less than one year [21]. Evidence indicates that PTSD has a high impact on obstetric and perinatal outcomes. Possible causes include poor prenatal care, excessive weight gain, and a high probability to engage in high-risk health behaviors, such as smoking, alcohol consumption and substance use [22].

Different authors have brought attention to the fact that PTSD increases the chances of a preterm birth. Yonkers et al. [23] have demonstrated that for each point increase on the Modified PTSD Symptom Scale (range, 0–110), the risk of preterm birth increases by 1% to 2%. A study conducted in New York City and upstate New York showed an adjusted odds ratio of 1.44 for births <1500 g and 1.67 for births 1500–1999 g one week after the September 11, 2001 World Trade Center disaster, compared to the two previous years [24]. Women with both PTSD and major depressive episode have a four-fold increased risk of preterm birth [23].

Schizophrenia and affective psychosis

Schizophrenia mothers have a 72% greater risk of thromboembolic disease than controls [25], even though ischemic brain lesions do not play a role in the pathophysiology of schizophrenia. A high prevalence of markers of low tPA activity was found in a sample of 70 schizophrenia patients [26]. For example, 20% of the patients had low free-protein S levels independent of hormones, of pregnancy, puerperium or inflammatory diseases, and 30% had persistent antiphospholipid antibodies, especially lupus anticoagulant or IgM anticardiolipin antibody. None of the 98 controls exhibited any of the two thrombophilias. It is possible that these thrombophilias could contribute to the increased risk of thrombotic events and to the high risk of miscarriages, preterm birth, and small-for-gestational-age newborns with low Apgar scores seen in schizophrenia pregnancies [27,28]. The risk of delivering a preterm small-for-gestational-age baby remains high after adjusting for confounders, such as maternal age, parity, socio-economic status and pre-pregnancy clinical morbidity [25].

Hyperhomocysteinemia, a condition that also impairs tPA catalytic activity, has been considered a risk factor for schizophrenia [29]. Low folate intake, which may increase homocysteine levels, is also associated with holoprosencephaly, spina bifida and other neural tube defects, prevalent in offspring of schizophrenia mothers [28].

Mothers with schizophrenia have higher rates of pre-existing type II diabetes mellitus and chronic hypertension than controls. Rates of gestational diabetes are also higher than in the general population, which might explain the increased frequency of large-for-gestational-age infants, compared to the general population [25].

Some authors have reported that schizophrenia women are at low risk of preeclampsia, which is possibly related to the high rate of cigarette smoking during pregnancy, up to 50% [27,28]. Cigarette smoking, which reduces psychiatric symptoms, stimulates secretion of multiple MMPs from inflammatory and epithelial cells [30]. Some of these MMPs mediate collagen and elastin proteolysis. Contrasting with its deleterious systemic effects, such as periodontitis, lung emphysema and aneurysms, elastolysis of placental vessels reduces susceptibility to preeclampsia. The low risk for preeclampsia in schizophrenia pregnancies has not been confirmed by Vigod et al., who have reported an 84% increased risk [24].

Cigarette smoking has also deleterious effects on pregnancy. Smoking is an established risk factor for placental abruption and for low-birth-weight infants. Except for these two adverse outcomes, mothers with a preexisting psychotic disorder have more obstetric complications than mothers who develop a psychotic disorder after birth [31]. Even after controlling for a high incidence of smoking during pregnancy, risks of delivering a low-birth-weight neonate or of having a stillbirth doubles if a psychotic episode occurs during pregnancy [26].

Mothers with affective psychosis, such as bipolar disorder, have more than twice the risk of preterm birth, of low-birth-weight or small-for-gestational-age babies. After adjustment for covariates, particularly smoking, the risks decrease but remain significant [32].

Eating disorders

Women with anorexia nervosa have very low fertility rates, but pregnancy may occur after recovery from the active phase of the disease, or occasionally during the active phase. In this case, women tend to deliver babies with significantly lower weight than the general population, which is mainly explained by lower pre-pregnancy body mass index [33,34].

Regardless of the diagnosis, any hospitalization during pregnancy because of medical issues related to an eating disorder increases the risk of small-for-gestational age babies and preterm deliveries [35]. One of these medical problems is hyperemesis gravidarum, a matter of great concern in patients with eating disorders [36]. Women with bulimia nervosa, with or without anorexia, have an increased rate of both miscarriages and preterm birth [34].

Rates of gestational diabetes are almost fivefold higher in the group with anorexia nervosa plus bulimia nervosa than in the general population [34]. As a group, mothers with eating disorders tend to gain more weight during pregnancy than controls, which may contribute to the increased risk of large-for-gestational-age infants. By its turn, macrosomia may increase the rate of cesareans described in these patients [35]. Mothers with binge eating disorders have the higher risk of large-for-gestational-age babies and cesarean section and a lower risk of small-for-gestational-age babies than mothers with other eating disorders [37,38].

Another risk factor for adverse obstetric events among women with eating disorders is cigarette smoking. The frequency of smoking during pregnancy has been reported to range from 14–37%, compared with 9% in women with no eating disorder. The rate is specifically high among patients with anorexia nervosa [37].

Conclusions

Mental disorders are highly prevalent in the general population. Special attention should be given to mothers with post-traumatic stress disorder, schizophrenia, anxiety, depressive, and eating disorders in order to decrease the risk of adverse obstetric and neonatal outcomes.

Conflict of interest statement

The authors report no conflict of interest.

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O PAPEL DO tPA NO TRATAMENTO DA PSICOSE

Considerando-se que a baixa atividade do tPA participa da fisiopatologia da esquizofrenia e suas comorbidades, e que pacientes com esquizofrenia têm alta prevalência de marcadores de baixa atividade do tPA, postulamos que intervenções que atenuam os sintomas psicóticos aumentariam a atividade do tPA.

De fato, intervenções no estilo de vida e suplementos nutricionais que costumam atenuar os sintomas psicóticos aumentam a atividade do tPA. A atividade física, conjugada a uma dieta equilibrada, ajuda a normalizar os níveis séricos de insulina e triglicerídeos, o que reduz o estímulo para produção de PAI-1. Suplementos de ômega 3 também ajudam a normalizar a trigliceridemia. A correção dos níveis da homocisteína com ácido fólico ajuda a restaurar a atividade do tPA.

O tPA também integra o mecanismo de ação de hormônios e medicamentos que melhoram os sintomas psicóticos, tais como a pregnenolona, o estrogênio, a ocitocina, antagonistas do receptor D3 da dopamina, o ácido retinóico, o ácido valpróico, o canabidiol, o nitroprussiato de sódio, a N-acetil cisteína e o warfarin. A eletroconvulsoterapia estimula as células cromafins da medula adrenal a liberarem tPA, junto com catecolaminas.

O papel do tPA no tratamento da psicose foi discutido em dois artigos:

Hoirisch-Clapauch S, Mezzasalma MA, Nardi AE. Pivotal role of tissue plasminogen activator in the mechanism of action of electroconvulsive therapy. J Psychopharmacol 2014; 28: 99-105.

Hoirisch-Clapauch S, Nardi AE. Improvement of psychotic symptoms and the role of tissue plasminogen activator. Int J Mol Sci 2015; 16: 27550-60.

- ✓ **O que os artigos têm de inovador**: Postulamos que condições que afetam a função do tPA, como anticorpos antifosfolipídio e hiperhomocisteinemia, prejudiquem a resposta aos procedimentos que aumentam os níveis do tPA, como a eletroconvulsoterapia. Estudos controlados ajudarão a determinar se o ácido fólico, corrigindo os níveis de homocisteína, melhora a resposta ao procedimento.
- ✓ Postulamos, ainda, que o rituximab possa ajudar no tratamento de pacientes com esquizofrenia refratária, que tenham anticorpos antifosfoli-

pídio em título moderado ou alto. O rituximab é um anticorpo monoclonal contra linfócitos que expressam o marcador CD (*cluster of differentiation*)-20, usado em pacientes com síndrome do anticorpo antifosfolipídio que não respondem ao tratamento convencional.

✓ Antipsicóticos atípicos, como a clozapina e a olanzapina, aumentam os níveis de pregnenolona no hipocampo. A pregnenolona é um precursor da progesterona. Na granulosa do ovário, a progesterona estimula a fusão do seu receptor com o receptor do PAI-1. Ao ocupar o receptor resultante, PGRMC1, a progesterona imortaliza as células da granulosa. Nossa hipótese, que precisa ser comprovada em modelos animais, é que, a progesterona também ocupe o receptor PGRMC1 no cérebro, impedindo que o PAI-1 promova apoptose neuronal.

✓ Nos modelos de acidente vascular cerebral isquêmico em roedores, o tPA intranasal aumentou a plasticidade, melhorando as funções cognitivas, sem causar sangramentos. Não existem artigos avaliando a ação do tPA intranasal em pacientes com esquizofrenia. À luz dos resultados vistos em roedores, sugerimos que o tPA intranasal seja testado em estudos controlados em pacientes com grave disfunção cognitiva.

Review

Pivotal role of tissue plasminogen activator in the mechanism of action of electroconvulsive therapy

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Abstract

Electroconvulsive therapy is an *important treatment option* for major depressive disorders, acute mania, mood disorders with psychotic features, and catatonia. Several hypotheses have been proposed as electroconvulsive therapy's mechanism of action. Our hypothesis involves many converging pathways facilitated by increased synthesis and release of tissue-plasminogen activator.

Human and animal experiments have shown that tissue-plasminogen activator participates in many mechanisms of action of electroconvulsive therapy or its animal variant, electroconvulsive stimulus, including improved N-methyl-D-aspartate receptor-mediated signaling, activation of both brain-derived neurotrophic factor and vascular endothelial growth factor, increased bioavailability of zinc, purinergic release, and increased mobility of dendritic spines. As a result, tissue-plasminogen activator helps promote neurogenesis in limbic structures, modulates synaptic transmission and plasticity, improves cognitive function, and mediates antidepressant effects.

Notably, electroconvulsive therapy seems to influence tissue-plasminogen activator metabolism. For example, electroconvulsive stimulus increases the expression of glutamate decarboxylase 65 isoform in γ-aminobutyric acid–releasing neurons, which enhances the release of tissue-plasminogen activator, and the expression of p11, a protein involved in plasminogen and tissue-plasminogen activator assembling.

This paper reviews how electroconvulsive therapy correlates with tissue-plasminogen activator. We suggest that interventions aiming at increasing tissue-plasminogen activator levels or its bioavailability – such as daily aerobic exercises together with a carbohydrate-restricted diet, or normalization of homocysteine levels – be evaluated in controlled studies assessing response and remission duration in patients who undergo electroconvulsive therapy.

Keywords

Electroconvulsive therapy, tissue plasminogen activator, neurogenesis, schizophrenia, major depressive disorders, N-methyl-D-aspartate, brain-derived neurotrophic factor, purinergic, zinc

Introduction

Electroconvulsive therapy (ECT) may be considered as *first-line treatment* when a rapid or a higher probability of response is necessary for patients with major depressive disorders, acute mania, mood disorders with psychotic features, catatonia or high suicide risk (American Psychiatric Association, 2006). Also, ECT can be used as a *secondary treatment when patients with* schizophrenia, major depression, mania or psychiatric syndromes associated with medical conditions have failed to respond to pharmacotherapy, or have shown intolerance to side effects of medication.

Several hypotheses have been proposed as ECT's mechanism of action, including improved N-methyl-D-aspartate (NMDA) receptor-mediated signaling, activation of both brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), increased bioavailability of zinc, purinergic release, and increased mobility of dendritic spines, neurogenesis in limbic structures, improved synaptic transmission and plasticity, improved cognitive function, and antidepressant effects. These different converging pathways might be facilitated by increased synthesis and release of tissue plasminogen activator (tPA) (Figure 1). For decades tPA, plasminogen and plasmin were known as proteins whose main role was to modulate coagulation, through intravascular fibrin degradation. However, this proteolytic system has numerous actions that go beyond the blood vessel. Indeed, tPA is a critical regulatory element in the neurochemistry.

tPA

Physiology and pathology

tPA and the end product of plasminogen activator-derived proteolysis, plasmin, are involved in synaptic plasticity, integrity of the blood-brain barrier, neurite outgrowth, cell migration, long-term potentiation and depression, neurogenesis, and excitotoxic cell death (Benarroch, 2007; Cesarman-Maus and Hajjar, 2005; Melchor and Strickland, 2005; Sappino et al., 1993).

It has been proposed that due to its proteolytic properties, tPA may be a key player in the biology of memory, learning, emotions, and the rewarding effect of licit or illicit substances (Madani et al., 2003; Nagai et al., 2006; Samson and Metcalf, 2006). Moreover, decreased levels of tPA or elevated levels of its main inhibitor,

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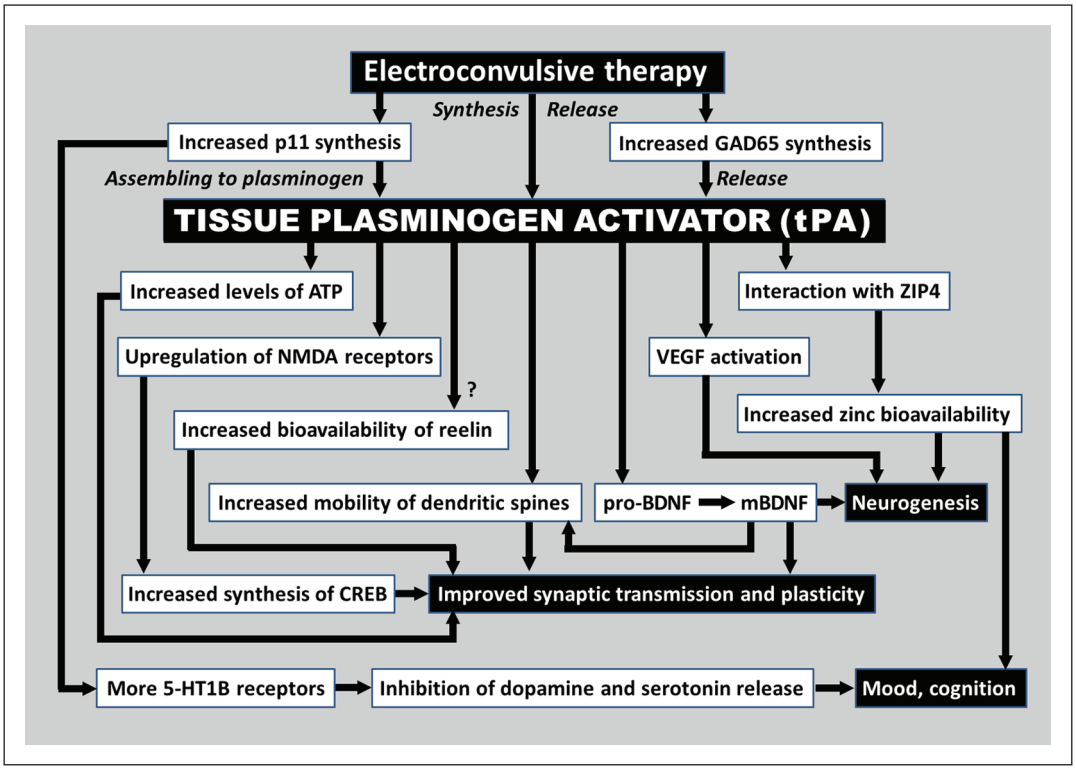

Figure 1. Electroconvulsive therapy and tissue plasminogen activator: a mechanistic proposal.
5-HT1B: serotonin receptor 1B; ATP: adenosine 5′-triphosphate; CREB: cAMP response element binding protein; GAD65: glutamate decarboxylase isoform 65; mBDNF: mature brain-derived neurotrophic factor; NMDA: N-methyl-D-aspartate; pro-BDNF: precursor of brain-derived neurotrophic factor; VEGF: vascular endothelial growth factor; ZIP4: zinc transporter 4.

plasminogen activator inhibitor 1 (PAI-1), have been described in patients with major depression and with schizophrenia (Eskandari et al., 2005; Harris et al., 2012).

Since tPA has antithrombotic and thrombolytic activity, if we assume that patients with psychosis and severe affective disorders have a significant imbalance of tPA, we could infer that these patients would have an increased risk of thromboembolism. As expected, elevated plasmatic levels of markers of thrombogenesis and thrombocyte activation, such as D-dimer and soluble P-selectin, have been demonstrated in antipsychotic-naive patients with acute psychosis, compared to healthy controls matched for age, gender and body mass index (Masopust et al., 2011).

Antipsychotic medications are associated with a dose-dependent risk for thromboembolic disorders (Allenet, 2012). Nonetheless, we are assuming that patients with severe mental illness have a pronounced deficit of tPA and that ECT restores tPA levels. Based on this premise, it may be possible that patients with severe mental illness would require higher-dose psychotropic medication than individuals with mild disease. In this setting, thrombotic tendency would be explained by severe tPA deficit, and not be related to the medication used by the patient.

tPA and electrically induced convulsions

tPA is synthesized by endothelial cells and neurons in most areas of the brain and stored in pre-synaptic vesicles (Gualandris et al, 1996; Sappino et al., 1993). The blood levels of tPA increase within minutes in response to a variety of stimuli that promote sympathoadrenal activation, such as physical and mental stress, or electrically induced convulsions (Fantl and Simon, 1948; Gualandris et al, 1996; Parmer et al., 1997). tPA activity rapidly increases by more than 50% and remains elevated for more than 24 h after a session of electroconvulsive shock (ECS, the animal variant of ECT) stimulus (Segawa et al., 2013). Although rapid increase of tPA blood levels after ECS is suggestive of secretion from stored pools into the circulation rather than de novo synthesis, it has also been proved that after a depolarization stimulus expression of tPA messenger RNA (mRNA) is upregulated (Carroll et al., 1994; Parmer et al., 1997). Peripheral tPA crosses the blood-brain barrier (Benchenane et al, 2005) and it is probable that plasmatic tPA could contribute to the increment of tPA in the central nervous system after an ECS session, such as observed in the hippocampus (Segawa et al., 2013).

How tPA modulates the mechanisms of action of ECT

Neurogenesis

Imaging studies of patients with schizophrenia or treatment-resistant depressive disorders show atrophy of limbic structures (hippocampus, amygdala and prefrontal cortex) (Madsen et al., 2000; Maller et al., 2012; Nakamura et al., 2013). Central nervous system regeneration or repair requires neurogenesis, angiogenesis and vascular remodeling. These processes depend on matrix

metalloproteinase activation by tPA or by tPA end product, plasmin (Benarroch, 2007; Carroll et al., 1994).

As reviewed in the sections on BDNF, VEGF and zinc, tPA participates in additional mechanisms implicated in neurogenesis that include activation of both BDNF and VEGF, and increased bioavailability of zinc.

A single ECS session promotes intense neurogenesis in the hippocampus of rats, as observed with bromodeoxyuridine (BrdU), a synthetic analogue of thymidine used to detect proliferating cells in living tissues. By day three, proliferation of BrdU-labeled hippocampal neurons doubled, then increased about threefold from day three to day five and returned to baseline levels by day seven (McCall, 2001). Similarly, ECT may reverse atrophy of limbic structures in humans (Nordanskog et al., 2010). *S*ince newly generated neurons have a limited lifespan, clinical relapse usually occurs within few *months following the last session of ECT*, when pharmacological treatment is not used (McCall, 2001; Nakamura et al., 2013).

BDNF

In central neurons, acute effects of BDNF may comprise enhancement of glutamatergic synaptic transmission, which is excitatory, and reduction in γ-aminobutyric acid–releasing (GABAergic) synaptic transmission, which is inhibitory. Chronic presence of BDNF stimulates formation and functional maturation of glutamatergic and GABAergic synapses (Gottmann et al., 2009).

tPA induces the production of plasmin, which mediates the extracellular cleavage of pro-BDNF (precursor of brain-derived neurotrophic factor) to its mature form, mBDNF (Pang et al., 2004). In contrast with pro-BDNF, which might be harmful to neurons, mBDNF not only regulates synaptic transmission and plasticity in the hippocampus and cortex – and as such takes part in several cognitive functions, but may also have antidepressant effects as well (Pang et al., 2004; Lu et al., 2005a).

A single administration of ECS rapidly increases hippocampal levels of pro-BDNF and stimulates glia to release tPA (Gottmann et al., 2009; Newton et al., 2003). Probably as a consequence of tPA release, accumulation of pro-BDNF and mBDNF levels is observed in rats receiving ECS for 10 days. By contrast, chronic administration of imipramine significantly increases mBDNF levels, but not pro-BDNF or tPA levels, indicating a different therapeutic mechanism from ECT (Segawa et al., 2013).

VEGF

VEGF has several functions: it stimulates endothelial mitogenic factor, restricts vascular porosity, stimulates the proliferation of neuronal precursors and possesses neuroprotective properties (Nowacka and Obuchowicz, 2012).

VEGF processing by physiologically relevant proteases, such as plasmin, is a key mechanism to convert extra-cellular matrix-bound VEGF into freely diffusible and active forms (Jin et al., 2002).

The VEGF gene is significantly upregulated after ECS in specific sub-regions of the hippocampus, especially the dentate gyrus (Yang et al., 2009). It has been shown that VEGF signaling is necessary for ECS induction of quiescent neural progenitor cell proliferation and is sufficient to produce this effect (Segi-Nishida et al., 2008).

N-methyl-D-aspartate (NMDA) receptors

The amino acid glutamate is the major excitatory neurotransmitter in central nervous system. Glutamate binding to different receptors, including NMDA, increases the influx of calcium through the receptors (Hardingham and Bading, 2003; Nicole et al., 2001). Although physiological calcium influx confers neuroprotection against the deleterious effects of electrical activity, either too little or too much calcium entry through *NMDA* receptors are detrimental to the neuron (Nicole et al., 2001).

The cleavage of NMDA receptor at arginine 67 is tPA-specific and plasmin-independent (Ng et al., 2012). It results in transient phosphorylation of the extracellular signal regulated kinases 1/2 and activation of cAMP response element binding protein (CREB; Wu et al., 2013).

