Plasma nitrite/nitrate concentrations in patients with schizophrenia

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Abstract

Background: Nitric oxide (NO) is known to be a signaling molecule with many physiological functions including apoptotic process regulation. Since apoptosis may contribute to the pathophysiology of schizophrenia, this study was undertaken to determine the plasma concentrations of NO in schizophrenia.

Methods: Nitrite/nitrate \((\text{NO}_2^-/\text{NO}_3^-)\) concentrations were measured in plasma from 40 patients with schizophrenia, and 36 age- and gender-matched healthy persons using a colorimetric test.

Results: Plasma \(\text{NO}_2^-/\text{NO}_3^-\) concentrations were significantly higher in patients with schizophrenia \((102.8 \pm 34.7 \mu\text{mol/L}, p < 0.0001)\) than in controls \((69.2 \pm 13.2 \mu\text{mol/L})\). Also, mean \(\text{NO}_2^-/\text{NO}_3^-\) values in female patients and controls were significantly higher \((118.2 \pm 44.7 \mu\text{mol/L}, p < 0.001; 74.8 \pm 16.1 \mu\text{mol/L}, p < 0.05\), respectively) compared to males \((94.7 \pm 25.3 \mu\text{mol/L}, 67.6 \pm 10.8 \mu\text{mol/L})\). Significant correlation was seen between plasma \(\text{NO}_2^-/\text{NO}_3^-\) concentrations and heredity, number of episodes and PBMC caspase-3 activity. These results suggest that NO could be considered an inducer or regulator of apoptosis in patients with schizophrenia.

Keywords: caspase-3 activity; nitrite/nitrate; schizophrenia.

Introduction

Although initially described as a mediator of endothelial relaxation, nitric oxide (NO) is recognized in numerous physiological processes including vasodilation, neurotransmission, immune and apoptosis regulation, and killing of intracellular pathogens. In the nervous system, NO is involved in corticotropin-releasing factor release, cytokine-mediated release of adrenocorticotropic hormone, cyclooxygenase and guanylate cyclase activation, and oxytocin and vasopressin secretion (1). NO is produced from the semi-essential amino acid L-arginine by two constitutive isoforms of nitric oxide synthase (NOS), endothelial NOS (eNOS, NOS3) and neuronal NOS (nNOS, NOS1). L-arginine is both the substrate and the regulator of NOS (2). The third isoform (nNOS, NOS2) is induced in inflammatory, endothelial, muscle and other cells by a wide range of inflammatory agents, especially cytokines (3), and bacterial endotoxines. eNOS can also be induced in some settings (4). However, unregulated NO synthesis, associated with either higher or lower NO availability, may have deleterious effects. Increased NO production may lead to nitrosylation of biomolecules and to reaction with superoxide, forming a very toxic peroxynitrite anion (5) capable of reacting with various biomolecules. Decreased NO may disturb physiological NO effects.

These toxic effects are involved in many human diseases including atherosclerosis, immune-mediated inflammatory processes, and ischemic cell death (6). NO is also suggested as a cytotoxic factor which induces apoptosis in neurodegenerative diseases as well as schizophrenia (7). Recently, it was shown that animals treated with a substance that blocks brain NO production became resistant to the schizophrenia-like effects of phencyclidine (8). In another study, the authors noted that plasma arginase activity and Mn were significantly decreased and total nitrite level was increased in patients with schizophrenia compared to controls (9). These findings suggest that a disturbed arginine-NO pathway may increase cell susceptibility to apoptosis, and may therefore be involved in the pathogenesis of schizophrenia. To test this hypothesis, we measured plasma nitrite/nitrate \((\text{NO}_2^-/\text{NO}_3^-)\) concentrations and peripheral blood mononuclear cell (PBMC) caspase-3 activity in patients with schizophrenia and correlated these parameters with patient number of episodes and PBMC caspase-3 activity. These results suggest that NO could be considered an inducer or regulator of apoptosis in patients with schizophrenia.
clinical characteristics, Positive and Negative Syndrome Scale (PANSS) scores and drug treatment.

Patients and methods

Patients were recruited at the Clinic of Psychiatry and the Clinic for Mental Health Protection of the Clinical Centre Niš. Subjects were assessed with the Diagnostic Interview for Psychosis by two independent psychiatrists, and diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th ed) and ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th revision). For disease evaluation and clinical management of patients, the PANSS was used for scoring positive symptoms (hallucinations, delusions of grandeur, disordered thought processes and bizarre behaviors), negative symptoms (social withdrawal, flat affect, loss of pleasurable feelings, reduced motivation, reduced speech output and cognitive disturbances) and general psychopathology scale which presented the structure of clinical disease manifestation. Each patient was tested and classified into one of three groups according to the score values: a group with PANSS positive score predominance (the values of score higher than 3), a group with PANSS negative score predominance (score values lower than –8), and a group showing positive and negative symptoms almost equally (score values near 0). Patients with immune, inflammatory and liver diseases were excluded from the study.

