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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.