CREB regulates the *BDNF* gene, which promotes *BDNF* transcription (Tao et al., 1998). It also induces the synthesis of factor 3 (Atf3), which protects neurons against excitotoxity, a pathological process by which neurons are damaged or killed when receptors like NMDA are over-activated by neurotransmitters such as glutamate (Wu et al., 2013). As a feed-forward mechanism, BDNF stimulates the synthesis of NMDA in cortical neurons through CREB (Kim et al., 2012).

Calcium influx via NMDA receptors depends on the cleavage of the amino-terminal domain of its GluN2A subunit. The process, mediated by an end-product of tPA, plasmin, removes zinc, as seen below (Cesarman-Maus and Hajjar, 2005; Yuan et al., 2009).

Although we can clearly recognize the findings as still speculative, some data have demonstrated that while low activity of NMDA receptors produces psychotic symptoms, ECS upregulates the receptors, probably as a consequence of increased levels of tPA released by the procedure (Hardingham and Bading, 2003; Watkins et al., 1998; Waxman and Lynch, 2005). These observations may be too simplistic and some other previously cited biochemical factors might also be involved in the emergence or in the prevention of psychotic symptoms.

Zinc

Glutamatergic neurons contain free zinc packaged into synaptic vesicles. Upon neuronal activation, the vesicular content is released into the synaptic space, whereby zinc modulates activity of postsynaptic neurons though interactions with receptors, transporters and exchangers (Emmetsberger et al., 2010).

Serum and brain zinc concentrations were found to be significantly decreased in patients with depression and schizophrenia (Grabrucker et al., 2011). Zinc contributes to increased levels of BNDF in the cortex; it also participates in synaptic transmission, neurogenesis and cortical plasticity (Kim et al., 1999; Levenson and Morris, 2011; Nowak et al., 2005).

tPA mediates zinc uptake and zinc sequestering in non-toxic lysosomes though the zinc influx transporter, ZIP4. Increased neuronal survival when zinc is sequestered in lysosomes supports the idea that sequestration results in neuroprotection (Emmetsberger et al., 2010). Indeed, zinc can be deleterious to neurons when it induces the synthesis of reactive oxygen species that can provoke oxidative stress. Additional deleterious effects of zinc result from increased permeability of ionotropic glutamate receptors NMDA, kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to extracellular ions, which

mediates excitotoxicity (Grabrucker et al., 2011; Kim et al., 2012; Nowak and Schlegel-Zawadzka, 1999).

Evidences indicate that tPA is involved in the robust increase of zinc levels in the hippocampus and a slight increase in the cortex and cerebellum observed after long-term use of ECT (Nowak and Schlegel-Zawadzka, 1999).

Purinergic release

Adenosine 5′-triphosphate (ATP) is released from many neural cell types in response to mechanical deformation, hypoxia or to some non-harmful agents, such as acetylcholine and thrombin (Burnstock, 2008).

In the central nervous system, ATP is a cotransmitter with glutamate, noradrenaline, GABA, acetylcholine and dopamine. Together with some of its enzymatic breakdown products (adenosine diphosphate and adenosine) and uracil nucleotides, ATP mediates neurotransmission, neuromodulation, cell proliferation, differentiation and death, *via* a number of channels and receptors (Burnstock et al, 2011).

The synthesis and release of neurotrophins, cytokines and chemokines in glial cells is controlled by purine receptors. For example, A_1 adenosine receptors inhibit release of excitatory transmitters, while P2Y adenosine receptors modulate the release of dopamine and enhance the glutamate concentration in the nucleus accumbens (Burnstock, 2008; Burnstock et al., 2011). Unsurprisingly, all four adenosine receptor subtypes, as well as P2X7 and P2Y receptors are expressed on glial cells (Jossin et al., 2007).

Abnormal purinergic signaling between neurons and glial cells impairs both learning and memory, and might be implicated in the neurobiology of schizophrenia and major depression (Burnstock et al., 2011; Lara et al., 2006; Lucae et al., 2006).

ECS promotes release of ATP by neurons, microglia and vascular glia cells, which may reduce the depressive behavior via stimulation of neurons and glia directly or after extracellular breakdown of ATP to adenosine (Sadek et al., 2011). Indeed, ECS increases the levels of adenosine in the brain substantially. Although adenosine A1 receptors are not altered after a single ECS session, there is a 20% increase of binding sites in cerebral cortex following repeated sessions, which can still be observed for at least 14 days after the last session (Gleiter et al., 1989). Also, sequential ECS increases the expression of P2X7 receptors on glial cells, which has been shown to increase the release of cytokines, chemokines and neurotrophins (Sadek et al., 2011).

Since blocking tPA synthesis in endothelial cells severely reduces the content of ATP, it seems that tPA contributes to increased neuronal content of ATP observed after ECS sessions (Eijnden-Schrauwen et al., 1995).

Dendritic spines

The micron-sized protrusions of the dendritic membrane that form the postsynaptic component for the vast majority of excitatory synapses of central nervous system are called dendritic spines. They are found on glutamatergic pyramidal neurons of the neocortex, on hippocampal neurons, on GABAergic cerebellar Purkinje neurons and medium-sized projection neurons of the striatum (Mataga et al., 2004). The glutamate receptors AMPA and NMDA expressed on dendritic spines surface promote excitatory inputs, required for memory storage and synaptic transmission (Calabrese et al, 2006). Neuropathological studies with autopsied brains from patients with schizophrenia have shown reduced numbers of dendritic spines (Bennett, 2011; Glantz and Lewis, 2000).

Repeated ECS increases the number of synapses, and especially the number of spine synapses in the hippocampus of rats (Chen et al., 2009; Tyler and Pozzo-Miller, 2003), a process that seems to require the participation of tPA. tPA promotes enzymatic break-down of chondroitin sulfate proteoglycan that favors mobility of dendritic spines, and also their pruning (Calabrese et al, 2006; Mataga et al., 2004). In addition, as already mentioned, tPA participates in BDNF activation, which is necessary to maintain normal spine density and behavior (Vigers et al., 2012).

How is TPA affected by ECT

GABAergic neurons

GABA, a major inhibitory neurotransmitter, is synthesized from glutamate by two isoforms of glutamate decarboxylase, *GAD65* and GAD67. GAD67 is widely distributed throughout the neuron whereas GAD65 lies primarily in axon terminals (Kaufman et al., 1991). Whereas GAD67 is constitutively active and produces >90% of GABA in the central nervous system, GAD65 is transiently activated and augments GABA levels for rapid modulation of inhibitory neurotransmission (Kanaani et al., 2010).

Substantial dysregulation of GAD mRNA expression in the hippocampus is observed in schizophrenia and bipolar disorder (Heckers et al., 2002). After repeated ECS administration, the expression of GAD65 increases, while the expression of GAD67 decreases in GABAergic hippocampal neurons (Jinno and Kosaka, 2009).

Curiously, release of tPA is under control of GABAergic neurons. Blocking GABAergic transmission prevents both the release of tPA and plasticity, whereas accelerating GABAergic synapse maturation increases the release of tPA (Hensch, 2005).

p11 (or S100A10)

Susceptibility to depression may be correlated with decreased levels of p11, a protein that increases the amount of serotonin receptor 1B (5-HT1B) receptors on the surface of neurons (Svenningsson et al., 2006). The function of the 5-HT1B receptor seems to depend on its location: in the frontal cortex it inhibits the release of dopamine, while in the striatum and the basal ganglia, it inhibits the release of serotonin. Another role of 5-HT1B receptors might be to control the secretion of other neurotransmitters, e.g. acetylcholine, glutamate, dopamine, norepinephrine and GABA (Pytliak et al., 2011).

It is very seductive to speculate that a single major neurotransmitter deregulation might be associated with a specific psychiatric disorder. Using this simplistic point of view, while deregulation of serotonin neurotransmission might be implicated in the pathophysiology of depressive disorders, schizophrenic patients seems to exhibit dopaminergic dysfunction in the mesolimbic and mesocortical systems (Kassam et al., 1998; Pytliak et al., 2011).

Reduction of p11 has been found both in post-mortem brain neurons from depressed individuals and in animal models of depression (Svenningsson and Greengard, 2007; Svenningsson et al., 2006). Reduction of p11 with adeno-associated virus–mediated RNA interference in the nucleus accumbens of mice results in depression-like behaviors (Alexander et al., 2010). Antidepressant medications, p11 gene therapy and repeated ECS sessions in rodents may increase p11 mRNA and p11 levels, which reverses depression-like behaviors (Alexander et al., 2010; Pytliak et al., 2011). Increased p11 mRNA and p11 levels were also demonstrated with antidepressants in humans (Melas et al., 2012). Interestingly, p11 is somehow related to tPA. When protein p11 binds to annexin A2, the resultant heterotetramer (annexin A2-p11)2 is able to assemble plasminogen and tPA and, as a consequence, plasmin is generated (Kassam et al., 1998; Svenningsson et al., 2006). As already mentioned, plasmin's numerous roles in the central nervous system include activation of VEGF and metalloproteinases involved in neurogenesis and angiogenesis. In the absence of annexin A2, p11 is unstable and rapidly degraded through a proteasome-dependent mechanism (He et al., 2008).

Future directions

Reelin

Reelin is a dimer capable of preventing entanglement of neurons, orientating them in a ventricular-to-pial disposition. Guidance of neuronal migration is achieved through proteolytic degradation of adhesion molecules fibronectin and laminin. Furthermore, reelin regulates NMDA receptor homeostasis and modulates synaptic function and plasticity in adult synapses (Reif et al., 2007).

In vivo, reelin is anchored to the extracellular matrix. To diffuse locally and send signals to target cells, reelin must be proteolytically fragmented in two main sites; one of them, C-terminal, is cleaved by tPA (Jossin et al., 2007).

Reelin expression was found to be decreased in all examined brain regions, including the hippocampus, in post-mortem tissue from schizophrenic patients (Doehner and Knuesel, 2010). It remains to be determined if ECT, promoting release and synthesis of tPA, activates reelin.

tPA levels in patients who respond or are refractory to ECT

To validate the hypothesis that tPA is paramount in the clinical response to ECT, it would be interesting to compare plasmatic levels of tPA in refractory patients and in patients who enter psychiatric remission. Some antiphopholipid antibodies might react against tPA (Lu et al., 2005b), and we recommend that patients refractory to ECT be screened for antiphospholipid antibodies.

Testing the hypothesis

Intranasal tPA has been shown to improve axonal remodeling and neuronal recovery in rats after stroke (Liu et al., 2012). Randomized studies in animal models of schizophrenia or severe affective disorders, comparing ECS to intranasal tPA might help confirm our hypothesis that tPA plays a pivotal role in the therapeutic mechanism of ECT.

Under the premise that increased levels of tPA would be required to obtain clinical response after ECT, conditions characterized by low tPA levels, such as hyperinsulinemia or hyperhomocysteinemia, would impair response to ECT or prevent long-term remission after the procedure. On the other hand, medications that increase tPA levels, such as anticoagulation, could prolong remission.

Insulin precursors increase in a dose-dependent manner the synthesis of PAI-1, (Nordt et al., 1994). We suggest that randomized studies be performed to evaluate whether decreased insulin synthesis could improve response and/or sustain remission to ECT. Natural candidates for such study would be physical activity, such as daily brisk walking, combined with a carbohydrate-restricted diet.

Another condition that might affect tPA is hyperhomocysteinemia. Homocysteine, a prothrombotic amino acid, impairs bioavailability of tPA through a mechanism that involves interaction of homocysteine with annexin II, the tPA receptor in endothelial cells (Hajjar et al., 1998). Although it has been shown that schizophrenic patients with hyperhomocysteinemia could benefit from the simple addition of folic acid, vitamins B12 or B6 (Levine et al., 2006), currently there are no recommendations to assess homocysteine levels as a routine in patients who undergo ECT.

Randomized trials are also required to assess whether medications that increase tPA, such as warfarin and other anticoagulants, could help sustain remission after ECT sessions. Since elevated blood pressure might increase the risk of intracranial hemorrhage, we recommend that warfarin not be given concomitantly to ECT (Hart, et al., 1995). It must be kept in mind that over half of those who receive warfarin are managed suboptimally, due to the complex pharmacodynamics and the several interactions with various foods, herbs and medications (Ansell et al., 2001). To ensure high-quality management of warfarin therapy, it is important that clinical investigators have skills in oral anticoagulation management.

Conclusions

This review suggests that the ECT mechanism of action may involve many converging pathways facilitated by increased synthesis and release of tPA. These complex pathways include the activation of both BDNF and VEGF; improved NMDA receptor-mediated signaling; increased bioavailability of zinc; purinergic release; and increased mobility of dendritic spines. ECT seems to modulate tPA metabolism, through increasing expression of both GAD65 isoform in GABAergic neurons, and p11 protein. Translational trials are needed to support our hypothesis.

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Conflict of interest

The author declares that there is no conflict of interest.

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Review

Improvement of Psychotic Symptoms and the Role of Tissue Plasminogen Activator

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Abstract: Tissue plasminogen activator (tPA) mediates a number of processes that are pivotal for synaptogenesis and remodeling of synapses, including proteolysis of the brain extracellular matrix, degradation of adhesion molecules, activation of neurotrophins, and activation of the *N*-methyl-D-aspartate receptor. Abnormalities in these processes have been consistently described in psychotic disorders. In this paper, we review the physiological roles of tPA, focusing on conditions characterized by low tPA activity, which are prevalent in schizophrenia. We then describe how tPA activity is influenced by lifestyle interventions and nutritional supplements that may ameliorate psychotic symptoms. Next, we analyze the role of tPA in the mechanism of action of hormones and medications effective in mitigating psychotic symptoms, such as pregnenolone, estrogen, oxytocin, dopamine D3 receptor antagonists, retinoic acid, valproic acid, cannabidiol, sodium nitroprusside, *N*-acetyl cysteine, and warfarin. We also review evidence that tPA participates in the mechanism by which electroconvulsive therapy and cigarette smoking may reduce psychotic symptoms.

Keywords: cognition; refractory; resistant; schizophrenia; tissue plasminogen activator

1. Introduction

Tissue plasminogen activator (tPA) is well known for its role in the coagulation pathway. Both endothelial and exogenous tPA convert plasminogen into plasmin. Plasmin dissolves the fibrin structure of thrombi, thus limiting thrombus formation to the site of vascular injury and restoring blood flow to ischemic territories [1].

Neurons, astrocytes, microglia, and oligodendrocytes also synthesize tPA. In these cells, tPA is stored in synaptic vesicles and released into the extracellular space by depolarization stimulus [2,3]. The expression of tPA is high in areas characterized by extensive remodeling of neuronal circuits throughout life, such as the hippocampus, the amygdala, prefrontal and cerebellar cortices, and the hypothalamus [3].

Until recently, it was assumed that once the brain was damaged, there was little, if any, possibility of axonal regeneration and formation of new synapses. Neurophysiological and neuroimaging studies support the notion that the human brain undergoes regeneration and synaptic plasticity. tPA plays an important role in both processes [4].

2. Tissue Plasminogen Activator and the Brain

Animal studies have demonstrated that tPA—itself or through activation of matrix metalloproteinases—mediates proteolysis of the extracellular matrix, which is a prerequisite for the

formation and elimination of synapses, and for synaptic strength changes [5]. Both mechanisms underlie cognitive processes. Cognitive functions, which are related to the outcome of schizophrenia and are little influenced by antipsychotic treatment, depend on tPA-mediated synaptic remodeling [3,5,6]. Cognitive decline may precede the onset of psychosis in schizophrenia by almost a decade [7].

Apart from extracellular matrix proteolysis, tPA catalyzes a number of processes that are usually defective in psychotic patients. For example, by cleaving the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor, tPA increases calcium influx that enhances NMDA receptor signaling [3,8]. Calcium entry through the NMDA receptor determines whether neurons will die or survive: it seems that too much NMDA receptor activity is harmful to neurons, but so is too little [9]. NMDA receptor is a key element in excitatory transmission and synaptic plasticity. Evidence that aberrant NMDA receptor signaling contributes to schizophrenia pathogenesis comes from the fact that antagonists of NMDA receptor produce neurocognitive dysfunction, such as seen in schizophrenia [3].

Another mechanism dependent on tPA proteolytic activity is the cleavage of neurotrophins. Neurotrophins may have opposite functions depending on their state: pre-cleavage and post-cleavage. For example, brain-derived neurotrophic factor (BDNF) precursor binding to the p75 receptor causes a long-lasting reduction in synaptic strength—referred to as long-term depression, and to neuronal apoptosis. By contrast, binding of mature BDNF to its tyrosine kinase receptor leads to a long-lasting increase in synaptic efficacy—known as long-term potentiation, and to neuronal survival [10].

Dopaminergic transmission also seems to be influenced by tPA. Plasmin, acting on pre-synaptic dopaminergic neurons via plasminogen activator receptor (PAR)-1, enhances depolarization-evoked release of dopamine in the nucleus accumbens [11]. As such, tPA mediates emotional cognitive functions, especially reward-related memory reconsolidation [11].

3. tPA Inhibition

In the brain, tPA is inhibited by plasminogen activator inhibitor (PAI)-1 and by neuroserpin. PAI-1 is released by endothelial cells in the presence of inductors such as glucocorticoids, transforming growth factor-β, angiotensin, glucose, insulin, and triglycerides [12]. A single nucleotide polymorphism in the PAI-1 promoter—known as PAI-1 4G/5G, results in elevated PAI-1 levels and, consequently, in decreased tPA activity [13].

Little is known about neuroserpin gene activation, apart from it being post-transcriptionally regulated by triiodothyronine [14]. Point mutations in the neuroserpin gene may cause an uncommon form of dementia, named familial encephalopathy with neuroserpin inclusion bodies [15].

4. Conditions that Inhibit tPA Function Are Prevalent in Schizophrenia

Markers of low tPA activity consistently described in schizophrenia include hyperhomocysteinemia and antiphospholipid antibodies, such as lupus anticoagulant and IgM isotype anticardiolipin antibody [16–18]. Importantly, both hyperhomocysteinemia and antiphospholipid antibodies may affect tPA activity without affecting tPA levels [19].

Homocysteine, for example, inhibits tPA interaction with a heterotetramer formed by two annexin A2 molecules and two molecules of protein p11 (also known as S100A10). Since the heterotetramer increases tPA-mediated plasmin generation, hyperhomocysteinemia impairs tPA activity [19].

In the central nervous system, protein p11 interacts with the serotonin 5-HT1B receptor, which might explain the positive correlation between homocysteine levels and the severity of schizophrenia negative symptoms [18].

Our group has recently searched 70 patients with schizophrenia or schizoaffective disorder and 98 controls without mental disorders for markers of reduced activity of tPA. Hyperhomocysteinemia

and antiphospholipid antibodies were highly prevalent in patients, but not in controls. Besides, we have identified a high prevalence of a not-previously described marker: free-protein S deficiency. Free-protein S and functional protein C are natural anticoagulants that form a complex, which inhibits tPA inhibitors. None of the controls had free-protein S deficiency and all participants had normal protein C levels, suggesting that protein S could have a role in schizophrenia independent of protein C [20]. In the same study, the association of the PAI-1 4G/5G polymorphism with hyperinsulinemia or hypertriglyceridemia was highly synergistic for acute episodes [20].

Based on the finding that chronic patients and those studied during acute episodes had more markers of low tPA activity (3–6 markers per patient, mean 3.1) than patients in remission (0–3, mean 0.9) or controls (0–2, mean 0.5), we have postulated that various concomitant conditions reducing tPA activity would be required for full expression of the disease [20].

It is possible that the number of markers per patient was underestimated. This is because some authors have noticed a negative correlation of anticardiolipin antibody levels with disease exacerbation, which is highly suggestive of antibody consumption during acute episodes [21].

Assuming that tPA has a crucial role in cognitive functions and that markers of low tPA activity are prevalent in patients with schizophrenia or schizoaffective disorder, it is not surprising that tPA plays an important role in the mechanism of action of pharmacological and non-pharmacological interventions that alleviate psychotic symptoms.

5. Lifestyle Interventions and Nutritional Supplements

5.1. Metabolic Interventions Effective in Decreasing Glucose and Insulin Levels

Since the PAI-1 promoter responds to glucose and insulin [12], one would expect that interventions effective in decreasing glucose levels and insulin synthesis, such as regular aerobic exercises and carbohydrate-restricted diets, could help normalize tPA activity. Confirming the expectations, obese patients with schizophrenia or schizoaffective disorder who achieve weight reduction with a program incorporating nutrition counseling and aerobic exercise may experience significant improvement of the mental symptoms [22]. Resolution of longstanding schizophrenia symptoms has been also reported after starting a low-carbohydrate, ketogenic diet [23].

Further evidence that interventions aimed at normalizing glucose and insulin levels may improve cognitive function comes from a study randomizing schizophrenia patients and healthy controls to exercise or non-exercise. Following aerobic exercise, hippocampal volume increased significantly in patients and healthy subjects, with no change in the non-exercising patients. Schizophrenia patients had modest cognitive improvement, which correlated with hippocampal volume changes [24].

5.2. Omega 3 Polyunsaturated Fatty Acids

Omega 3 polyunsaturated fatty acids increase tPA levels in the prefrontal cortex and hippocampus of rats submitted to chronic unpredicted mild stress to induce depressive behavior [25]. The impact of omega 3 in human tPA brain levels has not been determined, but omega 3 polyunsaturated fatty acids decrease serum triglycerides, with a magnitude proportional to triglyceride serum concentration [26]. Mice studies have shown that hypertriglyceridemia is an important element in the pathophysiology of learning and memory problems associated with obesity. The same studies have demonstrated that lowering triglyceride levels restores cognitive function [27]. Since the PAI-1 promoter responds to triglycerides, the reduction in PAI-1 levels observed in patients receiving omega-3 polyunsaturated fatty acids may be possibly related to the normalization of triglyceride levels [12,26].