The study included 40 schizophrenic patients (24 males, 16 females; mean age 30.2 ± 7.9) and 36 healthy controls (22 males, 14 females; mean age 29.8 ± 5.8) recruited at the Department for Blood Transfusion. The patients and controls were matched according to age, gender, marital status, education, living conditions, living settings and habits.

All subjects provided written informed consent and the study was approved by the Clinical Center Niš Ethics Committee.

Venous blood was collected in vacutainer tubes containing potassium EDTA as anticoagulant. Plasma samples were stored at –20°C until measurement of NO₂⁻/NO₃⁻. Concentrations of NO₂⁻/NO₃⁻ were measured using the modified cadmium-reduction method of Navaro-Gonzalez et al. (10) based on the Griss reaction. PBMCs were isolated from peripheral blood using lymphocyte separation medium (PAA, Pashing, Austria). Caspase-3 activity was measured using a colorimetric commercially available ELISA kit (Bio Source Europe S.A, Novellas, Belgium), and expressed per mg of protein.

Data analysis was performed using the SigmaStat computer program. Differences between groups were assessed using the Mann-Whitney rank sum test (ANOVA). Correlations between NO₂⁻/NO₃⁻ concentrations and demographic, clinical and therapeutic characteristics of patients were assessed using Pearson’s coefficient.

Results

The demographic characteristics of patients with schizophrenia and healthy controls are shown in Table 1. Patients and healthy subjects were matched for age and gender. Heredity was present in 16 of 40 schizophrenics. Age of disease manifestation was between 18 and 34 years. The duration of psychiatric disease was from 1 to >5 years. The patients had 1–7 episodes before inclusion in the study. In 20 patients, positive symptoms were predominant, in 12 negative symptoms predominated, and in eight both types of symptoms were almost equally expressed. Eighteen patients were treated with haloperidol and 22 with atypical antipsychotic drugs; 24 patients were smokers and 16 were non-smokers. The control group included 15 smokers and 21 non-smokers.

Plasma NO₂⁻/NO₃⁻ concentrations in patients with schizophrenia were significantly increased (102.8 ± 34.7 µmol/L, p < 0.001) when compared with controls (69.2 ± 13.2 µmol/L) (Figure 1). In both groups that were examined, we also observed a significant difference between males and females. In female control patients, plasma NO₂⁻/NO₃⁻ concentrations were 74.8 ± 16.1 µmol/L, which were significantly higher (p < 0.05) than in male controls (67.6 ± 10.8 µmol/L), but lower (p < 0.05) than in female patients (118.2 ± 44.7 µmol/L). These values were significantly increased compared to male patients (94.7 ± 25.3 µmol/L, p < 0.001) (Figure 1).

In addition, we observed that there was no significant difference in plasma NO₂⁻/NO₃⁻ concentrations between patients with PANSS positive score predominance, patients with PANSS negative score predominance, and the patients showing almost equally positive and negative symptoms (Table 2). Significant correlation was found between plasma NO₂⁻/NO₃⁻ concentrations and patient clinical characteristics.

Table 1 Demographic, clinical and pharmacotherapeutic characteristics of patients with schizophrenia.

<table>
<thead>
<tr>
<th></th>
<th>Schizophrenia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n</td>
<td>24/16</td>
<td>22/14</td>
</tr>
<tr>
<td>Age, years</td>
<td>30.2 ± 7.9</td>
<td>29.8 ± 5.8</td>
</tr>
<tr>
<td>Heredity (+/−)</td>
<td>16/24</td>
<td>−</td>
</tr>
<tr>
<td>Age of disease manifestaion, years</td>
<td>Between 18 and 34</td>
<td>−</td>
</tr>
<tr>
<td>Duration of psychiatric disease, years</td>
<td>1–&gt;5</td>
<td>−</td>
</tr>
<tr>
<td>Number of episodes</td>
<td>1–7</td>
<td>−</td>
</tr>
<tr>
<td>PANSS positive scores predominant (&gt;3)</td>
<td>20</td>
<td>−</td>
</tr>
<tr>
<td>PANSS negative scores predominant (&lt;−8)</td>
<td>12</td>
<td>−</td>
</tr>
<tr>
<td>PANSS positive and negative scores almost equally expressed (&gt;−8&lt;n&lt;3)</td>
<td>8</td>
<td>−</td>
</tr>
<tr>
<td>Haloperidol treated, n</td>
<td>18</td>
<td>−</td>
</tr>
<tr>
<td>Atypical antipsychotic drugs treated, n</td>
<td>22</td>
<td>−</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>59</td>
<td>43</td>
</tr>
<tr>
<td>Non-smokers, %</td>
<td>41</td>
<td>57</td>
</tr>
</tbody>
</table>
Figure 1 Plasma NO₂⁻/NO₃⁻ concentrations in patients with schizophrenia.
*p < 0.001 vs. control; *p < 0.05 vs. control male; **p < 0.05 vs. control female; ***p < 0.001 vs. schizophrenia male; ****p < 0.001 vs. control male.

