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Oxidative stress in schizophrenia patients treated with long-acting haloperidol decanoate

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ABSTRACT

In this study the role of oxidative stress in schizophrenia was investigated by evaluating the relationship of oxidative stress markers with neurochemistry, psychopathology, and extrapyramidal symptoms. Antioxidant activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and concentrations of malondialdehyde, protein carbonyls, nitrite, nitrate, glutathione, dopamine, noradrenaline, adrenaline, and serotonin were measured in 52 outpatients with DSM-IV diagnosis of schizophrenia treated with haloperidol decanoate. Psychopathology and extrapyramidal symptoms were assessed by positive and negative syndrome scale, global assessment of functioning, abnormal involuntary movement scale, Simpson Angus scale, and Barnes akathisia rating scale. Haloperidol dose was positively correlated with plasma protein carbonyls. Longer duration of illness was associated with decreased levels of glutathione peroxidase. Increased activity of superoxide dismutase was associated with increased levels of catalase, glutathione peroxidase, glutathione reductase and reduced glutathione, and decreased concentration of malondialdehyde, indicating joint action of various antioxidative systems. Increased levels of nitrite and noradrenaline were associated with decreased level of malondialdehyde. Akathisia was greater in patients with decreased catalase activity, indicating involvement of impaired antioxidant defense in developing extrapyramidal symptoms. These results confirm the hypothesis that oxidative stress is involved in pathophysiology of schizophrenia and severity of extrapyramidal symptoms.

1. Introduction

Schizophrenia is a mental disorder characterized by a range of symptoms generally classified into positive, such as delusions, hallucinations, and thought disorders, and negative symptoms, including deficits in cognitive and social ability, poverty of speech, affective flattening, and other (Yao and Keshavan, 2011). It has a poor outcome in spite of different treatment approaches. The exact etiology is still unknown. Significant body of evidence suggests that oxidative stress is implicated in schizophrenia. Studies in drug naive patients indicate an important role of reactive species (RS) and abnormal membrane essential polyunsaturated fatty acid (EPUFA) metabolism (Li et al., 2011; Wu et al., 2012). An increase in oxidative stress together with a decline in EPUFAs is leading to enhanced lipid peroxidation (Mahadik et al., 2001). The proposed mechanisms of increased oxidative stress involve dopamine catabolism (Hermida-Ameijeiras et al., 2004), nitric oxide metabolism (Akyol et al., 2002), abnormalities in the mitochondrial electron transport chain (Prabakaran et al., 2004) and genetic factors (Hori et al., 2000; Virgos et al., 2001). Oxidative stress appears to be more pronounced in treatment with classical compared to atypical antipsychotics, suggesting that antipsychotic treatment may contribute to the oxidative imbalance (Kropp et al., 2005). Oxidative stress has also been implicated as a contributing factor in the development of the adverse effects induced by classical antipsychotics (Kropp et al., 2005). Haloperidol is metabolized to a haloperidol pyridinium ion (HP^+) (Murata et al., 2007). HP^+ is toxic and can increase oxidative stress and induce plasma membrane damage which may partially explain pathogenesis of haloperidol induced extrapyramidal side effects such as parkinsonism symptoms (Boskovic et al., 2011; Murata et al., 2007; Sagara, 1998). On the other hand, in some studies oxidative stress was independent of antipsychotic treatment (Akyol et al., 2002; Raffa et al., 2009; Zhang et al., 2006; Zhang et al., 2012). Zhang et al. (2006) measured several biomarkers of oxidative stress including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GpX) in schizophrenia patients on long-term treatment with clozapine, risperidone and classical antipsychotics and found no difference between the three treatment groups.