One important trial supporting the hypothesis that omega-3 polyunsaturated fatty acids may influence cognition was conducted with young people at high risk of developing schizophrenia. This randomized, double-blind, placebo-controlled study has evaluated whether

omega-3 polyunsaturated fatty acid supplementation for 12-weeks could prevent progression to psychotic disorder [28]. During a median of 6.7 years of follow-up, almost 10% of the omega-3 group developed psychosis compared to 40% of the patients who received placebo. Furthermore, the placebo group had an earlier onset of psychosis. Severe baseline negative symptoms increased the chances of treatment response [28].

5.3. Folic Acid, Vitamin B12, and Pyridoxine

Hyperhomocysteinemia may result from deficiency of folate, vitamin B12, pyridoxine, from heavy cigarette smoking, alcohol abuse or dependence, or renal dysfunction [29].

Methylenetetrahydrofolate reductase (MTHFR) recycles folic acid. The homozygous MTHFR C677T polymorphism, present in 10%–12% of the world population, results in greater than 70% reduction in enzyme activity [30,31]. Since folic acid is required to metabolize homocysteine, this homozygous genotype may increase plasma homocysteine levels in individuals with low folate intake. In a cohort of first-episode drug-naïve schizophrenia, folate levels were directly related to hippocampal volume, while for homocysteine levels the correlation was negative [18].

In genome-wide association studies taking into account the association of the MTHFR C677T polymorphism with plasma homocysteine levels, the effect of homocysteine plasma levels on schizophrenia diagnosis was over two-fold [30]. Reduced folate levels in the absence of increased homocysteine levels do not increase the risk of schizophrenia [31]. Prolonged folic acid, vitamin B12, and pyridoxine supplementation was effective in alleviating symptoms of schizophrenia individuals with extremely high homocysteine levels (around 24 μmol/L) [29].

6. Hormones

6.1. Pregnenolone

Progesterone binds to different receptors in the rodent brain. One of them is progesterone receptor membrane component 1 (PGRMC1, also known as 25-dx), which is found in microglia, astrocytes, and neurons [32]. Unlike other progesterone receptors, PGRMC1 is uniformly expressed in all hippocampal neurons [32]. In neurons and glia, progesterone is synthesized from pregnenolone [33].

In the ovary, progesterone has been shown to possess antiapoptotic properties. In granulosa cells, PGRMC1 interacts with PAI-1 RNA binding protein (PAIRBP1). Progesterone, competing with PAI-1 for the PGRMC1/PAIRBP1 complex, immortalizes the granulosa cells [34]. Although the relationship between PGRMC1 and PAIRBP1 in the brain has not been demonstrated yet, as it has been in the ovary, evidence that progesterone may afford protection against psychotic symptoms comes from relapses of schizophrenia symptoms, rare during pregnancy, but common in the postpartum period [35].

In randomized, double-blind, placebo-controlled trials, adjunctive pregnenolone reduced the severity of negative symptoms of schizophrenia and schizoaffective disorder, especially if the patient has not been treated with mood stabilizers [36,37]. More evidence that progesterone improves schizophrenia symptoms comes from atypical antipsychotics. Clozapine, and to a lesser degree olanzapine, elevates pregnenolone levels in the hippocampus and cerebral cortex of rats [38]. Additional studies are required to evaluate if the benefits of clozapine, olanzapine, and pregnenolone in the treatment of mental disorders depend on the competition between pregnenolone and PAI-1 for the PGRMC1/PAIRBP1 complex.

6.2. Estrogen

Estrogen receptors α and β have opposite effects on the human PAI-1 promoter: while β receptor suppresses it, α receptor induces it [39]. In the human hippocampus, estrogen receptor β levels are greater than estrogen receptor α levels [40].

The Definitive Oestrogen Patch Trial showed that estradiol may be an effective adjunctive therapy for women with treatment-resistant schizophrenia or schizoaffective disorder [41]. In this double-blind trial, females aged 18–45 years were randomized for transdermal estradiol 100 μg, 200 μg or placebo. Estradiol groups had greater symptom improvement than the placebo group, particularly in positive symptoms. The higher effect was seen with 200 μg. Benefits have also been seen in male schizophrenia patients [42].

6.3. Oxytocin

Oxytocin is another hormone that influences tPA activity in the brain. In multiparae, a rise in tPA antigen occurs shortly after oxytocin infusion is started [43]. Intranasal oxytocin may improve psychotic symptoms and social cognition in patients with schizophrenia [44].

7. Pharmacological Interventions

7.1. Dopamine D3 Receptor Antagonists

Dopamine D3 receptors (D3R), localized in extrastriatal regions, possess the highest affinity for dopamine of all known dopamine receptors [45]. Studies with mice lacking functional D3R or treated with novel, potent D3R antagonists have indicated that the receptor is a key player in neurogenesis and learning [46]. These mice had enhanced activation of cyclic AMP response element-binding protein (CREB), which is responsible for tPA and BDNF transcription. An increased immunoreactivity of tPA and mature BDNF in the prefrontal cortex and hippocampus of animals using D3R antagonists supports the hypothesis that tPA is involved in cognitive functions [46]. ABT-925, a selective D3 antagonist tested in phase II studies in schizophrenia patients, has been shown to enhance cognition [47].

7.2. Retinoic Acid

Many genes coding for proteins whose expression is altered in the prefrontal cortex of schizophrenia are either directly or indirectly regulated by retinoic acid, a metabolite of vitamin A. Examples include pyruvate kinase muscle isozyme, mitochondrial aconitase 2, hexokinase 1, malate dehydrogenase 1, gelsolin, neuron-specific enolase, and actinin α4 [48].

Long-term exposure of human astrocytes and endothelial cells to retinoic acid induces tPA expression in both cells and decreases PAI-1 expression in astrocytes [49,50]. While retinoic acid does not cross the blood–brain barrier [51], bexarotene, a substance that interacts with retinoid X receptors, does. A study controlling for antipsychotic agents showed that adjunctive bexarotene improved positive symptoms of schizophrenia and schizoaffective disorder, especially in patients with mean or high baseline positive scale scores and those who were not on lipid-reducing agents [52].

7.3. Valproic Acid

Valproic acid—a histone deacetylase inhibitor currently prescribed for the treatment of epilepsy, bipolar disease, and schizoaffective disorder—promotes neurite extension and neuronal growth in cultures of neurons and astrocytes [53]. The mechanism seems to involve a reduction in PAI-1 activity in astrocytes [53]. In cultured endothelial cells, valproate significantly increases tPA expression, while PAI-1 is only marginally affected by the treatment [54].

7.4. Cannabidiol

Δ9-tetrahydrocannabinol and cannabidiol are two psychoactive constituents of cannabis. Cannabidiol, an endocannabinoid modulator, decreases expression and secretion of PAI-1 [55]. While Δ9-tetrahydrocannabinol is psychotomimetic, cannabidiol reduces psychotic symptoms of schizophrenia [56].

7.5. Sodium Nitroprusside

After infusion, the vasodilator sodium nitroprusside is converted to the neurotransmitter nitric oxide [57], which mediates tPA release by endothelial cells [58]. In a double-blind, placebo-controlled trial, schizophrenia patients who received a single intravenous administration of low-dose sodium nitroprusside presented significant improvement in cognitive performance, whereas those who received placebo did not [59].

7.6. Warfarin

Warfarin has fibrinolytic properties that depend on its ability to increase tPA activity in the vascular compartment. Although it is not known whether warfarin increases tPA synthesis in cells other than the endothelium, it has been demonstrated that tPA crosses the intact blood-brain barrier [60]. Five patients with schizophrenia or schizoaffective disorder on chronic warfarin therapy for recurrent deep-vein thrombosis showed remission of psychotic symptoms [61]. Three of them had persistent antiphospholipid antibodies. The appropriate dose of warfarin may be difficult to establish, due to the interaction of the anticoagulant with foods, herbs, dietary supplements, and caffeine-containing beverages [62,63].

7.7. N-Acetyl Cysteine

In a double-blind placebo-controlled trial, adjunctive *N*-acetyl cysteine reduced negative symptoms in patients with chronic schizophrenia [64]. While some authors have reported that *N*-acetyl cysteine reduces lipoprotein (a) levels [65], others have found a significant decrease in homocysteine levels, but an insignificant decrease in lipoprotein (a) levels [66]. Since lipoprotein (a) increases PAI-1 expression in endothelial cells [67], a reduction in lipoprotein (a) levels would increase tPA activity by reducing PAI-1 levels or homocysteine levels.

8. Electroconvulsive Therapy

tPA is synthesized by chromaffin cells, stored in catecholamine–containing vesicles and co-released with catecholamines in response to sympathetic stimulation [68]. Electroconvulsive therapy (ECT) results in a 3- and 15-fold increase in norepinephrine and epinephrine levels [69]. Seizures induced by electroconvulsive shock are accompanied by increased plasma levels of tPA and up-regulation of tPA in the rat hippocampus [70].

Of note, the mechanism of action of ECT seems to involve a number of pathways facilitated by increased synthesis and release of tPA. These pathways include the activation of BDNF and vascular endothelial growth factor, increased NMDA receptor mediated signaling, increased zinc bioavailability, purinergic release, and pruning of dendritic spines [71].

ECT is frequently considered first-line treatment when a quick response is necessary, such as for patients with catatonia, aggression, and suicidal behavior [72]. Individuals with positive symptoms, such as paranoid delusions and hallucinations, also have a high probability of response [73]. The procedure is recommended for schizophrenia patients who did not respond to at least two trials of antipsychotic drugs of different classes, at adequate dosages for at least four weeks each, and for those who have shown intolerance to medication side effects [73].

9. Cigarette Smoking

In mice, single nicotine treatment significantly increases tPA activity in the extracellular space of the nucleus accumbens. Then, through PAR-1 activation, tPA and plasmin regulate dopamine release and nicotine-induced reward [74].

Cigarette smoking is highly prevalent in schizophrenia. Among first-episode patients, it has been reported that smokers have significantly lower severity of negative and depressive symptoms in comparison with non-smokers. Besides, psychosis onset occurs later in life in patients with

severe nicotine dependence than in those with mild nicotine dependence or nonsmokers [75]. Smoking abstinence impairs attention and spatial working memory performance in smokers with schizophrenia, and smoking reinstatement reverses the impairment. Nicotine replacement therapy for schizophrenia patients who smoke very low nicotine content cigarettes may preserve cognitive function [76].

10. Future Directions

10.1. Intranasal tPA

In rats, tPA administrated intranasally during the subacute phase of traumatic brain injury or experimental stroke promotes neuroplasticity and significantly improves cognitive function [4]. Brain hemorrhage, a side effect of intravenous tPA, did not occur when animals were treated with intranasal tPA [77]. The role of intranasal tPA in psychotic disorders remains to be defined.

10.2. Rituximab

Patients with antiphospholipid antibody syndrome resistant to conventional medications may respond to treatment with rituximab, an anti-CD20 monoclonal antibody [78]. To date, no study has assessed the impact of rituximab in individuals with schizophrenia spectrum disorders refractory to antipsychotics.

11. Conclusions

tPA-mediated synaptic plasticity and neuronal regeneration are crucial processes for major brain functions. The hypothesis that tPA dysfunction might explain some obscure aspects of schizophrenia pathophysiology is supported by the antipsychotic armamentarium. The enhancement of tPA activity seems to be a common denominator of many interventions effective in mitigating psychotic symptoms and improving cognitive deficits, including lifestyle modifications, hormones, medications, electroconvulsive therapy, and even cigarette smoking. Controlled studies are needed to determine how interventions aiming specifically at correcting activity of tPA affect the outcome of psychotic disorders.

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LINHAS DE PESQUISA PARA TESTAR O MODELO PROPOSTO PARA A ESQUIZOFRENIA

A constatação de que pacientes com esquizofrenia ou transtorno esquizoafetivo têm alta prevalência de marcadores de baixa atividade do tPA ou deficiência da proteína S livre ajuda a integrar várias teorias propostas para explicar a patogênese destas doenças. Junto com as respostas, nos deparamos com mais perguntas. Estas foram enunciadas em um manuscrito, que propõe linhas de pesquisas para validar nossas hipóteses e replicar nossos resultados.

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- ✓ **O que o artigo tem de inovador**: Para validar a hipótese de que a deficiência da proteína S é um fator de alta relevância na etiopatogenia da esquizofrenia, recomendamos que os níveis de proteína S livre sejam avaliados em pacientes virgens de tratamento, no primeiro episódio psicótico. Identificando-se a deficiência, é importante estratificá-la em hereditária (ou seja, prevalente em parentes de primeiro grau), relacionada ao uso de hormônios ou a doenças inflamatórias, como a periodontite e a obesidade.
- ✓ Também consideramos importante que a prevalência de episódios psicóticos seja avaliada em pacientes com deficiência hereditária de proteína S, em um grande estudo multicêntrico.
- ✓ Além disso, recomendamos que a atividade do tPA e os níveis da proteína S livre sejam avaliados em pacientes com lupus eritematoso sistêmico, síndrome do anticorpo antifosfolipídio e outras doenças auto-imunes, comparando os pacientes que têm déficit cognitivo ou episódios psicóticos com os que não têm.
- ✓ De acordo com nosso modelo, a melhora dos sintomas psicóticos dependeria da correção concomitante de múltiplas condições capazes de reduzir a atividade do tPA, da plasmina e/ou da proteína S. Isto inclui mudanças no estilo de vida para normalizar os níveis de insulina, suplementação vitamínica para normalizar a homocisteinemia e o tratamento de doenças inflamatórias, como a periodontite e a obesidade.

Claro está que o reparo dos circuitos neuronais depende de várias etapas e, portanto, não se deve esperar que a melhora clínica ocorra logo após a normalização da atividade do tPA e da proteína S.

- ✓ É de suma importância avaliar como a esquizofrenia evolui em pacientes que começam a anticoagulação, estratificando os anticoagulantes em dois grupos: os que reduzem os níveis da proteína S, como o warfarin e o acenocoumarol, e os que não reduzem.
- ✓ Esse trabalho também reforça a necessidade de estudos que avaliem se exercícios de alto dispêndio calórico (por exemplo, caminhar 45–60 minutos, todo dia) são mais eficazes no alívio de sintomas psicóticos do que os de baixo dispêndio calórico (por exemplo, caminhar 25–40 minutos, algumas vezes por semana).

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HYPOTHESIS

Dysfunction in the coagulation system and schizophrenia

S Hoirisch-Clapauch[1], OB Amaral[2], MAU Mezzasalma[3,4], R Panizzutti[3,5] and AE Nardi[3,4]

Although different hypotheses have been formulated to explain schizophrenia pathogenesis, the links between them are weak. The observation that five psychotic patients on chronic warfarin therapy for deep-vein thrombosis showed long-term remission of psychotic symptoms made us suspect that abnormalities in the coagulation pathway, specifically low tissue plasminogen activator (tPA) activity, could be one of the missing links. Our hypothesis is supported by a high prevalence of conditions affecting tPA activity in drug-naive schizophrenia, such as antiphospholipid antibodies, elevated cytokine levels, hyperinsulinemia and hyperhomocysteinemia. We recently screened a group of schizophrenia patients and controls for conditions affecting tPA activity. Free-protein S deficiency was highly prevalent among patients, but not found in controls. Free-protein S and functional protein C are natural anticoagulants that form complexes that inhibit tPA inhibitors. All participants had normal protein C levels, suggesting that protein S could have a role in schizophrenia, independent of protein C. Chronic patients and those studied during acute episodes had between three and six conditions affecting tPA and/or protein S activity, while patients in remission had up to two, which led us to postulate that multiple conditions affecting tPA and/or protein S activity could contribute to the full expression of schizophrenia phenotype. This paper describes the physiological roles of tPA and protein S, reviewing how their activity influences pathogenesis and comorbidity of schizophrenia. Next, it analyzes how activity of tPA and protein S is influenced by biochemical abnormalities found in schizophrenia. Last, it suggests future directions for research, such as studies on animal models and on therapeutic approaches for schizophrenia aiming at increasing tPA and protein S activity.

Translational Psychiatry (2016) **6,** e704; doi:10.1038/tp.2015.204; published online 5 January 2016

INTRODUCTION

Schizophrenia has a substantial genetic basis, but environmental factors such as traumatic life events may increase the risk of psychotic symptoms.[1] The disorder is characterized by impaired cognition, by hallucinations and delusions, referred to as positive symptoms, and by social isolation, flat affect and low motivation, known as negative symptoms.[1]

Antipsychotic medications usually attenuate positive symptoms, but fail to improve negative features or cognitive function.[2] For example, 94% of 215 patients with schizophrenia spectrum disorder considered to be in remission had at least one residual symptom, mostly blunted affect, conceptual disorganization and social withdrawal.[3] Given that residual symptoms can impair performance in school, affect performance at work, disrupt relationships and substantially affect quality of life, the need for more effective therapies is obvious.

Although different hypotheses have been proposed to explain schizophrenia pathogenesis, a link connecting them is missing. Finding the molecular link would allow for a better understanding of schizophrenia pathophysiology, which could lead to new therapeutic targets and better prognostic outcomes.

After noticing that five psychotic patients on chronic warfarin therapy for deep-vein thrombosis showed remission of psychotic symptoms, we assumed that defective modulation of the coagulation pathway might contribute to schizophrenia pathogenesis.[4] In accordance with other studies, neuroimaging studies of our patients showed brain atrophy, but no ischemic lesions.[5,6]

Searching for elements that modulate the coagulation pathway and also participate in processes that help prevent brain atrophy, only one candidate emerged: tissue plasminogen activator (tPA).

Conditions affecting the activity of tPA, such as hyperhomocysteinemia and antiphospholipid antibodies, have been consistently described in drug-naive schizophrenia.[7–10] We recently screened 70 drug-treated schizophrenia patients and 98 controls for these and other conditions affecting tPA activity.[11] Persistent antiphospholipid antibodies were seen in 30% of the patients and none of the controls. Moreover, conditions that decrease tPA activity by increasing the activity of plasminogen activator inhibitor-1 (PAI-1, a major tPA inhibitor) were also highly prevalent among patients. Examples include the association of the 4G/5G polymorphism in the PAI-1 gene with hyperinsulinemia (20 vs 2%), hypertriglyceridemia (17 vs 5%) and hyperhomocysteinemia (24 vs 1%).

The same study detected a 22% prevalence of free-protein S deficiency in patients, while none of the controls presented the condition. As protein S is a cofactor of functional protein C, we were expecting to find a high prevalence of protein C deficiency among patients, but all participants had normal protein C levels. This and the 145-fold increased risk of having a first-degree relative with schizophrenia in patients with low free-protein S levels, compared with controls, led us to focus on protein S deficiency.[11]

Having observed that chronic schizophrenia patients and those studied during acute episodes had between three and six

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Table 1. Influence of low activity of tPA and/or protein S on schizophrenia features

Low activity of tPA and/or protein S	
Clinical comorbidity of schizophrenia	*Biochemical features of schizophrenia*
Cardiovascular risk	Hypercortisolemia
Thrombotic tendency	Elevated cytokine levels
Pregnancy complications	Hyperhomocysteinemia
Lower-than-expected risk of cancer	Hyperinsulinemia
Schizophrenia pathogenesis	Hypertriglyceridemia
Reduced neutrophil	Antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies)
Abnormal NMDA receptor activation	Low free-protein S levels?
Dopaminergic hypothesis	
Adverse fetal environment	

Abbreviations: NMDA, N-methyl-D-aspartate; tPA, tissue plasminogen activator.

conditions, while patients in remission had up to two, we postulated that simultaneous conditions affecting tPA and/or protein S activity could contribute to the full expression of the schizophrenia phenotype.[11,12]

In this review, we analyze the links between schizophrenia and elements that modulate the coagulation pathway, with particular emphasis on tPA and protein S. First, we describe the physiological roles of tPA and protein S, presenting evidence that the somatic comorbidity and laboratory abnormalities of schizophrenia can be related to decreased activity of tPA and/or protein S (Table 1). Next, we point out possible mechanisms by which low activity of tPA and/or protein S might contribute to schizophrenia pathogenesis. Finally, we suggest future directions for research, such as animal studies and therapeutic approaches based on normalization of tPA and protein S activity.

tPA: MECHANISM OF ACTION IN THE BLOOD AND THE BRAIN

tPA mediates both protective and toxic mechanisms in the brain. Endothelial or recombinant tPA catalyzes the conversion of plasminogen to plasmin (Figure 1) and plasmin degrades fibrin clots, thus protecting against ischemic injury.[13] On the other hand, an excessive amount of tPA may damage the blood–brain barrier, increasing the risk of edema and bleeding, both of which may exacerbate neuronal death.[14]

Neurons and glial cells also synthesize and release tPA, which is highly expressed in the cortex, amygdala, hippocampus and cerebellum.[13] Following brain insults such as epileptic seizure, trauma or stroke, increased synthesis of tPA can overactivate excitatory receptors such as the N-methyl-D-aspartate (NMDA) receptor, which increases calcium permeability, causing neuronal damage and death.[14,15] While excessive NMDA receptor activation can damage neurons, the same occurs with its reduced activity.[14,16]

Activation of NMDA receptors, reelin and neurotrophins, as well as dopamine release are some of the many neuroprotective mechanisms mediated by tPA or by plasmin that are defective in schizophrenia.[12,14–21] The NMDA receptor stimulates neuronal migration and is involved in mechanisms of synaptic plasticity, a prerequisite for learning and memory skills. Proteolytic processing of reelin by tPA is fundamental for its function. Reelin stimulates dendrite and dendritic spine development and has an important role in learning and memory.[17]

Cleavage of proneurotrophins, such as of pro-brain-derived neurotrophic factor (pro-BDNF), is also mediated by tPA. While proneurotrophins induce dendritic and synaptic deterioration of cultured neurons, and even neuronal apoptosis, mature