Table 2 Plasma nitric oxide concentrations in schizophrenic patients with different PANSS scores.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>NO₂⁻/NO₃⁻, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS positive score predominance</td>
<td>20</td>
<td>97.3 ± 36.4</td>
</tr>
<tr>
<td>PANSS negative score predominance</td>
<td>8</td>
<td>108.1 ± 25.4</td>
</tr>
<tr>
<td>PANSS positive and negative equally expressed</td>
<td>12</td>
<td>100.9 ± 26.4</td>
</tr>
</tbody>
</table>

The results are presented as means ± SD. There was no significant difference between tested groups.

including heredity (p < 0.01), number of episodes (p < 0.01) and PBMC caspase-3 activity (p < 0.05) (Table 3).

PBMC caspase-3 activity was significantly (p < 0.05) higher in patients compared with controls. No significant difference was found in NO₂⁻/NO₃⁻ concentrations or in PBMC caspase-3 activity between patient smokers (104.6 ± 32.3 μmol/L and 0.110 ± 0.064 μmol/mg protein, respectively) and non-smokers (105.1 ± 39.5 μmol/L and 0.123 ± 0.046 μmol/mg protein, respectively), as well as in control smokers (70.5 ± 17.9 μmol/L and 0.086 ± 0.035 μmol/mg protein, respectively) and non-smokers (68.4 ± 12.6 μmol/L and 0.086 ± 0.025 μmol/mg protein, respectively) (Figures 2 and 3).

Comparing plasma NO₂⁻/NO₃⁻ concentrations between patients treated with haloperidol (97.2 ± 31.2 μmol/L) and those in patients treated with atypical antipsychotic drugs (109.8 ± 33.7 μmol/L) revealed no significant difference (p > 0.05), although both groups had significantly higher values compared with controls (p = 0.00027) (Figure 4).

Discussion

We observed significantly higher NO₂⁻/NO₃⁻ concentrations in females than in males in both groups studied – healthy subjects and schizophrenics. Similarly, Peinado et al. (11) found that NO concentrations undergo age and gender changes that strongly correlated with serological markers, such as those related with cardiovascular function and lipids. Contrary to our findings, Ghasemi et al. (12) observed significantly higher NO metabolite concentrations in asymptomatic male non-smokers than in females, showing peak concentrations at the age of 50–59 years in both genders. A study conducted in monozygotic twin pairs following an overnight fast showed higher hereditability for females than for males, and higher values for non-smokers than smokers, but a low degree of heredity for NO (13). Cigarette smoking induced an increase in serum NO concentrations associated with increased aortic endothelial regeneration in an animal model (14). Significant correlation between serum NO and estradiol was observed in estrogenized males over 50 years of age (15). This finding could explain the results of this study considering that our studied groups consisted of sexually mature and active subjects. A discrepancy in serum NO

Table 3 Correlation between NO₂⁻/NO₃⁻ concentrations and clinical characteristics of patients.

<table>
<thead>
<tr>
<th>NO₂⁻/NO₃⁻, μmol/L</th>
<th>PBMC caspase-3 activity</th>
<th>Heredity</th>
<th>Number of episodes</th>
<th>Patient age at the disease onset</th>
<th>Duration of psychiatric disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>r = 0.264</td>
<td>r = 0.369</td>
<td>r = 0.408</td>
<td>r = 0.018</td>
<td>r = 0.037</td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
concentrations dependent on age and gender was published elsewhere (16).

To avoid circadian variations in blood concentrations of NO (17), as well as any influence from hyperlipidemia, we obtained blood samples in the morning following a 12-h fast. There was evidence that hypercholesterolemia itself significantly lowered NO concentrations. Although patients and controls in this study showed mean values of total cholesterol above the upper reference interval, there was no significant difference between males and females, or between patients and controls. Also, the urinary excretion rate of NO\textsubscript{2}/NO\textsubscript{3} was found to be decreased in uncomplicated male hypertensive patients, and unrelated to accompanying insulin resistance or dyslipidemia (18).