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RS destabilize phospholipid membrane structure and alter its fluidity and permeability. This impairs signal transduction and membrane receptor-mediated neurotransmitter uptake and release, which affects brain structure and function (Mahadik et al., 2001). Impaired signaling of multiple neurotransmitters, dopamine, adrenaline, noradrenaline, serotonin, and glutamate has been considered in schizophrenia (Lieberman and Koreen, 1993; Mahadik et al., 2001; Smythies, 2002; Yamamoto and Hornykiewicz, 2004). RS induced abnormalities may contribute to a number of clinically important consequences in schizophrenia, including psychopathology symptoms severity, tardive dyskinesia, and parkinsonism (Boskovic et al., 2011; Yao and Keshavan, 2011; Zhang et al., 2007).

Biomarkers of oxidative stress include antioxidants, markers of oxidative damage and markers of RS production (Boskovic et al., 2011). Antioxidant enzymes such as SOD, GpX, and CAT, along with glutathione (GSH) are most usually measured for quantifying antioxidant defense in schizophrenia (Yao and Keshavan, 2011). However, results of the clinical studies are conflicting. While the majority of studies reported decreased antioxidant defense in schizophrenia, there are also some studies where the opposite has been demonstrated (Altuntas et al., 2000; Dakhale et al., 2004; Kuloglu et al., 2002). In response to increased RS formation and related membrane damage due to lipid peroxidation, the concentration of antioxidant enzymes could rise as a compensatory mechanism (Boskovic et al., 2011). Oxidative damage is usually assessed by measuring concentration of MDA, 3-nitrotyrosine, protein carbonyls (PC), and 8-hydroxy-2-deoxyguanosine (Dietrich-Muszalska and Olas, 2009; Nishioka and Arnold, 2004; Yao and Keshavan, 2011). Markers of RS production include xantine oxidase (XO), homocysteine and nitric oxide, usually measured as a sum of nitrate (NO_3) and nitrite (NO_2) concentration (Akanji et al., 2007; Akyol et al., 2002; Dietrich-Muszalska et al., 2012).

A recent meta-analysis of biomarkers of oxidative stress in schizophrenia patients versus healthy controls provided an additional insight into the contribution of oxidative stress in pathophysiology of schizophrenia (Zhang et al., 2010). The subgroup analysis revealed that the ethnicity, number of patients and sample source may contribute to heterogeneity of the results. Additional factors, such as differences in measuring techniques, exposure to antipsychotic treatment, sampling of patients at different stages of the disease, differences in lifestyle and dietary patterns, may be responsible for discrepancy of the results among studies (Boskovic et al., 2011).

We have previously studied the association of genetic polymorphysms of antioxidant enzymes and tumor necrosis factor-alpha with pathophysiology of schizophrenia and extrapyramidal symptoms in patients treated with haloperidol depot (Boskovic et al., 2013). In the present study we aimed to further investigate the role of oxidative stress in schizophrenia in the same group of patients by a reanalysis of the biochemical and clinical data. We evaluated the relationship of various markers of oxidative stress with neurochemistry, psychopathology rating scales, and incidence of extrapyramidal symptoms. To the best of our knowledge these comprehensive relationships have not been tested in a treatment-homogenous group of patients with schizophrenia.