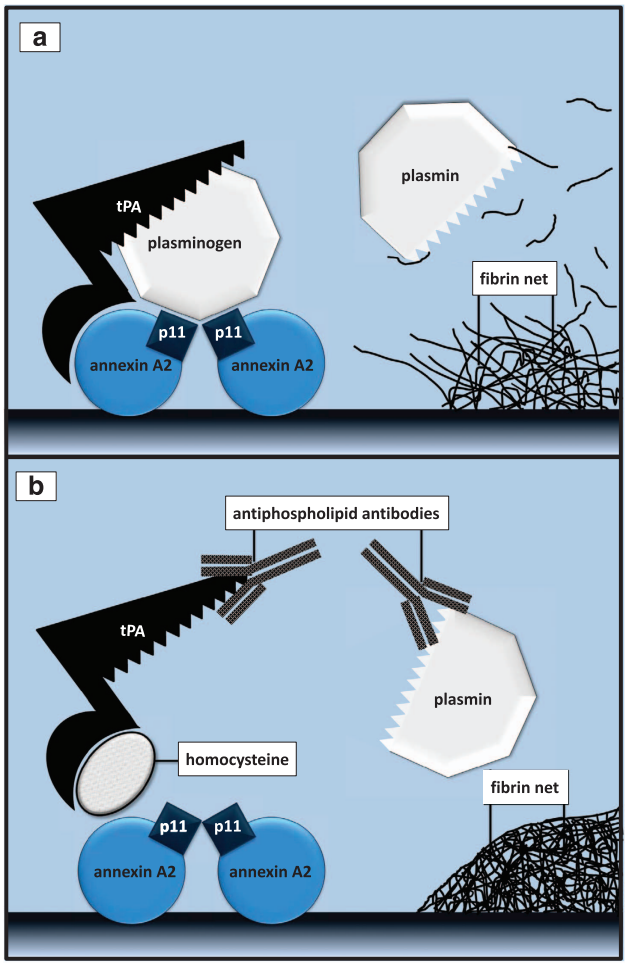

Figure 1. (**a**) Plasminogen and tPA must bind to the heterotetramer formed by two molecules of p11 and two molecules of annexin A2 to generate plasmin. Plasmin dissolves the fibrin net. (**b**). Antiphospholipid antibodies and homocysteine inhibit tPA-induced proteolysis and fibrinolysis. tPA, tissue plasminogen activator.

neurotrophins have opposite functions, promoting growth and remodeling of axons and dendrites, synaptic formation, function and plasticity, neuronal proliferation and survival.[18] In the limbic system, tPA regulates the release of dopamine, a neurotransmitter involved in reward-mediated learning and memory.[19]

Studies in animal models suggest that tPA plays a critical role in the formation of various forms of synaptic plasticity and cognition. For example, in open field object exploration task, tPA-knockout mice express deficits in habituation and reactivity to spatial change, decreased rearing and poor initial object exploration, consistent with altered hippocampal and striatal function.[22] In rats, subacute intranasal tPA treatment, initiated 7 days after traumatic brain injury, enhances neurogenesis in the dentate gyrus. In addition, intranasal tPA increases axonal sprouting of the corticospinal tract originating from the contralesional cortex into the denervated side of the cervical gray matter, and mature BDNF levels. As a result, treatment significantly improves cognitive and sensorimotor functional recovery.[23]

tPA ACTIVITY INHIBITION

In the brain, tPA is inhibited by PAI-1, PAI-2 and neuroserpin. PAI-1 is synthesized by arterial smooth muscle, fat cells, stroma cells of fat tissue and by hepatocytes, especially steatotic ones.[24]

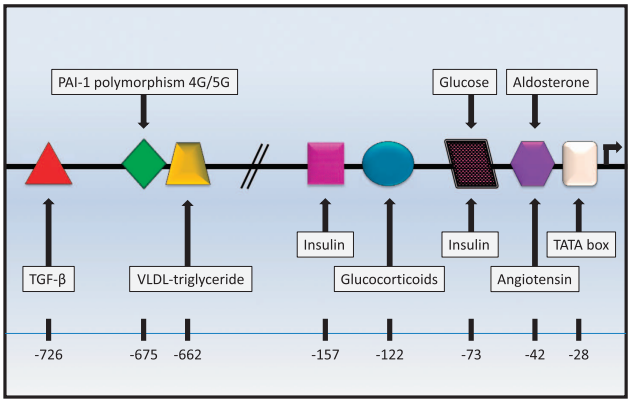

Figure 2. Schematic representation of PAI-1 promoter, showing the 4G/5G single nucleotide polymorphism (rs1799889) and enhancer elements. TATA box, the site of transcription initiation; TGF-β, transforming growth factor-β; VLDL, very-low-density lipoprotein.

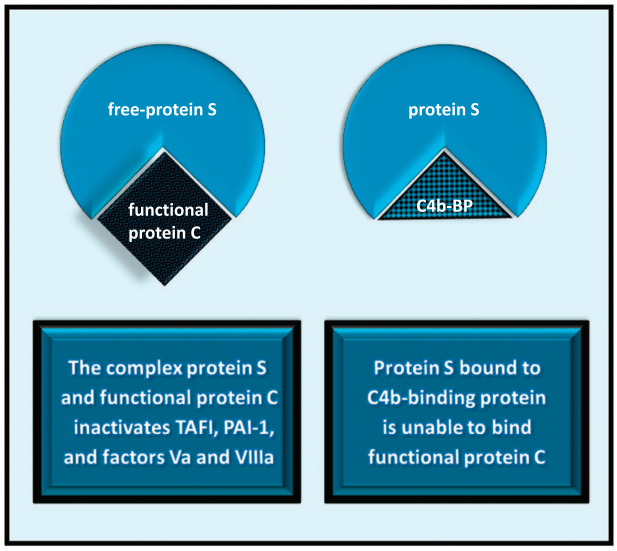

Figure 3. C4b-BP, C4b-binding protein; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor.

Although PAI-1 immunoreactivity has been observed in human neurons and some reactive astrocytes,[24] it has not been determined if these cells are able to synthesize PAI-1. PAI-2 is synthesized by keratinocytes, peritoneal macrophages, trophoblasts and microglia.[25]

The PAI-1 promoter is activated by insulin, glucose, triglycerides, angiotensin (Figure 2) and leptin, a hormone produced by adipocytes.[26,27] A meta-analysis has estimated that >20% of the first-episode or unmedicated schizophrenia patients are obese or overweight, with a high prevalence of elevated fasting glucose and insulin levels, hypertriglyceridemia and high blood pressure.[28] High levels of tumor necrosis factor-α, as seen in inflammatory disorders such as obesity, participate in the mechanism of insulin resistance in peripheral tissues.[29] Pancreatic β cells compensate for insulin resistance by increasing insulin production.

Neuroserpin co-localizes with the secretory protein chromogranin B in large dense core vesicles. Chromogranins seem to play an on/off switch role for secretory granule biogenesis.[30] An association between chromogranin B polymorphisms and schizophrenia has been reported in genome wide-association studies of the Chinese Han and Japanese populations. Although it is known that the polymorphism results in reduced levels of chromogranin B,[31] the relationship between chromogranin and tPA in schizophrenia has not been elucidated yet.

Polymorphisms that reduce tPA activity have been also identified in schizophrenia. In a large sample of Japanese schizophrenia individuals, the association between the mental disorder and two single nucleotide polymorphisms of PLAT, the human tPA gene, rs2020922 and rs8178817, was highly significant.[32]

PROTEIN S: MECHANISM OF ACTION IN THE BLOOD AND THE BRAIN

Protein S is a natural anticoagulant that circulates in an active free form, or bound to C4b-binding protein, one of the complement inactivator proteins (Figure 3).[33] Free-protein S forms a complex with activated protein C that inactivates factors Va and VIIIa, and two molecules that inhibit tPA: thrombin activatable fibrinolysis inhibitor and PAI-1.[33,34] Anticoagulant properties of protein S that do not depend on protein C include factor Xa and prothrombin inhibition.[35]

Roles for protein S beyond coagulation include neuroprotective and anti-inflammatory effects, which have been demonstrated in a murine *in vivo* model of ischemic stroke.[36] A direct neuronal protective effect has been also shown in cultured cortical neurons challenged with hypoxia and aglycemia, followed by reoxygenation.[37] Although it has previously been assumed that the phagocytosis of neurons is always preceded by their commitment to cell death, there is evidence that phagocytosis can mediate the death of viable neurons during development, inflammation and neuropathology.[38] Protein S supports survival, proliferation and differentiation of viable neurons and neuronal stem cells via a mechanism that involves Tyro3, Axt and Mer (TAM) receptors.[37,39] TAM receptors, expressed by both astrocytes and microglia, are tyrosine kinase receptors that regulate cell proliferation and survival, cell adhesion and migration. As a TAM receptor ligand, protein S increases the levels of protein Bcl-2, an apoptotic suppressor in a variety of cells, including neurons.[39]

PROTEIN S INHIBITION

Protein S plasma levels are determined by genetic and environmental factors, including sex, hormonal status, smoking, age and disease.[35] Inflammatory disorders are usually accompanied by decreased protein S activity, due to increased levels of C4b-binding protein. Hereditary deficiency of protein S deficiency is unlikely to be detected by genome wide-association studies because almost 200 mutations have been characterized in the PROS1 gene.[35]

LINKS BETWEEN CLINICAL COMORBIDITY OF SCHIZOPHRENIA AND LOW ACTIVITY OF tPA AND/OR PROTEIN S

Inherited and acquired conditions decreasing activity of tPA and/or protein S may affect clot lysis and extracellular matrix proteolysis, both of which may increase the risk of cardiovascular disease, thrombotic events and pregnancy complications. By affecting extracellular matrix proteolysis, low tPA activity may also contribute to a lower-than-expected risk of cancer.

Cardiovascular risk and thrombotic events

Meta-analyses have consistently shown that ischemic cardiovascular disease reduces the life expectancy of schizophrenia patients, which is about 15 years less than that of the general population. Besides, more than two-thirds of the patients with

schizophrenia die of coronary heart disease, compared with approximately half of the general population.[40,41] Increased cardiovascular risk has been ascribed to antipsychotic medication side effects, to cigarette smoking, to sedentary behavior and to immobility, such as in physical restraint or stupor.[42]

Low activity of tPA and/or protein S may also increase the risk of cardiovascular events. This is because a myocardial infarction may occur when an atherosclerotic plaque rupture triggers the formation of a blood clot that reduces blood flow through the coronary arteries. PAI-1, the major inhibitor of tPA, blocks the formation of plasmin, thereby preventing clot dissolution.[43] Individuals with unstable angina and myocardial infarction usually have increased PAI-1 levels and activity.[43]

Schizophrenia patients seem to be at increased risk of thromboembolic events. By impairing anticoagulation and fibrinolysis, low activity of tPA and/or protein S may contribute to the problem.

Pregnancy complications

Several studies have demonstrated that mothers with psychotic disorders are less likely to receive antenatal care and are at a higher risk of substance, alcohol and tobacco abuse than controls. However, even after controlling for these risk factors, the diagnosis of maternal schizophrenia remains predictive of adverse obstetric outcomes, such as intrauterine growth restriction, stillbirth and prematurity.[44,45]

Low tPA activity may possibly contribute to the adverse outcomes. A healthy pregnancy depends on embryo implantation, trophoblast invasion, placental angiogenesis and placental vessel remodeling, a process that accompanies exponential fetal growth.[45] tPA and/or plasmin participate in all these processes.[46] Hereditary protein S deficiency, increasing the risk of placental vessel thrombosis, may also increase the risk of intrauterine growth restriction and preterm delivery.[47]

Lower-than-expected risk of cancer

There seems to be a discrepancy between cancer incidence and exposure to cancer risk factors in schizophrenia, consistent with a protective effect.[48,49] The lower-than-expected risk of lung cancer is particularly impressive, considering the high prevalence of heavy smokers among schizophrenia individuals. Overall risk of neoplastic disorders is also significantly reduced among unaffected parents and siblings of patients.[50,51]

Tumor cells have the ability to penetrate the extracellular matrix, and, in the case of metastasis, the blood vessels. The degradation of surrounding tissues, a crucial step in tumor cell invasion and metastasis formation,[52,53] involves tPA and plasmin. In addition, proteolytic processing of extracellular matrix components by plasmin or tPA releases vascular endothelial growth factor and other molecules that regulate tumor angiogenesis.[52,53]

LINKS BETWEEN BIOCHEMICAL ABNORMALITIES OF SCHIZOPHRENIA AND LOW tPA AND/OR PROTEIN S ACTIVITY

Biochemical abnormalities commonly found in schizophrenia which may impair tPA and/or protein S activity include hypercortisolemia, increased cytokine levels, hyperhomocysteinemia and antiphospholipid antibodies, such as lupus anticoagulant and IgG and IgM anticardiolipin antibodies.

Hypercortisolemia

Individuals with first-episode psychosis are more likely to have experienced traumatic events than the general population.[54] Cortisol release from the adrenal glands increases several fold after exposure to a stressor.[55]

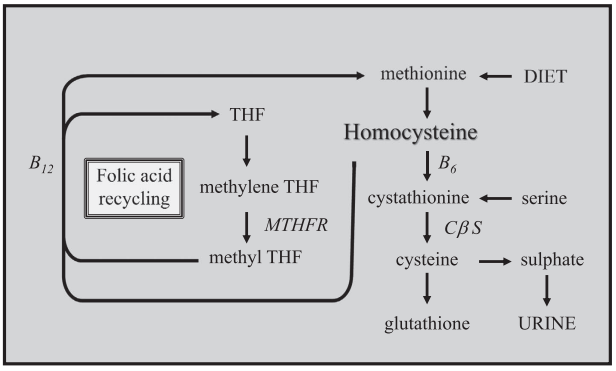

Figure 4. Homocysteine may be recycled to methionine or eliminated in urine as sulfate. Under conditions in which excess methionine is present, homocysteine condenses with serine to form cystathionine in a reaction catalyzed by the vitamin B6–dependent rate-limiting enzyme cystathionine β synthase. B12, vitamin B12; B6, vitamin B6; CβS, cystathionine β-synthase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate. Adapted from ref. 66.

First-episode patients, treated with antipsychotics for < 2 weeks, show significantly higher cortisol levels than controls.[54] PAI-1 promoter responds to cortisol (Figure 2).[26] Another mechanism by which cortisol decreases tPA levels involves glutamate. Glucocorticoids rapidly induce glutamate release in the hippocampus.[55] Extracellular glutamate inhibits release of tPA by brain cells responsible for its recycling: astrocytes.[56]

The hypothesis that glucocorticoids have a role in schizophrenia pathogenesis is supported by the fact that patients taking glucocorticoids and those with Cushing's syndrome may develop psychotic symptoms.[57] Moreover, it has been shown that subjects at high risk of schizophrenia who progress to the disorder have higher baseline cortisol levels than those who do not progress to psychosis or controls.[58]

Elevated cytokine levels

Acute psychotic episodes are usually characterized by increased levels of cytokines such as interleukin (IL)-1β, IL-6 and transforming growth factor (TGF)-β.[59] Cytokines are key regulators of inflammation that activate and recruit immune cells, increase blood supply and enhance vascular permeability.[60] Elevated cytokine levels are commonly found in schizophrenia,[59] accompanying obesity, periodontitis or other inflammatory disorders.

Components of the inflammatory response such as TGF-β may stimulate the synthesis of PAI-1 (Figure 2).[26] Inflammation also decreases protein S activity, either by increasing C4b-binding protein levels (Figure 3) or by facilitating the proteolytic inactivation of protein S, possibly through neutrophil proteases.[61] Conditions characterized by low protein S activity, such as systemic lupus erythematosus, Behçet's disease and Sjögren's syndrome may present with cognitive impairment, delusions and hallucinations.[9,62] In addition, puerperal psychosis occurs when protein S levels are low, regardless of systemic inflammation.[63]

Elevated homocysteine levels

Homocysteine is not a dietary constituent: it is formed upon demethylation of methionine. Homocysteine may be remethylated to methionine by N5-methyltetrahydrofolate in the presence of vitamin B12 or converted to cysteine by cystathionine-β-synthase, a vitamin B6-containing enzyme (Figure 4).[64] Homocysteine plasma levels correlate with homocysteine brain levels[64] and both folate deficiency and hyperhomocysteinemia are prevalent among first-episode, drug-naive schizophrenia patients.[65]

Different explanations for the association between elevated homocysteine levels and schizophrenia have been provided. Folate deficiency may induce neurodegeneration by increasing reactive oxygen species production and cytosolic calcium accumulation.[65] Besides, homocysteine may damage neuronal DNA, triggering apoptosis.[64] A third explanation fits into this model: homocysteine prevents tPA binding to annexin A2, which affects catalytic properties of tPA (Figure 1).

Elevated levels of antiphospholipid antibodies

Antiphospholipid antibodies such as anticardiolipin antibodies and lupus anticoagulant are highly prevalent in drug-naive schizophrenia.[9–11] The diagnosis of antiphospholipid antibody syndrome requires the persistence of antibodies, plus either a thrombotic event or an obstetric complication due to abnormal placentation.[67] Patients with antiphospholipid antibodies may present with cognitive dysfunction or full-blown psychosis, independent of brain ischemia.[4,8]

Based on the finding that antiphospholipid antibodies may increase blood–brain barrier permeability, it was postulated that the deleterious effects of these antibodies in the central nervous system were dependent on their binding to neurons, glia cells, oligodendrocytes and microglia.[9]

It is also possible that the link between antiphospholipid antibodies and mental disorders involves their inhibition of tPA and/or protein S activity. Antiphospholipid antibodies may directly inhibit tPA, plasminogen, plasmin or proteins that participate in the process of tPA and plasminogen assembling, such as β2 glycoprotein-1 and annexin A2 (Figure 1).[68] Antiphospholipid antibodies against protein S have been also described.[69]

LINKS BETWEEN SCHIZOPHRENIA HYPOTHESES AND LOW ACTIVITY OF tPA AND/OR PROTEIN S

Low activity of tPA and/or protein S might link different hypotheses for schizophrenia, including the neuropil hypothesis, the NMDA receptor hypothesis, the dopaminergic hypothesis and the hypothesis correlating an adverse fetal environment with an increased risk of developing schizophrenia.

Neuropil hypothesis

In schizophrenia, brain atrophy reflects loss of neuropil—the network of dense synaptic contacts formed by unmyelinated axons, dendrites and glial processes, rather than neuronal loss.[70] Neuropil atrophy, observed mostly in the prefrontal region, hippocampus and thalamus,[5] is thought to underlie changes in synaptic, dendritic and axonal organization that have been correlated to cognitive dysfunction.[6] Decreased neurotrophin availability and reduced reelin expression are important contributors to brain atrophy in schizophrenia.[6] The neuropil hypothesis supports the involvement of tPA in the pathogenesis of schizophrenia as tPA mediates proteolytic processing that activates neurotrophins and reelin.

Reduced activity of NMDA receptor

Evidence indicates that hypofunction of the NMDA receptor, impairing synaptic plasticity, may contribute to the pathophysiology of schizophrenia. tPA may also have a role in the NMDA receptor hypothesis, because proteolytic activity of tPA and plasmin enhances NMDA receptor signaling.[15,16]

Dopaminergic hypothesis

Positron emission tomography and single-photon emission computed tomography have shown that impairment of dopaminergic transmission in schizophrenia is mostly pre-synaptic, and affects dopamine synthesis capacity, baseline synaptic dopamine levels and dopamine release.[71] Dopamine-activated post-synaptic neurons release tPA into the extracellular space. tPA converts plasminogen to plasmin, and plasmin acts on pre-synaptic dopaminergic neurons via plasminogen activator receptor-1 to potentiate the activity-dependent release of dopamine in the nucleus accumbens.[72]

Adverse fetal environment

Individuals born prematurely or with low-birth weight are at increased risk for developing schizophrenia.[73] Assuming a high prevalence of hereditary protein S deficiency or inherited conditions reducing tPA activity among mothers of schizophrenia patients, one would expect in this group an increased prevalence of pregnancy complications related to abnormal placentation. In this setting, a preterm delivery or a small-for-gestational-age offspring would be a parallel occurrence, not an element belonging to schizophrenia pathogenesis.

RECOMMENDATIONS FOR FUTURE RESEARCH

Protein S deficiency

We recommend that free-protein S levels be assessed in a large series of first-episode drug-naive patients with schizophrenia and their first-degree relatives. The prevalence of psychosis in thrombophilia patients with hereditary protein S deficiency also remains to be determined.

Risk of thromboembolic events

A large multicentric study is needed to compare the prevalence of thrombotic events before the diagnosis of schizophrenia with that of the general population. The Computerized Registry of Patients with Venous Thromboembolism (RIETE, www.riete.org) is currently looking for a possible association between mental disorders and thrombotic tendency.

Autoimmune disorders

We recommend that tPA activity and free-protein S levels be assessed in patients with autoimmune disorders with and without psychosis, especially those with lupus erythematosus or antiphospholipid antibody syndrome.

Interventions aiming at increasing tPA or protein S activity

Controlled studies are required to determine how interventions aiming specifically at increasing tPA and protein S activity affect the course of schizophrenia. It should be highlighted that because restoration of hippocampal and prefrontal cortex circuitry in schizophrenia patients requires multiple sequential steps, short-term improvement after normalization of tPA and protein S activity is not expected to occur. Interventions effective in increasing tPA levels include lifestyle modifications, vitamin supplementation aiming at normalizing homocysteine levels, treatment of inflammation, anticoagulants and electroconvulsive therapy. Intervention studies should stratify patients as users of anticoagulants that inhibit protein S synthesis, such as warfarin or acenocoumarol, and users of other anticoagulants. Although promising, intranasal tPA has not been tested in humans.

Evidence indicates that lifestyle interventions, such as exercise,[74,75] a low-carbohydrate diet[76] and diets for weight loss,[77] can alleviate psychotic symptoms. It has not been defined whether higher-calorie-expenditure exercises (45 to 60 min per session, almost daily walking) are more effective than short distance walking (25 to 40 min, few times per week, or multiple exercise modalities) in alleviating psychotic symptoms.[78] We

suggest that protein supplementation be tested in controlled studies, as a strategy to correct hyperinsulinemia and restoring tPA activity.