With respect to schizophrenia, it was shown that NOS activity is significantly higher in platelets of drug-naive schizophrenic subjects compared to controls, drug-treated schizophrenics and subjects with panic disorders (19). In addition, it has been documented that the total amount of NOS-immunoreactive paraventricular neurons in post mortem hypothalami were smaller in depressed and schizophrenic patients compared with normals (20). This might be related to the presumed regulatory function of NO in the release of corticotroin-releasing hormone and arginine-vasopressin and/or oxytocin, which had been reported to be over-expressed in endogenous psychoses. An increase in NOS activity and protein in the hippocampus and cerebellum was found in rats that were subjected to prenatal stress, and this was correlated with behavioral changes in adults (1). Plasma nitrate concentrations were found to be significantly decreased, while asymmetric dimethylarginine (ADMA) was increased in drug-naïve schizophrenic patients compared to control subjects. In only three of these patients, treatment with neuroleptics for 3 months lowered ADMA concentrations and increased nitrate in plasma (21). Contrary to these findings, a systematic review by Bernstein et al. (22) suggested that alterations in NO metabolism are not unique to, or indicative of, schizophrenia. Our results showed a highly significant increase in plasma NO\textsubscript{2}/NO\textsubscript{3} concentrations in patients with schizophrenia compared with controls. Yilmaz et al. (23) also found significantly higher NO in males with chronic schizophrenia (p < 0.03) compared with male controls. However, Lee and Kim (24) showed significantly lower NO concentrations in schizophrenics both before and after treatment compared with controls. Similarly, Suzuki et al. (25) noted no difference in plasma nitrite concentrations, but found significantly lower plasma nitrate in schizophrenic patients with deficit syndrome. Also, in our study there was no significant difference in plasma NO\textsubscript{2}/NO\textsubscript{3} concentrations between patients treated with typical or atypical antipsychotic drugs, or between smokers and non-smokers. With respect to smoking status, we also measured NO\textsubscript{2}/NO\textsubscript{3} in patients with ischemic heart disease and found no significant difference between smokers and non-smokers, although smokers in this study were much older than smokers with schizophrenia and had a longer smoking status (6). Increases in NO\textsubscript{2}/NO\textsubscript{3} concentrations in our patients may be partially explained by the ability of atypical antipsychotic drugs to up-regulate the expression of copper/zinc superoxide dismutase mRNA and down-regulate p75NTR mRNA (26, 27), as well as the ability of haloperidol to strongly increase the expression of copper/zinc superoxide dismutase mRNA (27). This enzyme is a known regulator of NO concentrations because it scavenges superoxide anion radical and prevents formation of toxic peroxynitrite with NO, thereby increasing bioavailability of NO. That could be a potential cause of increased NO\textsubscript{2}/NO\textsubscript{3} concentrations observed in our patients.

The second source of NO noted in our patients might be NOS1. NOS1 is highly expressed in the brain as well as in the airways, intestine, kidney and heart. Recently, Kremeyer et al. (28) found a significant association between eight single nucleotide polymorphisms (SNPs) in the NOS1AP gene region and schizophrenia. Others (29) have shown that some SNPs in schizophrenics were characterized by increased gene expression leading to higher NO production. The finding that
the exon 1c promoter polymorphism is linked to schizophrenia indicates that regulatory rather than coding variants convey a genetic risk for psychosis (30). These results show that NOS1 may be responsible for the uncontrolled NO production in patients with schizophrenia.

To date, a number of neuronal cell culture models that were treated with different apoptotic agents such as protein S100B, cytokines, 3,4-methyl-enedioxy-methamphetamine or NO donors, show that the cells undergo apoptosis with the formation of NO (31). NO can release cytochrome C from mitochondria (32) and the apoptotic mechanism includes caspase-3 activation following down-regulation of Bcl-2 and up-regulation of Bax protein levels (33). The evidence for progressive clinical deterioration and subtle neurostructural changes following the onset of schizophrenic psychosis has led to the hypothesis that apoptosis may contribute to the pathophysiology of schizophrenia (34). Observed morphological brain changes in schizophrenics, reduced cortical Bcl-2 protein (35), a 50% higher Bax/Bcl-2 ratio in the temporal cortex of schizophrenics compared with non-psychiatric subjects suggest that cortical cells are vulnerable to apoptosis (36). In favor of these postmortem findings is our in vivo finding of increased PBMC caspase-3 activity in patients with schizophrenia. Further studies are needed to clarify the mechanisms underlying the increase in plasma NO and caspase-3 activity in patients with schizophrenia.

In conclusion, the results of this study show that plasma NO$_2^{-}$/NO$_3^{-}$ concentrations are significantly increased in patients with schizophrenia, being significantly higher in female than male patients and healthy controls. The absence of any correlation between patient NO$_2^{-}$/NO$_3^{-}$ concentrations and drug treatment and the existence of a significant correlation between NO$_2^{-}$/NO$_3^{-}$ concentrations and heredity, number of episodes, as well as PBMC caspase-3 activity suggests that NO could be considered an inducer or regulator of apoptosis in patients with schizophrenia.

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Conflict of interest statement

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