2. Methods

2.1. Patient population and clinical assessments

We recruited 52 outpatients with schizophrenia treated with long-acting haloperidol decanoate in naturalistic study as they were coming for their regular monthly check-ups. Outpatients were diagnosed on the basis of the structured clinical interview for DSM-IV. Patients with minimum 3 years duration of illness were receiving a stable intramuscular dose of haloperidol decanoate for at least 6 months. They were treated with intramuscular haloperidol decanoate (4.2–50 mg/week), and concomitantly 14 patients were given oral dose of haloperidol (2–30 mg/day). In patients concomitantly treated with haloperidol tablets, bioavailability of 65% of the oral dose relative to the intramuscular dose was assumed (Holley et al., 1983). All subjects were of the Caucasian origin and physically healthy, based on complete medical history and physical examination. Demographic data and data on smoking status, alcohol consumption, use of food supplements containing antioxidants and all concomitant medication were obtained from the available hospital records and patient interview. At the inclusion in the study a written informed consent to participate was obtained. The patients were recruited from the 4 main psychiatric hospitals in Slovenia, University Psychiatric Clinic Ljubljana, University Psychiatric Clinic Maribor, Psychiatric Hospital Celje and Psychiatric Hospital Begunje between July 2010 and March 2011. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Psychopathology was assessed by two experienced psychiatrists by positive and negative symptoms scale (PANSS) and global assessment of functioning (GAF) scale. Extrapyramidal symptoms were evaluated with abnormal involuntary movement scale (AIMS) for tardive dyskinesia, Simpson Angus scale (SAS) for parkinsonism and Barnes akathisia rating scale (BARS) for akathisia. Tardive dyskinesia was diagnosed if at least "moderate" movements in one or more of the seven body areas, or at least "mild" movements in two or more areas were observed on the AIMS scale. Parkinsonism was diagnosed if a patient scored more than 0.3 on the SAS scale. Akathisia was diagnosed if according to BARS the patient was scored as "mild" or worse on the global clinical assessment of akathisia item. Clinical ratings were obtained on the day of the regular ambulatory visit for drug dosing. At the same time a blood sample was drawn for biomarker measurements and neurochemistry.

2.2. Blood sampling and assays

Venous blood was drawn and plasma samples were prepared immediately by centrifugation. Whole blood and plasma samples were aliquoted into separate tubes and stored at −80 °C before analysis.

Enzyme antioxidant activities were determined in whole blood using Ransod® for SOD, Ransel® for GpX and glutathione reductase (GR) kits from Randox (Crumlin, United Kingdom) and CAT according to the method by Aebi (1984). The protocols were followed as per the direction in the manufacturer's manual, and activities of SOD, GpX and GR were expressed as units per gram of hemoglobin. Hemoglobin was measured by Randox colorimetric assay.

Reduced and oxidized forms of glutathione (GSH and GSSG, respectively) concentrations in whole blood were measured with a Chromsystems Diagnostic kit for HPLC (Munich, Germany).

Oxidative damage of macromolecules was assessed by measuring PC and MDA. PC was determined in plasma using a kit from Cayman Chemical Company (Ann Arbor, MI, USA) and the concentration was expressed as nmol per milligram of plasma proteins. Total plasma proteins were measured by a kit from Sentinel Diagnostics (Milan, Italy). Plasma concentration of MDA was measured with a Chromsystems Diagnostic kit for HPLC.

Nitric oxide production was assessed by measuring concentration of NO_3 and NO_2 in plasma using Cayman colorimetric kit.

Chromsystems assays (Catecholamine in plasma and Serotonin) were used for determination of noradrenaline, adrenaline, dopamine and serotonin in plasma.

All samples were measured at least in duplicate and measurements were within the method range. Intra- and inter-assay precision was less than 10%.

2.3. Statistical analysis

For descriptive purposes, continuous variables were presented as medians (ranges), and frequencies (percentages) were used to describe categorical variables. Continuous data between two independent groups were compared by Mann–Whitney *U* test (two-tailed). Spearman's rank-order coefficient of correlation was used to evaluate the associations between two continuous variables. Significance level was set at $p < 0.05$. Additionally, as this is a multidimensional problem with many confounding factors a principal component analysis (PCA) with varimax rotation was performed to provide an unbiased structure of the associations of biochemical markers with neurochemistry, psychopathology, and extrapyramidal symptoms. Since the various measurement values are on different scales we chose to use a correlation rather than a covariance matrix. SPSS version 20 (IBM SPSS, Chicago, IL, USA) was used for statistical analyses.

3. Results

3.1. Demographic and clinical data

Demographic and clinical characteristics of the 52 patients included in the study are presented in Table 1. The majority, 32 patients (61.5%) had paranoid type schizophrenia, 18 (34.6%) had shizoaffective disorder, and 2 (3.8%) had disorganized type