Many physicians will be reluctant to conduct studies with anticoagulant medications in therapeutic doses, due to the risk of bleeding. Patients with a previous thrombotic event and persistent antiphospholipid antibodies have a high risk of recurrence and most authorities agree that they should be placed on long-term anticoagulation.[67] Schizophrenia patients with such profile are thus ideal candidates for preliminary studies. Although it is known that tPA crosses the intact blood–brain barrier,[79] whether warfarin increases tPA synthesis in the central nervous system remains to be defined.

Electroconvulsive therapy may alleviate psychotic symptoms. In rats, tPA activity rapidly increases by over 50% after electroconvulsive shock, and remains elevated for more than 24 h.[80] tPA mediates a variety of chemical reactions underlying the mechanism of action of electroconvulsive therapy.[81] We recommend that patients refractory to the procedure be screened for conditions affecting tPA activity, such as hyperhomocysteinemia or antiphospholipid antibodies (Figure 1).

Animal studies

Animal studies are very suitable to generate evidence of a direct causal relationship between tPA or protein S and schizophrenia-like symptoms. More careful examination of the tPA-knockout mice should be performed, focusing on schizophrenia-related findings and behaviors such as social cognition. Another strategy would be to study the developmental and behavioral effects of transient and/or conditional tPA gene knockdown. Protein S knockout mice are not viable,[82] but protein S-deficient mice can reach adulthood. Studies of brain development and behavior should be performed in protein S-deficient mice, as well as in animals with transient and/or conditional protein S gene knockdown. The reversal of developmental and behavioral findings in those animal models with antipsychotic drugs and/or warfarin should also be investigated.

We also recommended that tPA levels, tPA activity, and total- and free-protein S levels be evaluated in animal models of schizophrenia with good etiologic, phenotypic and predictive validity. Assuming that low tPA activity will be detected in these animals, it would be interesting to assess if warfarin therapy can change behavioral phenotypes with or without metabolic interventions aiming at preventing hyperinsulinemia and hypertriglyceridemia. It would be also important to assess if intra-cerebral levels of tPA and protein S correlate with their peripheral levels. It is well-known that cortisol, insulin and triglycerides increase PAI-1 plasma levels (Figure 2),[26] but their effect on neuronal PAI-1 levels has not been reported.

It has been reported that tPA-null mice are resistant to neuronal destruction after intra-hippocampal injections of excitotoxins,[83] but it is unknown whether these animals are more vulnerable to stress-induced neuronal damage.

CONCLUSION

Future research is needed to elucidate the exact role of tPA and protein S in the pathogenesis of schizophrenia and to determine the impact of interventions aiming specifically at correcting activity of tPA and protein S on the course of schizophrenia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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O tPA E A PATOGÊNESE DA PÚRPURA TROMBOCITOPÊNICA TROMBÓTICA

Tudo indica que a baixa atividade do tPA contribua para a patogênese de outras doenças acompanhadas de disfunção cognitiva. Uma delas é a púrpura trombocitopênica trombótica (PTT), uma doença rara, potencialmente fatal e caracterizada por oclusão microvascular generalizada.

A patogênese da PTT tem sido relacionada à grave deficiência da ADAMTS13, uma desintegrina e metaloproteinase com domínio de trombospondina 13. Esta enzima cliva multímeros do fator von Willebrand, um fator que promove a adesão das plaquetas ao endotélio lesado. Se os multímeros não forem clivados em moléculas menos adesivas, poderão promover agregação plaquetária intravascular, consumo de plaquetas e hemólise microangiopática. A PTT também pode cursar com febre, lesão renal e transtornos do sistema nervoso central.

Há vários dados contrariando a hipótese de que a deficiência da ADAMTS13 seja o único substrato fisiopatológico da PTT. Um deles é que os níveis de ADAMTS13 têm fraca correlação com a gravidade da trombocitopenia ou da hemólise, com sintomas do âmbito neurológico ou com a resposta ao tratamento, que consiste na troca plasmática. Além disso, alguns pacientes com grave deficiência congênita de ADAMTS13 cursam com frequentes recaídas desde a infância, enquanto outros são assintomáticos até os 40 anos. Como as alterações cognitivas são prevalentes, tanto nos pacientes com PTT em atividade, quanto nos que permanecem em remissão por muito tempo, postulamos que a baixa atividade do tPA seria um elemento importante na fisiopatologia da PTT.

A hipótese é reforçada pelo fato de que diversas condições que podem desencadear recaídas da doença cursam com baixa atividade do tPA. São exemplos: a gestação e o puerpério, infecções bacterianas, a infecção por HIV com alta carga viral, e o uso da mitomicina, da gemcitarabina, da ciclosporina, do everolimus e do tacrolimus.

A gestação, o puerpério e infecções bacterianas cursam com resistência insulínica, que estimula a produção de insulina. Infecções bacterianas e por HIV, com alta carga viral, costumam cursar com hipercortisolismo. A insulina, o cortisol e medicações que desencadeiam a PTT aumentam a síntese de PAI-1.

Duas doenças autoimunes podem cursar com um quadro clínico indistinguível do da PTT: o lupus eritematoso sistêmico e a síndrome do anticorpo antifosfolipídio catastrófica. Nas duas doenças, a baixa atividade do tPA pode resultar tanto da inflamação sistêmica, quanto de autoanticorpos.

O tPA e a plasmina têm propriedades análogas ao do ADAMTS13, ou seja, promovem proteólise do fator von Willebrand. A hipótese de que a baixa atividade do tPA e da plasmina, impedindo o funcionamento de um *back-up* do ADAMTS13, contribuiria para a patogênese da PTT e para as alterações cognitivas evidenciadas na fase aguda e na remissão a longo prazo da doença, foi defendida no artigo:

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- ✓ **O que o artigo tem de inovador**: Esse é o primeiro trabalho a associar condições que podem deflagrar a PTT à baixa atividade do tPA.
- ✓ Recomendamos que tratamentos capazes de restaurar os níveis de tPA, como a anticoagulação, sejam testados, em estudos controlados, na prevenção da PTT, em pacientes com deficiência hereditária da ADAMTS13.
- ✓ Se o hiperinsulinismo reduz a atividade do tPA, é provável que a hidratação com soro glicosado e dietas com alto índice glicêmico possam piorar o curso clínico da doença. Recomendamos, portanto, que intervenções metabólicas capazes de manter a produção de insulina dentro dos limites fisiológicos sejam testadas em estudos controlados, na prevenção e tratamento dos ataques.

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A role for tissue plasminogen activator in thrombotic thrombocytopenic purpura

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ABSTRACT

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterized by generalized microvascular occlusion. TTP has been related to severe deficiency of ADAMTS13, an enzyme that cleaves von Willebrand factor multimers into less adhesive molecules. However, ADAMTS13 deficiency correlates poorly with severity of thrombocytopenia or microangiopathic hemolysis, with the frequency of neurologic complications or the response to plasma exchange. Also, some patients with severe hereditary ADAMTS13 deficiency consistently relapse every few weeks, whereas others remain asymptomatic into their forties. Taken together, these findings suggest that an additional element is missing in the pathophysiology of TTP. We postulate that both low ADAMTS13 activity and low tissue-plasminogen activator activity are required to trigger TTP attacks. Tissue-plasminogen activator end product, plasmin, extensively degrades von Willebrand factor, breaking-down the bonds between platelets and the blood vessel wall, so that low tissue-plasminogen activator activity prevents a mechanism similar to that of ADAMTS13. The hypothesis that low tissue-plasminogen activator activity plays an important role in TTP pathogenesis is further substantiated by TTP comorbidity. Problems prevalent in patients with TTP attacks or with long-term TTP remission, including increased body mass index, major depression, cognitive abnormalities, hypertension, and premature death, are somehow associated with low tissue-plasminogen activator activity.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare but life-threatening disease, characterized by intravascular platelet clumping in arterioles and capillaries, leading to thrombocytopenia, microangiopathic hemolytic anemia, fever and ischemic organ dysfunction [1]. In clinical practice, thrombocytopenia, fragmentation of erythrocytes, and an impressively elevated serum lactate dehydrogenase value are sufficient to suggest the diagnosis [2].

Widespread microvascular occlusion has been related to severe deficiency of a disintegrin-like and metalloprotease with thrombospondin type 1 repeats (ADAMTS13) an enzyme that cleaves large von Willebrand factor (VWF) multimers into smaller and less adherent proteins [3]. When ADAMTS13 levels are severely reduced or the enzyme is dysfunctional, ultra-large VWF molecules accumulate in the circulation or on the vessel wall. These VWF multimers are capable of agglutinating platelets, causing generalized thrombotic events.

Different findings suggest that an element is missing in TTP pathogenesis. One is the observation that some patients with severe hereditary ADAMTS13 deficiency consistently relapse every few weeks, whereas others remain asymptomatic into their forties [1]. Also, ADAMTS13 deficiency correlates poorly with the severity of thrombocytopenia, microangiopathic hemolysis, or neurologic abnormalities, with the response to plasma exchange, or the number of plasma exchange treatments required to achieve remission [4]. Moreover, patients in long-term remission of TTP are at high risk of mental and physical disabilities [2] that are not explained by ADAMTS13 deficiency alone.

We postulate that low tissue-plasminogen activator (tPA) activity is the missing element in TTP pathophysiology. The role of ADAMTS13 in congenital or acquired TTP has been exhaustively discussed. This paper focuses on the relationship between low tPA activity and obscure aspects of TTP (Fig. 1).

Plasmin as a natural backup of ADAMTS13

Studies performed on mice deficient in ADAMTS13 showed that plasminogen activation acts as a natural backup for ADAMTS13 to

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degrade obstructive platelet-VWF complexes on endothelial cells [5]. Plasmin can detach platelets anchored in a VWF-dependent manner to collagen matrix or inner layers of arteries [6]. Furthermore, at sites of thrombus formation or vascular injury Glu-plasminogen is converted by plasmin to Lys-plasminogen, which is the binding protein to ADAMTS13 [7].

Although mutations encoding the plasminogen gene are prevalent in complement-mediated thrombotic microangiopathy [8], plasmin deficiency alone is not sufficient to trigger TTP, as seen in patients with congenital deficiency of plasminogen [9].

Comorbidity of TTP

Individuals in long-term TTP remission have decreased survival [10]. Assuming that the patient is in remission, one would not expect that microangiopathy would explain the increased risk of premature death. In the other hand, reduced plasmin levels, preventing the dissolution of intraluminal thrombus, increase the chances of an ischemic stroke or a myocardial infarction, which increase the risk of death [11].

Many patients in TTP remission complain of persistent fatigue, cognitive abnormalities, and problems with memory and concentration. Additionally, a significant percentage of survivors are diagnosed with major depression [10,12]. Two findings suggest that low ADAMTS13 levels could be implicated in the pathogenesis of these psychiatric disorders. First, astrocytes and microglia are able to synthesize ADAMTS13. Second, both expression and activity of ADAMTS13 are significantly increased in the rat spinal cord after injury, indicating that the protease might have a critical role in central nervous system repair, particularly after neuronal injury [13]. However, the role of ADAMTS13 in neurochemistry is insignificant, while the role of tPA in cognitive and emotional processing and in mood regulation is prominent [14–16].

tPA itself, or via plasmin activation, catalyzes cleavage of brain-derived neurotrophic factor (BDNF) precursor into mature BDNF [17]. In contrast with pro-BDNF, which is harmful to neurons, mature BDNF has antidepressant effects. tPA also participates in the mechanism of synaptic transmission and neuronal plasticity in the hippocampus and cortex, and as such improves cognitive performance [16,18].

TTP triggers

It is possible to classify TTP triggers under four groups: hyperinsulinemia, hypercortisolism, autoimmune disorders, and medication side effect. Low tPA activity is commonly seen in all these situations.

Hyperinsulinemia

An insulin response element has been identified in the promoter of plasminogen activator inhibitor (PAI)-1, a potent inhibitor of tPA [19,20]. Causes of hyperinsulinemia include sedentary lifestyle in conjunction with a high-carbohydrate diet, and insulin resistance. Although there is no evidence that unhealthy lifestyle increases the chances of TTP attacks, conditions characterized by insulin resistance, such as pregnancy, puerperium and acute bacterial infection, can trigger TTP [21,23]. Insulin resistance of pregnancy, which persists up to 16 weeks postpartum, is considered a physiological adaptation that reduces maternal glucose uptake in peripheral muscles, in order to save nutrients for the fetus and the neonate [21,22]. In its turn, it has been postulated that insulin resistance observed in the setting of bacterial infections diverts glucose from muscle towards fueling immunity [23].

Since insulin promotes fat storage, increased body mass index, a common finding in patients with TTP attacks [4,10], is highly suggestive of chronic hyperinsulinemia. Aldosterone produced by perivascular adipocytes [24] links hyperinsulinemia to a prevalent condition in TTP patients in remission: hypertension with normal kidney function [10].

Women with a history of TTP seem to be at high risk of preeclampsia in subsequent pregnancies [25]. Mothers with preeclampsia have higher PAI-1 levels than controls, which can be associated to polymorphisms or to metabolic problems, such as hyperinsulinemia [26,27]. Hyperinsulinemia increases endothelin-converting enzyme-1 expression in trophoblasts, which has been implicated in the physiopathology of preeclampsia [27].

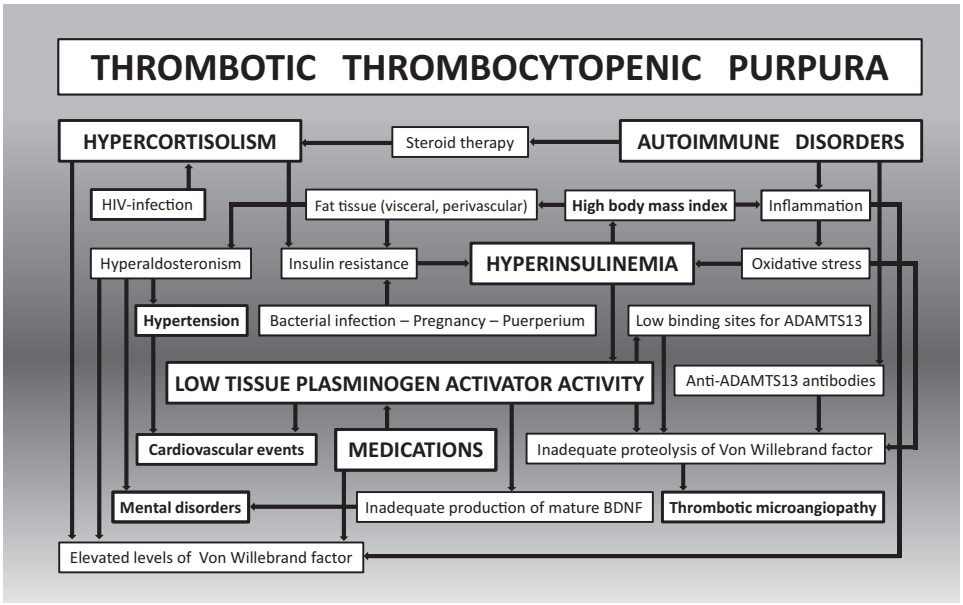

Fig. 1. ADAMTS13: a disintegrin-like and metalloprotease with thrombospondin type 1 repeats; BDNF: brain-derived neurotrophic factor.

Chronic insulin resistance increases the risk of depression and cognitive disorders, which are prevalent in patients in TTP remission. In fact, severity of these mental disorders is positively correlated to impaired glucose tolerance [28,29]. Elevated PAI-1 levels, affecting neurochemical signaling pathways or increasing the risk of cerebrovascular insults, are only part of the explanation [28,29]. Hyperinsulinemia is usually associated with hyperaldosteronism, and aldosterone treatment induces depression-like behavior in rats [30].

Hyperaldosteronism may also contribute to the thrombotic tendency of TTP. Physiological concentrations of aldosterone stimulate endothelial exocytosis of Weibel–Palade bodies, a process that releases VWF and can be antagonized by spironolactone, a mineralocorticoid receptor blocker [31]. Different studies have consistently demonstrated that VWF levels are elevated in patients with insulin resistance and other inflammatory disorders. However, the thrombotic tendency related to elevated VWF levels has been overshadowed by the strong association between insulin resistance and inflammatory disorders and elevated PAI-1 levels [32,33].

Another mechanism that might account for the increased thrombotic risk observed in patients with chronic insulin resistance is oxidative stress. Oxidative damage of VWF may cause resistance to ADAMTS13-mediated proteolysis, but does not affect VWF binding to platelets [14].

Hypercortisolism

Some disorders characterized by hypercortisolism, such as human immunodeficiency virus (HIV) or bacterial infections, are at increased risk for TTP. TTP may be the initial presenting feature of patients with high HIV viral load or with low CD4+ T-cell count following non-compliance with antiviral treatment. During HIV infection and acute bacterial infections, activated immune cells secrete proinflammatory cytokines that stimulate the release of glucocorticoids [34,35]. Also, corticosteroids are often prescribed as first-line therapy for systemic lupus erythematosus, a disease that is not uncommon among TTP survivors.

Both overt and subclinical hypercortisolism are well-recognized causes of hypertension, high body mass index [36], and mental disorders. Glucocorticoid excess may impair mental health through a mechanism that involves cortisol binding to mineralo- and glucocorticoid receptors in the hippocampus, amygdala and hypothalamus. After being activated by cortisol, glucocorticoid receptor dimerize with mineralocorticoid receptor. Dimers are transported to the nucleus where they bind to glucocorticoid response element in the serotonin promoter, inhibiting gene expression [37]. Serotonin levels are significantly lower in patients with depression than in the general population. Some authors speculate that glucocorticoids can attenuate serotonin post-synaptic receptor activity, which can also induce depression [38].

Even though a positive correlation between cortisol and PAI-1 levels exists [39], elevated PAI-1 levels persist after normalization of cortisol levels [40]. It seems that acquired insulin resistance at the level of the muscle may account for the elevated PAI-1 levels, commonly seen in individuals with hypercortisolism [41]. A positive correlation also exists between plasma cortisol and VWF levels, especially high-molecular-weight VWF multimers [42]. VWF promoter response to glucocorticoids depends on particular polymorphisms: while haplotype 1 (–3268G/–2709C/–2661A/–2527G) influences corticosteroid-mediated increase in VWF levels, haplotype 2 (–3268C/–2709T/–2661G/–2527A) represents a protective genetic element [43].

Autoimmune disorders

A chronic state of inflammation that interferes with insulin signaling [44] is not the only mechanism responsible for the low tPA activity observed in autoimmune disorders. Antiphospholipid antibodies, for example, are able to react against all components of the fibrinolytic mechanism, including tPA, annexin A2, and β_2-glycoprotein [45].

Antiphospholipid antibodies are produced by about one third of the patients with systemic lupus erythematosus [44] and it is known that a significant percentage of patients with TTP develop lupus erythematosus [10]. Most patients with systemic lupus erythematosus present with a mild-to-moderate degree of cognitive dysfunction, and a few have severe cognitive dysfunction and major depression [46], which is not surprising, since tPA mediates cognitive and affective behaviors.

Some patients with systemic lupus erythematosus or with catastrophic antiphospholipid syndrome have a clinical presentation similar to TTP [47,48]. In these patients, systemic inflammation combined with autoantibodies may result in elevated levels of VWF and decreased activity of both tPA and ADAMTS13 [49].

Medications

Different medications that may precipitate TTP attacks have been shown to decrease tPA activity [2,50–52]. The mechanism by which immunosuppressive agents cyclosporine, everolimus and tacrolimus decrease tPA activity involves increased PAI-1 levels [53,54]. Increased synthesis of PAI-1 seems to participate in the pathogenesis of TTP triggered by mitomycin and gemcitabine, as well [55,56]. Of note, silencing urokinase and its receptor inhibits the growth of pancreatic tumors in animal models and sensitizes pancreatic cancer cells to gemcitabine-induced apoptosis [56].

The mechanism by which oral contraceptives reduce tPA activity depends on increased PAI-1 activity. Protein S multimers have the ability to inhibit PAI-1 and total- and free-protein S concentration are considerably reduced during oral contraceptive use [57,58].

Consequences of the hypothesis

Further studies are needed to corroborate the hypothesis that decreased tPA activity must occur along with low ADAMTS13 activity to trigger TTP attacks. Additional studies are also necessary to: (1) evaluate whether full anticoagulation together with metabolic intervention can prevent TTP attacks; (2) determine if such interventions can prevent comorbidity of TTP; (3) to assess if glucose infusion worsens the course of the disease.

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O tPA E A FISIOPATOLOGIA DAS DIFICULDADES NO APRENDIZADO

A hipoglicemia neonatal protraída ou refratária aumenta consideravelmente o risco de que a criança tenha disfunção cognitiva, como dificuldades no aprendizado e transtornos do espectro autista.[25,26] A explicação clássica é que a hipoglicemia neonatal privaria os neurônios de sua fonte principal de energia. Além disso, na hipoglicemia há consumo de outras fontes energéticas, que não a glicose. Altos níveis de cetonas, lactato e piruvato seriam tóxicos para o cérebro.[27]

Nossa explicação envolve a baixa atividade do tPA, inibindo mecanismos importantíssimos para a neurogênese e a plasticidade sináptica. Se a baixa atividade do tPA influencia a fisiopatologia das alterações do aprendizado e dos transtornos do espectro autista, e se hipoglicemia neonatal aumenta o risco de alterações do aprendizado e dos transtornos do espectro autista, era de se esperar que crianças com hipoglicemia neonatal tivessem uma atividade deficiente do tPA e maior risco de trombose. De fato, o acidente vascular cerebral não é incomum em crianças com hipoglicemia sintomática, nascidas a termo ou pré-termo.[28,29]

A relação entre a baixa atividade do tPA e a hipoglicemia passa pela hiperinsulinemia, que aumenta os níveis do PAI-1. Decidimos criar um modelo para explicar a hipoglicemia neonatal, envolvendo a baixa atividade do tPA na mãe e no bebê.

HIPERINSULINISMO SUPRAFISIOLÓGICO E HIPOGLICEMIA NEONATAL

Os níveis de glicose no período neonatal correlacionam-se inversamente com os níveis do peptídeo-C no cordão umbilical. O peptídeo C é liberado de um precursor junto com a insulina, o que significa que a hipoglicemia neonatal resulta da secreção excessiva de insulina pelas células β do feto e do neonato.

Se a insulina é um hormônio anabólico, que faz com que a gordura seja estocada, em vez de ser usada para produzir energia, era de se supor que a hiperinsulinemia crônica fetal se traduzisse em macrossomia. A constatação de que muitos neonatos pequenos para a idade gestacional têm hipoglicemia sugere que o estímulo suprafisiológico para produção de insulina ocorra próximo ao parto. Nós postulamos que um estímulo potente para a produção materna de insulina perto do parto, também seria um potente estímulo para a produção fetal e neonatal de insulina, o que aumentaria o risco para hipoglicemia neonatal.

Para provar a hipótese, recrutamos 155 mães com marcadores de produção suprafisiológica de insulina (acantose ou obesidade grau III) ou evidência de aumento das necessidades de insulina (qualquer infecção bacteriana invasiva ou uso de corticóides sistêmicos na semana antes do parto; falta de atividade física ou dieta rica em carboidratos nas 24 horas antes do parto, incluindo refeições de alto índice glicêmico ou >50 g de glicose para tratar episódios de hipoglicemia iatrogênica, em gestantes diabéticas).

Os níveis glicêmicos dos 158 bebês foram avaliados 1, 2 e 4 horas após o nascimento. A menor glicemia de cada um foi correlacionada com os parâmetros maternos e com preditores clássicos de hipoglicemia neonatal, como baixo peso ao nascer, baixo peso para a idade gestacional e parto prematuro.

Os únicos preditores independentes de hipoglicemia neonatal foram o sedentarismo e uma dieta rica em carboidratos, nas 24 horas antes do parto. O risco aumentou cinco vezes com o sedentarismo, onze vezes com a dieta rica em carboidratos e 329 vezes com os dois fatores de risco. Nenhuma mãe sem os dois fatores de risco teve um filho com hipoglicemia neonatal (Tabela 3).

Tabela 3. Crianças nascidas de mães sedentárias, cuja dieta era rica em carboidrato nas 24 horas antes do parto, de mães com um ou nenhum fator de risco, estratificadas de acordo com o valor mínimo da glicemia, aferida com 1, 2 e 4 horas de vida: 155 mães e 158 bebês (Med Hypotheses 2016; 87: 80-6.)

	Dois fatores de risco	Um fator de risco	Nenhum fator de risco
≤40 mg/dL (48 crianças)	19 (86%)	29 (29%)	0
41–45 mg/dL (24 crianças)	2 (9%)	17 (17%)	5 (14%)
≥46 mg/dL (86 crianças)	1 gemelar (5%)	53 (54%)	32 (86%)

Neste estudo, os 43 neonatos com hipoglicemia apontados por preditores clássicos também foram identificados usando-se como critério de predição os fatores de risco materno. Por outro lado, cinco neonatos com hipoglicemia só foram identificados por fatores de risco materno. Todos eram filhos de mães não diabéticas, sem obesidade, nascidos a termo, com peso adequado para a idade gestacional.

Considerando-se que o hiperinsulinismo materno, reduzindo a atividade do tPA, possa afetar a angiogênese placentária, é provável que dietas de alto índice glicêmico, aliadas ao sedentarismo, aumentem o risco de partos prematuros e de bebês pequenos para a idade gestacional. Neste caso, a prematuridade e o crescimento intrauterino restrito decorreriam do mesmo problema que aumenta o risco de hipoglicemia neonatal: o estímulo para produção suprafisiológica de insulina na mãe. A alta prevalência de macrossomia em filhos de mães diabéticas reforça esta hipótese. A baixa produção de insulina restringiria a produção de PAI-1, e nem os níveis do tPA, nem a angiogênese placentária seriam afetados.

O artigo propondo novos fatores de risco para a hipoglicemia neonatal e defendendo que o hiperinsulinismo patológico materno, reduzindo os níveis do tPA, seja uma causa importante de prematuridade, foi publicado como:

Hoirisch-Clapauch S, Porto MA, Nardi AE. May maternal lifestyle have an impact on neonatal glucose levels? Med Hypotheses 2016; 87: 80-6.

O que o artigo tem de inovador: É provável que a predição da hipoglicemia com base em fatores de risco maternos melhore o aproveitamento escolar, por aumentar as chances de se corrigir precocemente os níveis glicêmicos.

- ✓ São necessários estudos controlados para determinar se uma dieta balanceada, combinada com atividade física regular perto do parto, é capaz de prevenir a hipoglicemia neonatal. Também é importante definir o impacto de um estilo de vida saudável nas proximidades do parto, em cada trimestre e durante toda a gestação nas funções cognitivas da criança.
- ✓ Há grandes chances de que um estilo de vida saudável durante toda a gestação, normalizando os níveis de insulina e tPA maternos, ajude a prevenir a prematuridade.

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May maternal lifestyle have an impact on neonatal glucose levels?

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ABSTRACT

Neonatal glucose levels correlate negatively with umbilical cord levels of C-peptide, a polypeptide secreted with insulin. In other words, neonatal hypoglycemia results from excessive insulin secretion from fetal/neonatal beta cells. Given that insulin causes fat to be stored rather than to be used for energy, one would expect that chronic hyperinsulinemia would result in large-for-gestational-age neonates. The finding that many small-for-gestational-age neonates have hypoglycemia suggests that the stimulus for insulin production occurs close to delivery. We postulated that a potent stimulation of maternal insulin production close to delivery would also provide a potent stimulus for fetal and neonatal insulin production, causing neonatal hypoglycemia. This study has evaluated 155 mothers with markers of excessive insulin production (such as acanthosis or grade III obesity), or with situations characterized by increased insulin requirements (such as an invasive bacterial infection or use of systemic corticosteroid within a week before delivery; or sedentariness or high-carbohydrate intake within 24 h before delivery) and their 158 neonates who were screened for glycemic levels at 1, 2 and 4 h after birth. The minimum glucose level was correlated to the maternal parameters, and to classical predictors of neonatal hypoglycemia, such as low-birth weight and preterm delivery. The only independent predictors were sedentariness and high-carbohydrate intake within 24 h before delivery. The risk of neonatal hypoglycemia increased five-fold with sedentariness, 11-fold with high-carbohydrate intake, and 329-fold with both risk factors. The risk of neonatal hypoglycemia seems to be highly influenced by maternal lifestyle within 24 h before delivery. Controlled randomized trials may help determine whether a controlled carbohydrate diet combined with regular physical activity close to delivery can prevent neonatal hypoglycemia and all its severe complications to the newborn.

Introduction

Low plasma glucose has been reported in as much as 15% of the newborns [1]. Even though many authors regard transitory neonatal hypoglycemia as a physiological process, experimental and clinical data suggest that blood glucose levels ⩽40 mg/dL during the neonatal period represent a risk factor for learning disabilities, attention difficulties, developmental delays, behavior problems, hyperactivity, partial epilepsy, and autistic features [2].

Neonatal hypoglycemia has been strongly associated with elevated cord levels of C-peptide [3], a polypeptide produced by the pancreas along with insulin. Neonatal hyperinsulinemia may be harmful to the brain because glucose uptake by the brain is mediated by glucose transporters (GLUT) that do not depend on insulin [4]. As such, hyperinsulinemia diverts glucose from neurons to cells whose glucose uptake is regulated by insulin, such as myocytes and adipocytes [5].

Early diagnosis and treatment of neonatal hypoglycemia is crucial because, although glycogen stored in astrocytes provides short-term energy to neural elements, persistent, recurrent or severe hypoglycemia deprives the brain of its main energy source [6], causing irreversible brain damage [7].

The diagnosis of neonatal hypoglycemia requires a high index of suspicion, because it may present with nonspecific signs such as irritability, lethargy, poor feeding, eye-rolling, seizures, apnea, cyanosis, hypothermia, or tachypnea, and it may even be asymptomatic [4].

Neonatal hypoglycemia should be suspected in infants born to diabetic mothers, in preterms, in those with birth weight above the 90th percentile for gestational age (large for gestational or LGA) or below the 10th percentile for gestational age (small for gestational age or SGA). Since hyperinsulinemia provides a potent stimulus for excessive fetal growth, an explanation is needed as to why SGA neonates can also have high insulin levels that contribute

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to hypoglycemia [8]. For unknown reasons, neonatal hypoglycemia has been also described in full-term infants born to non-diabetic mothers, without panhypopituitarism, congenital adrenal hyperplasia, or other endocrinopathies [8].

The hypothesis

Based on the assumption that neonatal hyperinsulinemia usually accompanies neonatal hypoglycemia, we hypothesized that a strong stimulus for maternal insulin production close to delivery would also provide a strong stimulus for fetal and neonatal insulin production. In this study, we sought to determine whether markers of hyperinsulinemia or situations that increase maternal insulin requirements would help predict neonatal hypoglycemia.

Evaluation of the hypothesis

Mothers were selected if they had at least one of the following indicators: grade III obesity [9], acanthosis *nigricans* [10] (surrogates of chronic maternal hyperinsulinemia), any invasive bacterial infection or if they had used corticosteroid within seven days before delivery (surrogates of subacute insulin resistance), if they were classified as a high-carbohydrate intake or sedentariness within 24 h before delivery (conditions that could increase maternal insulin requirements close to delivery). Exclusion criteria consisted of oral hypoglycemic agent use during pregnancy or neonatal death before four hours of life.

Grade III obesity is defined by the World Health Organization as weight divided by square height $\geqslant$40 kg/m^2 [9]. In this study, it was defined as maternal weight minus 12 kg for singleton and minus 18 kg for twin pregnancy divided by square height $\geqslant$40 kg/m^2. Subtracted weight corresponds to the average amount of retained fluid, the weight of the baby, the placenta, and the amniotic fluid.

Acanthosis *nigricans* [10] was identified in the nape of the neck at the time of delivery. We assumed that acanthosis is usually the cutaneous manifestation of pathological insulin resistance and compensatory hyperinsulinemia, while congenital and paraneoplastic acanthosis are rare exceptions.

Corticosteroid use included corticosteroids for accelerating fetal lung maturation.

Invasive bacterial infections included urinary infection, pneumonia or severe periodontal disease diagnosed by a physician, within a week before delivery.

High-carbohydrate intake was defined as >75% of calories from carbohydrates within 24 h before delivery, providing they were not fasting or >50 g of glucose as high-glycemic index meals (snacks, candies, soft drinks or any other high-carbohydrate, low-fiber meal) or >50 g of glucose to treat iatrogenic hypoglycemia within 24 h before delivery. In general, mothers classified as high-carbohydrate intake reported eating less than 2 g/kg of meat, fish, poultry or equivalent per meal, in two meals within 24 h before delivery. Mothers who fasted for at least 12 h before delivery were not classified as high-carbohydrate intake.

Mothers who reported <40 min of moderate or intense physical activity, such as housework or walking within 24 h before delivery were classified as sedentary.

The six categorical variables were correlated to the minimum blood glucose concentration in their neonates at 1, 2 or 4 h after birth, and significant predictors of hypoglycemia were identified. The same was done with neonatal parameters widely acknowledged as predictors of hypoglycemia, such as low-birth weight or prematurity [11].

After identification of significant maternal and neonatal predictors, a stepwise regression analysis was performed with all predictors to determine independent predictors. Next, to identify the most sensitive method for screening neonatal hypoglycemia, we sought to determine if any hypoglycemic neonate identified according to the maternal predictors was left unidentified with neonatal predictors and vice versa.

Although significant predictors were identified in the whole population of neonates, we decided to stratify neonates according to the presence or absence of maternal diabetes, so as to evaluate the impact of the maternal endocrinopathy on neonatal outcomes.

This evaluation was carried out from February 2011 through June 2013 at Hospital Federal dos Servidores do Estado do Rio de Janeiro (HFSE), a public tertiary hospital. All mothers provided written informed consent. Maternal and neonatal data were obtained blindly by independent observers.

All expectant mothers who met any inclusion criterion were consecutively selected. Of 157 women approached, one declined and 156 were enrolled upon informed consent, 50 of whom were diabetic and 106 non-diabetic and none of them were vegan or vegetarian. Maternal characteristics according to the inclusion criteria are shown in Table 1. Mothers were interviewed before delivery and a second interview was conducted within 24 h after delivery. They were asked to detail their intake of food and fluids within 24 h before delivery. Then, the amount of protein, carbohydrate and lipid intake was determined based on the U.S. Department of Agriculture Food Composition Database [12].

One neonate died two hours after birth and the mother-son pair was excluded from analysis. Neonates were considered euglycemic when blood glucose was >40 mg/dL at 1, 2, and 4 h after birth, and hypoglycemic when blood glucose was $\leqslant$40 mg/dL at 1, 2, or 4 h after birth [13].

Infants were classified as SGA, appropriate for gestational age (AGA) or LGA, based on Olsen growth curves [14]. Babies weighting less than 2500 g at birth were classified as low birth weight (LBW) [15]. Samples for neonatal glucose measurement obtained from heelstick (98%) or arterial puncture (2%) were immediately assessed using Accu-Chek, Roche. Glucose was analyzed by the hexokinase method in euglycemic neonates with symptoms suggestive of hypoglycemia.

Breastfeeding was initiated within 2 h of life, except for three infants born to HIV-positive mothers and eight babies whose mothers were considered to have insufficient breast milk. These eleven babies and other neonates diagnosed with hypoglycemia were offered cow-milk formula (10 mL, 70 kcal/100 mL). Blood glucose levels were measured again 20 min after feeding in neonates with hypoglycemia, and formula was given immediately to those whose blood glucose levels remained $\leqslant$40 mg/dL, and after two hours when it was >40 mg/dL. A 10% glucose solution was infused at the rate of 200 mg/kg followed by 8 mg/kg/min for $\geqslant$4 h into hypoglycemic neonates unable to swallow, when glucose levels were $\leqslant$30 mg/dL, or when hypoglycemia persisted despite oral feeding.

Table 1
Maternal characteristics according to the inclusion criteria.

	Non-diabetic mothers (n = 105)	Diabetic mothers (n = 50)
Grade III obesity close to delivery	21 (20%)	13 (26%)
Acanthosis *nigricans* close to delivery	20 (19%)	15 (30%)
Corticosteroid use within the week preceding delivery	17 (16%)	8 (16%)
Bacterial infection within the week preceding delivery	2 (2%)	3 (6%)
High-carbohydrate intake within 24 h before delivery	21 (20%)	16 (32%)
Sedentariness within 24 h before delivery	48 (46%)	41 (82%)

The amount of glucose infused and laboratory results were retrieved from medical records. As an obstetric routine, intravenous glucose was not given peripartum. Diabetic mothers with glucose levels <60 mg/dL within 24 h before delivery were given oral or parenteral glucose, regardless of symptoms. No dietary or exercise counseling were given to the mothers by the authors.

There were no missing data, except for glucose levels within 24 h before admission from three diabetic mothers (6%). The protocol was approved by HFSE Institutional Review Board as #416.2010.

Continuous variables are presented as mean ± standard deviation and compared by Student's *t*-test. Categorical variables are presented as percentage and compared using Pearson's chi-square or Fisher's exact tests. Alpha error level was set to 0.05. At the High-Risk Neonatal Unit, neonates screened according to current guidelines show a 25% incidence of hypoglycemia. For a desired power level of 0.8 at a significance level of 0.05, and assuming the new screening guidelines would be able to identify ⩾40% of hypoglycemic neonates, the study required an a priori sample of 119 neonates. Statistical analyses were conducted with R statistical software.

Baseline characteristics

A total of 105 non-diabetic women who gave birth to 106 neonates, including a pair of twins and 50 diabetic mothers who gave birth to 48 singletons and two sets of twins were included. Grade III obesity was more frequent in diabetic mothers who gave birth to hypoglycemic neonates (38% vs 10%, $P = 0.02$), but the difference was not significant concerning non-diabetic mothers who delivered hypoglycemic and euglycemic neonates. Acanthosis *nigricans* was more frequent among non-diabetic mothers who delivered hypoglycemic neonates than in non-diabetic mothers who delivered euglycemic neonates (35% vs 14%, $P = 0.01$). The difference was not significant between diabetic mothers of hypoglycemic and euglycemic infants.

One non-diabetic woman with severe orthopedic problems reported to be very active, and did not fulfill any inclusion criterion. She was considered to be sedentary due to her physical limitations and agreed to participate in the study. Five mothers (10%) had type 2, 13 (26%) had type 1, and 32 (64%) had gestational diabetes, diagnosed according to the recommendations of the International Association of Diabetes and Pregnancy [15]. Sixteen mothers were classified as high-carbohydrate intake, eight of whom had iatrogenic hypoglycemia corrected with ⩾50 g of oral or parenteral glucose. All non-diabetic mothers classified as high-carbohydrate intake reported to have consumed <4 g/kg of meat, fish, poultry or equivalent within 24 h before delivery.

A total of 39/48 (81%) hypoglycemic and 81/110 (74%) euglycemic neonates were delivered by C-section ($P = 0.08$). Maternal and neonatal characteristics are shown, respectively, in Tables 2 and 3.

Predictors of neonatal hypoglycemia

The only maternal parameters that increased the risk of neonatal hypoglycemia were a high-carbohydrate intake and sedentariness within 24 h before delivery (Table 2). Neonatal characteristics that increased the risk of neonatal hypoglycemia were birth weight, gestational age at birth, and newborns classified either as SGA or as LBW (Table 3). All significant predictors were entered a stepwise regression to identify independent predictors (Table 4). Assuming an overlap between neonatal characteristics related to prematurity, a stepwise regression analysis was performed excluding one, two or three neonatal characteristics. The results did not change: only a high-carbohydrate intake and sedentariness remained as independent predictors.

Table 2
Maternal characteristics according to the presence or absence of neonatal hypoglycemia.

	Hypoglycemic neonates (47 mothers)	Euglycemic neonates (108 mothers)	*P* value
Age (years)	29 ± 7	31 ± 7	0.07
Afro-descendants	30 (64%)	57 (53%)	0.2
Grade III obesity	7 (15%)	27 (25%)	0.2
Acanthosis *nigricans*	14 (30%)	22 (20%)	0.2
Corticosteroid use	10 (21%)	16 (15%)	0.4
Bacterial infection	2 (4%)	3 (2%)	0.4
High-carbohydrate intake	25 (53%)	11 (10%)	<0.0001
Sedentariness	41 (87%)	50 (56%)	<0.0001

The risk for neonatal hypoglycemia increased 11-fold with high carbohydrate intake (95% CI: 4–24; 56% sensitivity, 90% specificity, $P < 0.001$), five-fold with sedentariness (95% CI: 2–11; 85% sensitivity, 50% specificity, $P < 0.001$), and 34-fold with either high-carbohydrate intake or sedentariness (95% CI: 4–255; 40% sensitivity, 100% specificity, $P < 0.001$). The two risk factors were highly synergistic, increasing the risk 329-fold (95% CI: 32–3362; 86% sensitivity, 100% specificity, $P < 0.001$). Mothers who gave birth to hypoglycemic neonates reported to rest more continuous days before delivery than mothers who delivered euglycemic neonates (5 ± 1 vs. 3 ± 3, $P = 0.01$). The risk of neonatal hypoglycemia decreased with a balanced diet plus ⩾12-h fast before delivery: OR = 0.2 (95% CI: 0.08–0.4; 44% sensitivity, 85% specificity, $P < 0.001$).

Independent predictors were entered into a linear regression model, to evaluate the relative impact of each predictor on minimum glucose levels. Considering a constant of 57 mg/dL, a high-carbohydrate intake decreased glucose levels by 13 mg/dL ($P < 0.001$), and sedentariness by 9 mg/dL ($P < 0.001$) (Table 5). A balanced diet plus ⩾12-h fasting before delivery increased minimum glucose level by approximately 5 mg/dL ($P = 0.02$).

All 37 mothers who were not classsified as high-carbohydrate intake or as sedentariness gave birth to euglycemic neonates. A total of 19 (86%) neonates born to 22 mothers classified as both sedentariness and high-carbohydrate intake had hypoglycemia. Hypoglycemia was detected in 48 of 158 (30%) neonates, 22 born to diabetic and 26 to non-diabetic mothers. A mother of twins, who reported both sedentariness and a high-carbohydrate intake, had one euglycemic baby and one baby with minimum blood glucose level of 41 mg/dL.

Two neonates with borderline hypoglycemia (minimum glucose concentration at 1, 2 or 4 h >40 and <45 mg/dL) identified according to the screening based on neonatal parameters were not identified with a screening based on the two maternal parameters: a 2540-g boy born at 36 gestational weeks to a grade III obese mother with preeclampsia, and a 1840-g girl born at 35 gestational weeks, whose mother, a chronic corticosteroid user, had lupus and antiphospholipid antibody syndrome.

All hypoglycemic neonates identified according to neonatal parameters were also identified with the screening based on two maternal parameters: a high-carbohydrate intake or sedentariness. The reverse was not true. Five (4%) infants with hypoglycemia did not have any conventional risk factor: all were term babies born to non-diabetic mothers, weighting 3000–3350 g – including the child born to the mother with orthopedic problems (Table 6). Importantly, two of these five neonates were asymptomatic.

Table 3
Neonatal characteristics according to the presence or absence of neonatal hypoglycemia.

	Non-diabetic mothers			Diabetic mothers			All mothers		
	HN (26 infants)	EN (80 infants)*	*P* value	HN (22 infants)*	EN (30 infants)*	*P* value	HN (48 infants)	EN (110 infants)	*P* value
Gestational age at birth (weeks)	35 ± 4	37 ± 6	0.0002	36 ± 2	37 ± 2	0.1	35 ± 3	37 ± 6	0.0001
Birth weight (grams)	2260 ± 975	3154 ± 858	<0.001	3023 ± 889	3126 ± 639	0.6	2609 ± 1003	3128 ± 805	0.004
Low birth weight	14 (54%)	19 (25%)	0.005	5 (23%)	8 (27%)	0.1	19 (40%)	27 (25%)	0.03
Small for gestational age	8 (31%)	8 (10%)	0.01	1 (5%)	1 (3%)	1	9 (19%)	9 (8%)	0.04
Blood glucose level, 1 h after birth	37 ± 10	60 ± 14	<0.0001	34 ± 14	59 ± 12	<0.0001	36 ± 12	60 ± 12	<0.001
Blood glucose level, 2 h after birth	56 ± 21	61 ± 14	0.1	44 ± 15	63 ± 19	0.0002	50 ± 19	62 ± 16	<0.0002
Blood glucose level, 4 h after birth	64 ± 15	65 ± 28	0.4	47 ± 12	65 ± 15	0.0005	57 ± 24	65 ± 16	0.03
Severe hypoglycemia 1, 2 or 4 h after birth	1 (4%)	0	–	3 (14%)	0	–	4 (8%)	0	–
Refractory hypoglycemia	4 (15%)	0	–	9 (41%)	0	–	13 (27%)	0	–

HN: hypoglycemic neonates; EN: euglycemic neonates. * Includes a pair of twins.

Table 4
A stepwise regression analysis depicting how a high carbohydrate intake and sedentariness maternal the day before delivery perform in predicting neonatal hypoglycemia, compared to neonatal risk factors (155 mothers and 158 infants).

	Coefficient	*P* value
Minimum neonatal blood glucose level	21	–
Gestational age at birth (weeks)	1	0.05
Birth weight (grams)	0.002	0.4
Low-birth-weight	−2	0.5
Small-for-gestational-age neonate	−3	0.3
High-carbohydrate intake	−13	<0.0001
Sedentariness	−9	0.0008

Correlation coefficient (r^2): 0.32.

Table 5
Impact of independent predictors on neonatal glucose levels: linear regression analysis (155 mothers and 158 infants).

	Coefficient	*P* value
Minimum neonatal blood glucose level	**57**	–
High-carbohydrate intake	**−13**	<0.0001
Sedentariness	**−9**	<0.0001

Correlation coefficient (r^2): 0.29.

The impact of maternal diabetes on neonatal hypoglycemia

Of the eight mothers who experienced at least one hypoglycemic episode (i.e. glucose level <60 mg/dL) within 24 h before delivery, six (75%) delivered hypoglycemic neonates. Compared to diabetic mothers without hypoglycemia, mothers who required ⩾50 g of intravenous/oral glucose to correct iatrogenic hypoglycemia within 24 h before delivery had an eight-fold increased risk of having an hypoglycemic neonate (95% CI: 2–37; 38% sensitivity, 93% specificity, $P = 0.02$). Diabetic mothers who gave birth to hypoglycemic neonates received more intermediate acting (NPH) insulin within 24 h before delivery than diabetic mothers who gave birth to euglycemic neonates (60 ± 44 IU vs 41 ± 41 IU, $P = 0.04$). The difference was not significant concerning regular/lispro insulin (10 ± 19 IU vs 10 ± 16 IU, $P = 0.9$).

Compared to hypoglycemic infants born to non-diabetic mothers, hypoglycemic neonates born to diabetic mothers had lower glucose levels at 2 and 4 h of life, and an increased prevalence of refractory hypoglycemia (Table 3). Additionally, hypoglycemic episodes were more frequent in type 1 diabetic mothers' offspring than in infants born to mothers with gestational or type 2 diabetes (62% vs. 35%).

Table 6
Presence of sedentariness and/or high-carbohydrate intake according to minimum neonatal glucose level at 1, 2 or 4 h (155 mothers and 158 infants).

	Two risk factors	One risk factor	No risk factor
⩽40 mg/dL (48 infants)	19 (86%)	29 (29%)	0
41–45 mg/dL (24 infants)	2 (9%)	17 (17%)	5 (14%)
⩾46 mg/dL (86 infants)	1 (5%)*	53 (54%)	32 (86%)

* One of a pair of twins.

Consequences of the hypothesis and discussion

Two important points were drawn from this clinical evaluation. The first was that high-carbohydrate intake and sedentariness in the pre-partum period seems to have a direct effect on newborn's blood sugar levels, independent of the mother's diabetes status. The two maternal predictors seem to be especially useful when it comes to screen AGA term infants born to non-obese, non-diabetic mothers for neonatal hypoglycemia.

The second was that neonatal characteristics related to placental insufficiency do not seem to be independent predictors of neonatal hypoglycemia. Our data suggest that, in non-diabetic pregnancies, adverse perinatal outcomes, such as preterm deliveries, SGA, and LBW could derive from the same maternal conditions that increase the risk of neonatal hypoglycemia – high-carbohydrate intake and sedentariness.

Physiological and pathological insulin metabolism in pregnancy

The placenta has a tremendous influence on maternal metabolism [16]. Physiological insulin resistance of pregnancy, mediated by placental hormones and evidenced especially in the second and third trimesters, ensures that maternal glucose will be diverted adequately to the fetus to meet the nutritional and growth demands [16]. Insulin resistance stimulates maternal β-cells to increase insulin secretion [17]. In its turn, insulin stimulates the production of PAI-1, a major tissue plasminogen activator inhibitor [18,19]. Both placental angiogenesis and placental vessel remodeling depend on activation of matrix metalloproteinases by tissue plasminogen activator. Physiological insulin resistance of pregnancy, increasing PAI-1 levels [20], seems to favor physiological placental senescence that reduces the risk of hemorrhage at delivery. We postulate that pathological hyperinsulinemia, related or not to insulin resistance, would be an important contributor to accelerated placental maturation, increasing the chances of preterm delivery and intrauterine growth restriction (Fig. 1).

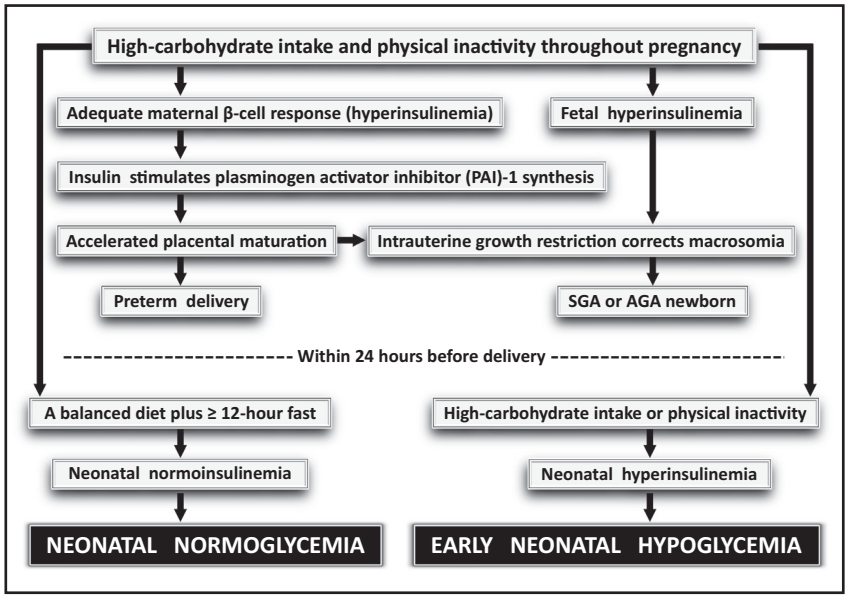

Fig. 1. SGA: small-for-gestational-age, AGA: appropriate-for-gestational-age.

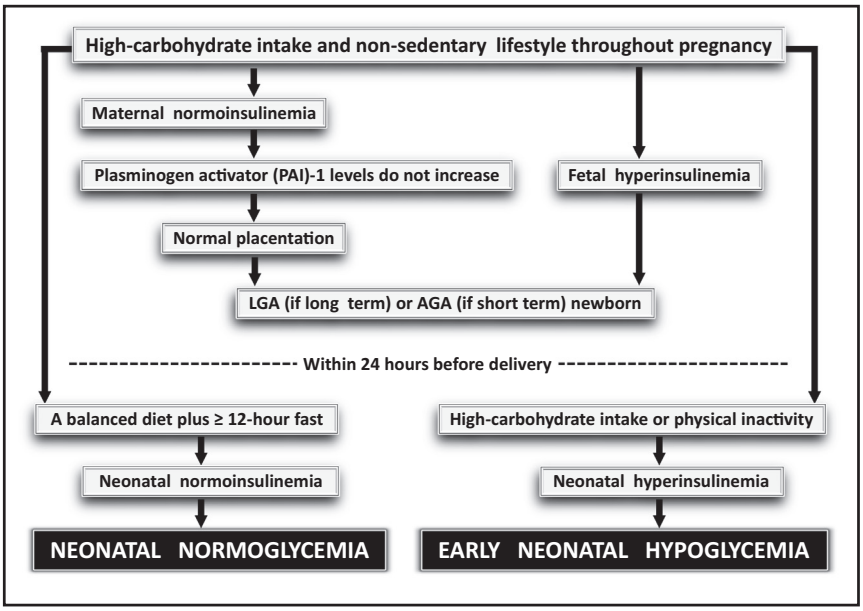

Fig. 2. LGA: large-for-gestational-age, AGA: appropriate-for-gestational-age.

When active mothers consume large amounts of carbohydrates, physical activity prevents overstimulation of pancreatic β-cells [21]. Insulin levels remain within normal limits, thus preventing overstimulation of PAI-1. As a result, normal tissue plasminogen activator levels allow the placenta to mature physiologically (Fig. 2). Large amounts of glucose cross the placenta and stimulate fetal β-cells to produce insulin. Fetal hyperinsulinemia is a potent stimulus for production of growth hormone and growth factors, responsible for fetal overgrowth [22]. In order to avoid shoulder dystocia, many macrosomic infants are delivered by elective cesarean, and maternal fasting before delivery may help normalize neonatal insulin levels. In our clinical data, hypoglycemia was detected only in one neonate born to a mother not classified as high-carbohydrate intake, who fasted for at least 12 h before delivery. She was a non-diabetic, chronic corticosteroid user, who reported to be sedentary.

A high-carbohydrate intake combined with lack of physical activity or TNF-α excess, as from obesity or inflammation, provides a strong stimulus for maternal insulin production [23]. In this scenario, either β-cells are dysfunctional and diabetes supervenes, or an excessive amount of insulin is produced, providing pathological stimulation of PAI-1 synthesis. PAI-1 excess may cause premature placental senescence, increasing the risk of preterm deliveries and intrauterine growth restriction [24]. One would expect all babies nourished with large amounts of carbohydrate to be LGA, but placental insufficiency seems to "correct" macrosomia (Fig. 1).

Diabetic pregnancies

The placentas of patients with gestational diabetes mellitus who deliver term infants are characterized by delayed maturation [25,26]. One of the reasons is that when insulin secretion is inadequate, PAI-1 synthesis is not affected. In this setting, placental

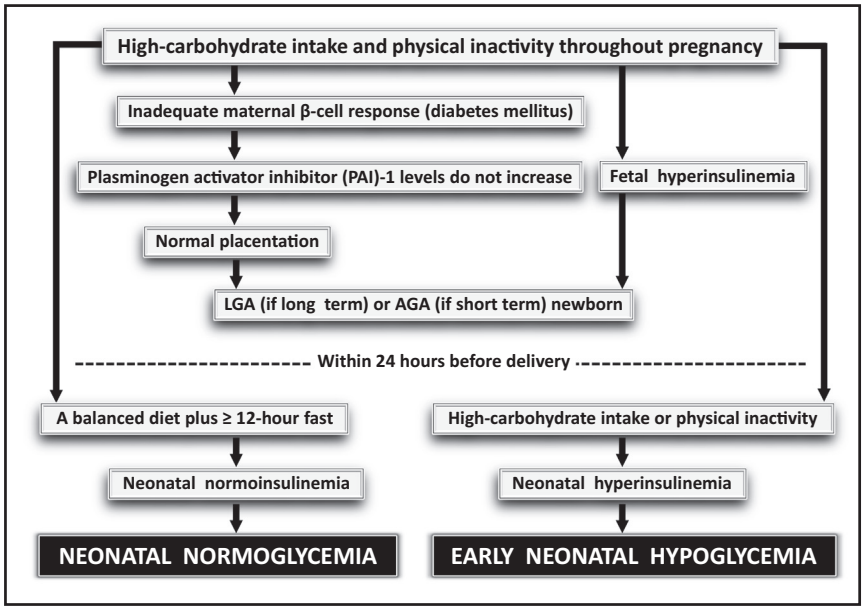

Fig. 3. LGA: large-for-gestational-age, AGA: appropriate-for-gestational-age.

angiogenesis occurs physiologically, which reduces the risk of intrauterine growth restriction and preterm delivery (Fig. 3).

Neonates born to mothers with type 1 diabetes have a higher incidence of hypoglycemic episodes than offspring of mothers with type 2 or gestational diabetes [27], possibly because episodes of iatrogenic hypoglycemia corrected with oral or intravenous glucose are not unusual in expectant mothers with type 1 diabetes, but are infrequent in mothers with non-insulin dependent diabetes. Four pregnant women with type 1 diabetes of in our sample reported bringing candies to the hospital to correct hypoglycemia, which are not usually computed as dietary intake. Besides that, all mothers with type 1 diabetes reported that they were not encouraged to engage in physical activity during prenatal visits.

One possible hypothesis for the finding that LGA neonates born to diabetic mothers have a higher prevalence of neonatal hypoglycemia than LGA infants born to non-diabetic mothers [27] is that diabetic mothers are usually given intravenous glucose when they undergo prolonged fasting before delivery.

Future directions

Our findings need to be confirmed in larger studies, particularly with infants with no conventional risk factors for neonatal hypoglycemia, but with one or both of the two maternal risk factors. It is also recommended that studies be performed to assess how corticosteroid use or invasive bacterial infections influence neonatal glucose levels, since the small number of patients in this study with any of these conditions precludes any conclusion about their impact on neonatal glucose levels. Considering that Accu-Chek tends to overestimate glucose levels at low glucose values [29], it would be important that our hypothesis could be confirmed using the hexokinase method.

In order to assess whether extreme prematurity itself is a prerequisite for neonatal hypoglycemia, we recommend that neonates born prematurely due to cervical incompetence, whose mothers were neither sedentary nor on a high-carbohydrate diet within 24 h before delivery, be screened for neonatal hypoglycemia.

It has been demonstrated that antenatal nutritional education may reduce the risk of having a preterm birth and low-birthweight offspring [28]. Further studies are needed to determine whether a controlled carbohydrate diet combined with regular physical activity close to delivery can prevent neonatal hypoglycemia and all its severe complications to the newborn.

Conclusions

Lack of physical activity combined with a high-carbohydrate diet was highly synergistic, leading to a 329-fold increased risk of having a baby with neonatal hypoglycemia. The high prevalence of refractory hypoglycemia among offspring of diabetic mothers, despite feeding or parenteral glucose, indicates the need to prevent both maternal hyperglycemia and iatrogenic hypoglycemia close to delivery. Our study has demonstrated that the risk of neonatal hypoglycemia is highly influenced by maternal lifestyle within 24 h before delivery.

Disclosure

The authors declare no conflict of interest.

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Contribution of each author

Silvia Hoirisch-Clapauch – designed the project, collected and analyzed the data, wrote the first draft of the manuscript.

Maria Amelia S. Porto – designed the project, collected and analyzed the data, wrote the first draft of the manuscript.

Antonio E. Nardi – reviewed the project, discussed the data, wrote the final version of the manuscript.

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OS SEROTONINÉRGICOS E TRANSTORNOS DO ESPECTRO AUTISTA

Como a angiogênese placentária depende do tPA, é provável que intervenções capazes de corrigir os níveis de tPA na mãe ajudem a prevenir complicações relacionadas à insuficiência placentária, como partos prematuros e recém-natos de baixo peso. Postulamos que, se estas intervenções não prevenirem o estímulo para a síntese de insulina no feto ou no neonato, há uma grande chance de que crianças nascidas a termo, sem restrição de crescimento, não sejam rastreadas para hipoglicemia neonatal por critérios clássicos de predição da endocrinopatia. A não correção dos níveis glicêmicos poderia provocar lesões cerebrais, aumentando o risco para transtornos do espectro autista e dificuldades no aprendizado.

Nossa hipótese para a fisiopatologia de transtornos do espectro autista envolve a produção suprafisiológica de insulina em fetos e neonatos, aumentando os níveis do PAI-1, o que impediria a ativação de neurotrofinas e da relina pelo tPA. O achado de que a atividade da relina e neurotrofinas no cérebro desses indivíduos é baixa, reforça a hipótese.[30] Sendo a relina responsável pela migração neuronal, a migração caótica prejudicaria as conexões neuronais e causaria aumento do volume do cérebro.[30,31]

Os antidepressivos do tipo ISRS inibem a liberação do PAI-1, o que ajuda a corrigir os níveis do tPA. Vários autores relataram um aumento do risco de transtornos do espectro autista em filhos de mães que usaram ISRS na gestação.[32-39]

Transtornos do espectro autista também foram prevalentes nos filhos de mães que usaram valproato e nos filhos de mães com síndrome do anticorpo antifosfolipídio, mas não nos filhos de mães com lupus.[40,41] Diferente das mães com lupus sem anticorpos antifosfolipídio, as com síndrome do anticorpo antifosfolipídio têm indicação de anticoagulação plena. O valproato e a anticoagulação plena aumentam os níveis de tPA,[42] o que ajudaria a prevenir a prematuridade, mas não corrigiria o estímulo para a hiperinsulinemia fetal e neonatal.

É provável que estudos de populações, que não tenham por hábito descansar e ingerir uma dieta rica em carboidrato nas proximidades do parto, não detectem um maior risco para autismo em filhos de mulheres que usaram ISRS na gestação.

A hipótese de que os ISRS melhoram os sintomas mentais por um mecanismo que envolve o aumento dos níveis de tPA foi defendida no artigo:

Hoirisch-Clapauch S, Nardi AE, Gris JC, Brenner B. Are the antiplatelet and profibrinolytic properties of selective serotonin-reuptake inhibitors relevant to their brain effects? Thromb Res 2014; 134: 11-6.

O que o artigo tem de inovador: É o primeiro trabalho a sugerir que a melhora dos sintomas depressivos pelos ISRS tenha, como pré-requisito, o aumento da atividade do tPA.

- ✓ Considerando-se que o tPA também participa da angiogênese placentária e da modificação dos vasos da placenta que acompanham o crescimento fetal, é provável que o uso dos ISRS na gestação, aumentando os níveis do tPA, ajude a prevenir partos prematuros por insuficiência placentária.

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Review Article

Are the antiplatelet and profibrinolytic properties of selective serotonin-reuptake inhibitors relevant to their brain effects?

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ABSTRACT

The serotonin transporter (SERT) is found in neuron and platelet membranes. Selective serotonin-reuptake inhibitors (SSRIs) are widely prescribed for severe depression. They may at least partly counteract the effects of serotonin on the vascular biology system, can lower agonists-induced platelet activation, aggregation and procoagulant activity *in vitro*, thus modulating platelet thrombogenicity. Other effects, such as those mediated through PAI-1 modulation, may indirectly influence neurobiology-relevant mechanisms involved in depression. Patients receiving SSRIs are at increased bleeding risk and decreased risk of arterial occlusive events, such as myocardial infarction, compared to those using non-SSRI antidepressants. The objectives of this review were to highlight antiplatelet and profibrinolytic properties of SSRIs and discuss the potential role of these activities in the context of SSRI brain effects.

Contents

Introduction

Selective serotonin-reuptake inhibitors (SSRIs), including fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and escitalopram, inhibit the serotonin transporter (SERT, SLC6A4), a plasma membrane integral glycoprotein, which mediates the uptake of serotonin across the plasma membrane before its storage in specific organelles. SERT is found in neurons and platelets.

SSRIs are one of the most prescribed drug classes in adults, adolescents and children [1,2]. Although these antidepressants have been used in the treatment of mood, anxiety, eating, and some personality disorders, the best results can be observed when SSRIs are indicated for severe depression [3]. In a large meta-analysis, an advantage of these antidepressant medications over placebo was nonexistent to negligible among depressed patients with mild, moderate, and even severe

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0049-3848/

baseline symptoms, whereas it was considerable in patients with very severe symptoms [3].

Although SSRIs have better overall safety and tolerability than older antidepressants, their side effects include akathisia, sexual dysfunction, weight gain, sleep disturbance [4], and in patients with SSRI intoxication, increased levels of oxidative stress markers are reported [5].

It has been also observed that patients on SSRIs are at increased risk for abnormal bleeding, which includes perioperative, gastrointestinal and brain hemorrhage, and at decreased risk of arterial occlusive events, such as myocardial infarction, compared to those who use non-SSRI antidepressants [6–9]. SSRIs, with their pleotropic actions involving serotonin metabolism, induce hemostatic alterations that may be relevant to the effects of SSRIs on the brain. The aim of the current review is to highlight the main features of the anti-hemostatic effects of the SSRIs, discussing their risks and benefits.

Neurobiology of Major Depressive Disorders and SSRI Effects on the Brain

Understanding of the neurobiological basis of a major depressive disorder has deepened with advance in neuroimaging, molecular and genetic studies, albeit the contribution of these advances to the clinical outcome of patients with such disorders is still uncertain [10].

Neural systems that regulate emotion processing and reward seeking are dysfunctional in a major depressive disorder. Specifically, the medial prefrontal-limbic network, including amygdala, anterior cingulate cortex, and medial prefrontal cortex, is modulated by serotonin neurotransmission, and the reward network, centered on ventral striatum and interconnected orbitofrontal and medial prefrontal cortices, is modulated by dopamine. Two informative neural systems were identified in a meta-analysis of neuroimaging studies [11]. The first one, centered on the dorsolateral prefrontal cortex and more dorsal regions of the anterior cingulate cortex, was characterized by reduced activity in the resting state, which returned to normal with treatment. The second network, centered on the medial prefrontal cortex and subcortical regions, was hyperactive in response to emotional stimuli in the depressed state, but returned to normal after antidepressant therapy. This provided further evidence of increased activity in the neural systems supporting emotion processing (amygdala and medial prefrontal cortex), and reduced activity in the neural systems supporting regulation of emotion (e.g., dorsolateral prefrontal cortex).

Three groups of peripheral mediators/messengers may be involved in the pathophysiology of the illness: (1) neurotrophic growth factors, including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1); (2) proinflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α); and (3) impaired regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Based on these findings, some diagnostic and therapeutic approaches have been suggested. For example, mature form of BDNF is decreased in individuals with major depression [12,13], and antidepressant therapy reverses this decrease. The production of proinflammatory cytokines is increased in stressed and depressed individuals and antidepressant drugs can normalize their concentrations or suppress their synthesis in major depression. Notably, the anti-inflammatory effects are not restricted to cells of the peripheral circulation; SSRIs were found to potently inhibit microglial TNF-α and nitric oxide production [14].

The serotonin transporter (SERT) gene (SLC6A4) can have a functional insertion-deletion promoter variant (serotonin transporter-linked polymorphic region, 5HTTLPR). A recent meta-analysis [15] showed an association between the 5HTTLPR long allele and increased response to SSRIs, and their reduced side effects. It also demonstrated connection between the 5HTTLPR short allele and increased paroxetine-induced adverse effects. Several single-nucleotide polymorphisms of the serotonin type-2a receptor (5-HT_{2A}) are related to the outcome of SSRI treatment. Particularly, the Met allele of the functional Val/Met polymorphism (rs6265) in the BDNF gene is associated with response to SSRIs [16].

Serotonin and Platelets

Some proteins that participate in the vascular biology are stored within distinct vesicle-like secretory platelet organelles, which include lysosomes, α and dense granules. Among them are signaling molecules like ADP and serotonin. Serotonin is synthesized and secreted into the blood stream by enterochromaffin cells in the gastrointestinal tract and is rapidly taken up and stored in the dense granules.

Serotonin storage in platelets requires uptake across the platelet plasma membrane with further transport across the dense granule membrane [17] (Fig. 1). The uptake is mediated by the SERT SLC6A4, a member of the SLC6 gene family of Na^+/Cl^--dependent neurotransmitter transporter proteins exhibiting a cluster of 12 membrane-spanning segments, which is apparently identical in brain and platelets. The actual uptake process involves binding of serotonin to its recognition site within the transporter and its transport across the membrane together with a Na^+ ion. The second step involves the translocation of a K^+ ion across the membrane to the exterior of the cell. Both tricyclic antidepressants and the SSRIs bind to SERT and inhibit serotonin uptake into neurons and platelets. In addition, platelets exhibit uptake mechanisms for several amino acid transmitters, including γ-aminobutyric acid, glutamate, aspartate, and glycine.

The transport of serotonin across the dense granule membrane is supposed to be mediated by a reserpine-sensitive vesicular monoamine transporter (VMAT, SLC18), which is also present in the secretory vesicles of monoaminergic neurons and neuroendocrine cells in the gut. This transport is driven by an electrochemical proton gradient across the vesicular membrane, which is generated by the vacuolar H^+-ATPase and is potently inhibited by reserpine and tetrabenazine.

The release of serotonin promotes platelet functions via the serotonin receptor (5-HT_{2A}), a $G_{\alpha q}$-protein coupled receptor on platelets that activates phospholipase C and induces the phosphatidylinositol-calcium second messenger system. The serotonin-dependent intracellular signaling pathway facilitates platelet activation and is inhibited by SSRIs [18]. When platelets adhere and aggregate at a site of vessel injury, serotonin is secreted and directly accelerates platelet aggregation and potentiates the response of platelets to other agonists such as adenosine diphosphate, collagen, and thromboxane A_2 [19].

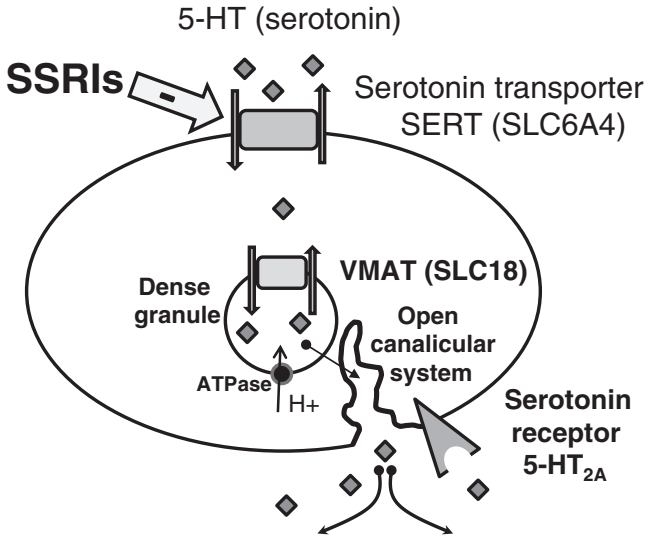

Fig. 1. The platelet serotonin uptake pathway.

Serotonin stimulation also accelerates the exocytosis of platelet α-granules, which secrete their ingredients, the procoagulant molecules, into the plasma [20]. One of these molecules, PAI-1, is released at the site of thrombus formation [21,22]. PAI-1levels in arterial clots are 2-3-times higher than those observed in venous clots and the relative PAI-1 content determines their resistance to thrombolysis [22]. PAI-1 inhibits the bioavailability of free active tissue-plasminogen activator (tPA) and plasmin, which are serine proteases mediating cleavage of brain-derived neurotrophic factor precursor (proBDNF) to its mature form (mBDNF). Multiple lines of evidence have shown that cleavage of proBDNF is pivotal both for the pathophysiology of major depressive disorder and for the mechanisms of action of antidepressants [23].

Serotonin released from activated platelets was shown to increase tissue factor and PAI-1 mRNA expression in rat aortic endothelial cells. Since it has no significant effect on the expression of tissue-factor pathway inhibitor and tPA, serotonin causes endothelial cells to be procoagulant and hypofibrinolytic [19]. Serotonin also enhances the interaction of platelets with circulating tissue-factor rich microvesicles, thus potentiating their overall procoagulant activity [24,25].

As to other effects on vascular biology, serotonin exhibits strong vasoactive properties, possibly attributable to the stimulation of serotonin receptors on endothelial cells and to nitric oxide production [26]. Being an angiogenic factor, at low micromolar concentrations, serotonin could induce endothelial cell proliferation, migration and tube formation *in vitro* [27]. Serotonin was also found to stimulate extracellular matrix synthesis in interstitial fibroblasts and promote tissue fibrosis [28].

Serotonin and Neuroendocrine Mechanisms

Serotonin is synthesized and secreted by adipocytes [29]. Evidence indicates that serotonin regulates adipocyte function in a direct manner via blood circulation and/or paracrine and autocrine mechanisms. Hyperserotoninergic rats, for instance, have lower leptin levels and tend to lose weight [29].

In β-cells, serotonin helps regulate insulin secretion, through a mechanism that shares similarities with trafficking of proaggregatory α-granules in thrombocytes and results in their exocytosis: serotonylation [30]. Serotonylation is covalent coupling of serotonin to target proteins, a process that activates specific small GTPases, which in turn promote glucose-mediated insulin secretion [31]. Interestingly, both insulin and its precursors — proinsulin and split products of proinsulin — stimulate synthesis and release of PAI-1 [32].

BDNF and TPA-Plasmin Pathway

A traumatic event induces hypersecretion of cortisol and adrenaline, which can damage specific brain areas responsible for emotional processing, such as the hippocampus [33]. A number of substances help prevent hippocampal atrophy, including neurotrophin mBDNF, which has been shown to enhance neurogenesis and neurite sprouting [34].

Abnormal hippocampal neurogenesis throughout life has been identified as one of the neurobiological mechanisms underlying depressive disorders. In paradigms of depression using experimental models such as rodents, reduced levels of hippocampal neurogenesis have been associated with increased levels of depressive behavior [35]. Furthermore, it is known that the effectiveness of antidepressants such as SSRIs depends on adequate levels of neurogenesis [36]. Treatment with SSRIs enhances hippocampal neurogenesis, improving depressive phenotype of the animals [37]. Such evidence suggests that hippocampal function is an important biological target for the improvement of depression symptoms by pharmacological agents such as SSRIs.

Transcription of the BDNF gene results in the production of proBDNF by different tissues. proBDNF is stored within platelet granules, released through thrombin stimulation [38] and proteolytically cleaved to mBDNF through the tPA–plasmin pathway. ProBDNF and mBDNF have opposing effects on both neuronal morphology and physiology; while mBDNF promotes dendritic growth, cell survival, and long-term potentiation, proBDNF induces dendritic retraction, long-term depression, and apoptosis. Long-term potentiation and long-term depression are two common forms of synaptic plasticity, with the first strengthening neuronal synapse, and the second selectively weakening it. Since the precursor proBDNF and the mature protein mBDNF can elicit opposite effects on cellular functions, cleavage of proBDNF is an important regulatory mechanism of neurochemistry and inadequate cleavage may increase the risk for mood disorders [39]. Indeed, patients with major depression have increased levels of proBDNF and decreased levels of mBDNF [12].

Given that plasmin is required to convert proBDNF into mBDNF, elevated PAI-1 synthesis reduces the production of mBDNF. Since PAI-1, inhibiting the production of plasmin, prevents the dissolution of blood clots formed on the top of the ruptured atherosclerotic plaque, one would expect that patients with depressive disorders could have an increased risk for cardiovascular events.

Depressive Disorders and Cardiovascular Risk

Depression is a common comorbid condition in patients with coronary artery disease (CAD) [40]. While 17-27% of patients with CAD have major depression, a significantly larger percentage present with symptoms of depression [41]. The relative risk for the development of CAD particularly associated with major depression, is also significant [42].

In a large meta-analysis, the risk of developing coronary heart disease in patients with depression was 1.8, as was the relative risk of death due to cardiovascular events [43]. Although the highest mortality rate was observed in patients with most severe depressive symptoms, compared to those without depression, a higher mortality rate was also observed at very low levels of depressive symptoms, which are not generally considered clinically significant [44].

Patients with metabolic syndrome or type 2 diabetes, who are known to have an elevated risk for cardiovascular disorders, are also at a high risk for depressive disorders [45,46]. Likewise, depression predicts a 1.66-time greater risk of diabetes [47]. Of note, compared to controls, insulin sensitivity was found to be significantly lower in non-obese young males with major depression and bipolar depression, but not in patients with stress-related depression (i.e., reactive depression) [48].

These findings suggest that cardiovascular disorders and depression might have a common denominator, which probably derives from abnormal insulin metabolism. One possibility is that increased levels of PAI-1, resulting from hyperinsulinemia, would inhibit plasmin synthesis. The consequences would not be limited to inadequate cleavage of proBDNF into mBDNF and to abnormal dissolution of intraluminal coronary thrombi. Since plasmin is required to activate metalloproteinases responsible for angiogenesis and neurogenesis [49], elevated PAI-1 levels might prevent not only myocardial remodeling, but also limbic system regeneration.

Patients with bipolar disorders are known to be at high risk of premature death, comorbid cardiovascular disease being the leading cause of excess mortality [50]. These disorders are currently conceptualized as an early manifestation of a multi-systemic inflammatory disease favoring obesity, diabetes mellitus, hypertension, cerebrovascular and cardiovascular diseases [51].

SSRIs, Oxidative Stress and the Endothelium

Oxidative stress may play a role in the pathogenesis of depression. Urinary excretion of F2 isoprostanes, a marker of oxidative stress, was found to be elevated in patients with depression compared to control subjects, albeit without correlation with depression severity scores.

These results suggest that alternative mechanisms, beyond oxidative stress, may be involved in the development of depression and subsequent responses to treatment [52]. Using specific hippocampus tissue testing in a rat model, long-term treatment with venlafaxine could protect against stress-induced oxidative cellular and DNA damage potentially through antagonizing oxidative stress and enhancing antioxidant defense mechanisms [53]. SSRIs may modulate endothelial functions. In major depression patients, the low peripheral production of nitric oxide (NO) was increased by administration of paroxetine [54].

In-vitro, several SSRIs (paroxetine, fluoxetine and sertraline) enhance caspase 3/7 activity, a process that triggers beta cell apoptosis, therefore inhibiting insulin secretion. Therefore, it would be expected that these drugs could accelerate the transition from an insulin-resistant state to an overt diabetes [55]. In this setting, hyperglycemia would cause oxidative stress that could increase vascular risk. Nonetheless, increased levels of oxidative stress markers have been demonstrated only in patients with SSRI intoxication [5]. In addition, chronic use of fluoxetine or sertraline has led to improved glucose control in diabetic individuals [56]. A plausible explanation is that depressive episodes are associated with sedentary lifestyle. After remission, a patient has more chances to engage in regular aerobic physical activity, which increases glucose uptake by skeletal muscles via an insulin-independent mechanism [57].

SSRIs and Inflammation

Coagulation and inflammation interactions are well-established players in physiological and pathological states, such as thrombosis, sepsis, etc. In major depression, elevations in the level of proinflammatory cytokines and other inflammation-related proteins were observed in plasma and cerebrospinal fluid as well as in postmortem specimens. Increased rates of proinflammatory cytokines persist after clinical symptoms of depression subside and can also predict the onset of a depressive episode [58]. SSRIs exert anti-inflammatory effects on T-lymphocytes, dendritic cells and neutrophils. These agents have been shown to reduce the levels of TNF-α and IL-6, thereby contributing to their anti-inflammatory properties [59]. Sertraline and paroxetine diminish stimulated TNF-α release from T-lymphocytes while fluoxetine reduces stimulated proliferation [60]. Fluoxetine attenuates bacterial antigen presentation to effector T lymphocytes and can inhibit the release of the free radical superoxide from neutrophils stimulated with platelet activating factor and formyl-methionyl-leucyl-phenylalanine. SSRIs can limit microglial and astroglial inflammatory processes. The impacts of antidepressants on astrocytes are dependent upon the model of study, with anti-inflammatory effects observed in models replicating neuropathology. Fluoxetine, paroxetine and sertraline are found to inhibit the ability of murine microglia to produce TNF-α and the free radical NO. A comparative analysis of the SSRIs showed similar anti-inflammatory potencies, with the exception of citalopram which was found to be 2.5 times less potent than sertraline [60].

Additionally, inflammation induces insulin resistance, which is related to a hyperinsulinemic state that promotes synthesis and release of PAI-1 [61]. Interestingly paroxetine, fluoxetine, and sertraline inhibit insulin-induced Tyr phosphorylation of insulin receptor substrate (IRS)-2 protein and the activation of its downstream targets Akt and the ribosomal protein S6 kinase-1 (S6K1). Inhibition of insulin signaling is dose-dependent, and is associated with a marked inhibition of glucose-stimulated insulin secretion from pancreatic islets [55].

How SSRIs Affect Coagulation and Fibrinolysis

Patients on SSRIs may present with a mild bleeding tendency manifesting primarily with easy bruising and skin hematomas. The increased bleeding risk has been related to the lower platelet serotonin content and lower ADP, collagen or epinephrine-induced platelet aggregation detected in platelet-rich plasma of SSRI-medicated patients [62]. In studies performed in humans all SSRIs have consistently shown a drastic decrease in platelet serotonin content after several weeks of treatment, reaching levels around or below 10% of the pretreatment serotonin levels [63]. While mucosal bleeding is uncommon with SSRIs, some patients experienced increased bleeding following surgical procedures [64].

Several mechanisms have been suggested to explain the decreased risk for cardiovascular disorders seen in patients receiving SSRIs [65]. One potential mechanism could be related to the normalization of elevated platelet aggregation observed in CAD patients. A number of studies have reported inter-individual variability in platelet response to aspirin and clopidogrel, and as such, could identify patients who did not achieve platelet inhibition as low-responders or resistant to these drug. For example, after percutaneous coronary intervention with stenting, high post-treatment platelet reactivity was found to predict recurrent cardiovascular events at 30 days [66].

The anti-hemostatic properties of SSRI also involve their inhibition of fibrinolysis. Patients on serotonergic antidepressants appear to have fibrinogen and PAI-1 plasma levels that were similar to those of healthy controls, and lower than in depressed patients receiving non-serotonergic antidepressants [67].The hypothesis that SSRIs decrease cardiovascular illness severity through attenuation of the effect of anxiety or depression on coagulation was refuted by the fact that depressed patients on serotonergic and non-serotonergic antidepressants had similar scores of depression and trait anxiety [67]. Cognitive-behavioral stress management can reduce stress behavior and vital exhaustion, but does not improve intermediate biochemical targets related to the metabolic syndrome and ischemic heart disease [68]. The Sertraline Anti-Depressant Heart Attack Trial (SADHAT) examined the use of sertraline in depressed subjects experiencing MI or unstable angina. Sertraline was found to reduce platelet activation in addition to that afforded by co-administered anti-platelet regimens and was associated with a lower risk of reinfarction and/or mortality [69].

The premise that the restoration of hippocampal circuitry in depressive disorders would require the following multiple sequential steps: (1) serotonin inhibition; (2) decreased insulin synthesis and release; (3) decreased PAI-1 synthesis and release; (4) increased plasmin production; (5) cleavage of proBDNF into mBDNF; (6) neurogenesis, may provide at least in part an explanation for the known delayed onset of patient's response to SSRIs.

Different findings support the hypothesis that PAI-1 is an important intermediary in response to SSRI. First, the prevalence of haplotype 4G (derived from the rs2227631-G and rs1799889-4G polymorphisms), which encodes for increased PAI-1 synthesis, appeared to be lower in patients with major depressive disorders who responded to SSRI than in non-responders [70]. Second, exercise also produced an antidepressant response [71]. Although regular moderate physical training does not decrease PAI-I activity, regular exercise may be effective in preventing hyperinsulinemia, which contributes to controlling elevated PAI-1 level in subjects homozygous for the 4G allele [72].

Conclusions

The direct or indirect involvement of the haemostatic system in the pathophysiology of severe depression is still a matter of investigation. Patients with untreated depressive disorders are at higher risk of cardiovascular disease than depressive patients treated with SSRIs. Serotonin vascular effects can, at least partially, be counteracted by SSRIs, thereby potentially decreasing thrombogenicity. We hypothesize that SSRI effect in severe depression may be mediated via modulation of haemostatic and vascular biology pathways. Further research should focus on the role of SSRIs in vascular pathologies and on the potential capability of antithrombotics to increase plasminogen activator levels in severe depression.

Author Contribution Statement

SH-C: wrote the paper, approved the final version of the paper
AEN: wrote the paper, approved the final version of the paper
J-CG: wrote the paper, approved the final version of the paper
BB: wrote the paper, approved the final version of the paper

Conflict of Interest Statement

The authors have no conflicts to declare.

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CONSIDERAÇÕES FINAIS

Há evidências de que a baixa atividade do tPA participe da fisiopatologia de outras doenças caracterizadas por disfunção cognitiva. Por exemplo, em ratos, a plasmina foi capaz de degradar as fibrilas do peptídeo β amilóde, impedindo a progressão para doença de Alzheimer.[43,44] Além disso, em um relato de caso, um paciente com Alzheimer obteve melhora acentuada das funções cognitivas quando usou warfarin. Três vezes a medicação foi suspensa e o paciente piorou. Duas vezes o warfarin foi reintroduzido, com melhora das funções cognitivas.[45]

Intervenções que aumentam a atividade da plasmina também têm sido propostas como nova abordagem terapêutica da doença de Parkinson.[46] A disfunção cognitiva na doença de Parkinson abrange um grande espectro, que vai de pouco acentuada até um quadro demencial grave. A doença se caracteriza por inclusões de α-sinucleína, na forma de corpos de Lewy. Considera-se que agregados de α-sinucleína sejam responsáveis pela morte de neurônios dopaminérgicos. Como também existe α-sinucleína no liquor e no plasma de indivíduos saudáveis, postula-se que o defeito na degradação da α-sinucleína extracelular possa contribuir para a fisiopatologia da doença.[46] A plasmina, mas não o tPA, é capaz de degradar monômeros e agregados de α-sinucleína.[45]

CONCLUSÕES

A esquizofrenia se acompanha por tendência trombótica e atrofia de áreas que processam a cognição, como o hipocampo e o córtex pré-frontal. Das proteínas que regulam a coagulação, três também participam de processos que previnem e revertem a atrofia cerebral: o tPA, a plasmina e a proteína S.

O tPA, diretamente ou catalisando a produção de plasmina, ativa neurotrofinas, a relina e o receptor N-metil-D-aspartato. Como resultado, esta serino protease participa da tolerância contra danos causados por situações altamente estressantes, promove remodelação sináptica e neurogênese. A proteína S estimula a proliferação e diferenciação de células tronco, independente do tPA.

Estamos propondo um novo modelo para a fisiopatologia de transtornos do espectro da esquizofrenia, onde a atividade aberrante do tPA, da plasmina e/ou da proteína S não só tornaria os neurônios mais vulneráveis, como também inibiria a remodelação sináptica e a neurogênese. Nossa hipótese é reforçada pelo achado de alta prevalência de condições que afetam a atividade do tPA em pacientes com estes transtornos, incluindo hiperinsulinemia, hipertrigliceridemia, hiperhomocisteinemia, anticorpos antifosfolipídio e deficiência da proteína S livre. Também corrobora a hipótese a constatação de que várias intervenções eficazes no tratamento da psicose corrigem a atividade do tPA, da plasmina e/ou da proteína S.

Há fortes evidências de que a atividade defeituosa do tPA e da plasmina também influencie a fisiopatologia de outras condições acompanhadas por disfunção cognitiva, como a púrpura trombocitopênica trombótica, a doença de Alzheimer, a doença de Parkinson, dificuldades no aprendizado e transtornos do espectro autista. No nosso modelo para explicar as dificuldades no aprendizado e os transtornos do espectro autista, o hiperinsulinismo fetal e neonatal, reduzindo os níveis do tPA, prejudicaria a neurogênese e a conectividade neuronal.

Nossos resultados sugerem que intervenções objetivando especificamente a correção da atividade do tPA, da plasmina e da proteína S possam criar novas perspectivas terapêuticas para as psicoses e as disfunções cognitivas. Por serem propostas inovadoras, recomendamos que nossas hipóteses sejam validadas em modelos animais e estudos controlados em humanos.

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