Quetiapine, Olanzapine and Haloperidol Affect Human Plasma Lipid Peroxidation in vitro

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Key Words

Oxidative stress · Lipid peroxidation · Antipsychotics · Quetiapine · Olanzapine · Haloperidol

Abstract

Objective: Oxidative injury in schizophrenia may be caused not only by pathophysiological processes but partly also by treatment with antipsychotics. The purpose of the present study was to examine and to compare the effects of quetiapine (QUE), olanzapine (OLA) and haloperidol (HAL), at final concentrations corresponding to doses used for treatment of acute episodes of schizophrenia, on plasma lipid peroxidation in vitro, measured by the level of thiobarbituric acid-reactive substances (TBARS). **Methods:** Blood from 30 healthy volunteers was collected into ACD (citric acid/citrate/dextrose) solution. The drugs in form of active substances were dissolved in 0.01% dimethyl sulfoxide, added to plasma at the final concentrations [QUE (175 and 275 ng/ml), OLA (20 and 40 ng/ml), HAL (4 and 20 ng/ml)] and incubated for 1 and 24 h at 37°C. The level of TBARS was measured spectrophotometrically (according to the Rice-Evans method, 1991). **Results:** The comparative study in vitro showed that QUE causes a decrease in the TBARS level in plasma, whereas HAL increases the plasma TBARS level. After 24 h of incubation of plasma with QUE or HAL (at lower and higher concentrations), the differences in TBARS levels between the drugs were significant ($p = 5.9 \times 10^{-4}$, $p = 2.2 \times 10^{-5}$, respectively).

Conclusion: QUE and OLA, contrary to the prooxidative action of HAL, did not induce oxidative stress; moreover, QUE has antioxidant properties.

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Introduction

Antipsychotics are extensively used in the treatment of schizophrenia and other psychiatric disorders, psychotic mainly. Oxidative stress may contribute to specific aspects of schizophrenic symptomatology and complications of its treatment such as prominent negative symptoms, tardive dyskinesia (TD), and parkinsonian symptoms. The first-generation antipsychotics such as haloperidol (HAL) often cause extrapyramidal side effects: motor disturbances of TD and parkinsonism [1]. TD occurs in 20–40% of the patients, who are treated chronically with antipsychotics [2, 3]. In spite of its high incidence, the pathophysiology of TD remains elusive. Chronic treatment with antipsychotics probably increases free radical production and oxidative stress [4]. A role that increased reactive oxygen species (ROS) and oxidative stress in the etiopathology of antipsychotics induced TD has been proposed [5, 6]. Chronic use of antipsychotics is also reported to cause a decrease in the activity of antioxidant enzymes, superoxide dismutase and catalase [7].

The second-generation antipsychotics (SGAs) may also have some effects on oxidative stress, measured by the level of lipid peroxidation in plasma. The effects of olanzapine (OLA) and quetiapine (QUE) on this process are explained only partially. QUE and OLA belong to the chemical class of benzisoxazole derivatives and have been found to be effective in the treatment of schizophrenic disorders, with therapeutic activity mediated through a combination of D2 and 5HT2 receptor antagonism [8]. Moreover, it has been shown that they have a very low potential for causing extrapyramidal symptoms (EPS) across the full dose range [9, 10]. QUE and OLA may improve cognitive functions (attentional, motor, and visuo-motor skills) as well as executive functions connected to the psychopathology of schizophrenia [10]. It seems that the antioxidant mechanism of QUE and OLA may contribute to the low incidence of EPS and movement disorders caused by these drugs in patients with schizophrenia, and may be effective in the treatment of TD caused by other antipsychotics [10, 11].

The aim of the study was to evaluate the effects of SGAs and establish whether there is a difference among OLA, QUE and HAL action regarding their influence on lipid peroxidation in human plasma, measured by the level of thiobarbituric acid-reactive substances (TBARS).

Materials and Methods

Inclusion Criteria of Healthy Subjects

Blood samples were taken from 30 healthy males aged between 25 and 29 years (average: 28.3, SD = 1.5 years), without psychiatric, neurological or somatic disorders or a history of head injuries, lipid or carbohydrate metabolism disorders, with a normal body mass index, and not being treated with any drugs. Healthy subjects (no smokers) did not use any addictive substances and antioxidant supplementation, and their diet was balanced (meat and vegetables). They lived in similar socioeconomic conditions. Psychiatric examination (using the M.I.N.I. – Mini International Neuropsychiatric Interview [12]), and neurological and somatic examinations were performed.

All subjects signed a consent to the participation in the study, according to the protocol accepted by the Committee for Research on Human Subjects of the Medical University of Lodz (No. RNN/899/2000).

Isolation of Plasma

Human blood (3 × 7.5 ml) was collected into ACD solution (citric acid/citrate/dextrose; 5:1 v/v) between 8.00 and 8.30 a.m. and centrifuged for 20 min at 2,500 rpm and 20°C in a Sigma 3K30 centrifuge to obtain plasma. The drugs obtained from the manufacturers (QUE: Celon Pharma, Poland; OLA: Adamed, Poland; HAL: Polfa-Warsaw, Poland) in the form of active ingredients were dissolved in 0.01% dimethyl sulfoxide (Sigma). Drug

solutions were added to 0.5 ml of plasma (QUE at the final concentrations of 175 and 275 ng/ml; OLA 20 and 40 ng/ml; HAL 4 and 20 ng/ml) and incubated for 1 and 24 h at 37°C. The controls were plasma samples containing 0.01% dimethyl sulfoxide without drug. Metabolites of QUE and OLA were not investigated.

Evaluation of Lipid Peroxidation Level

In control samples and samples of plasma after the incubation with the drug, the concentrations of TBARS were measured spectrophotometrically, according to the Rice-Evans method [13]. The absorbance was measured in a SEMCO spectrophotometer at 535 nm in 1-cm cuvettes. The TBARS expressed in micromoles per liter were calculated based on the absorbance value, using the molar extinction coefficient for TBARS ($\epsilon = 1.56 \times 10^5 \text{ M}^{-1} \times \text{cm}^{-1}$). All estimations were performed twice, including control plasma samples, in which spontaneous lipid peroxidation, without the influence of the drug, was measured.

Statistical Analysis

The results were subjected to statistical analysis (mean values and standard error of the mean). The significance of differences in TBARS levels (drug-treated samples vs. control) was calculated using the paired Student's t test. The two-way ANOVA test was used. Post hoc comparisons for the TBARS levels were carried out with the NIR test. Statistica v. 6.0 by Statsoft, Inc. was used.

Results

The comparative study in vitro has shown that after incubation of plasma with tested antipsychotics, plasma lipid peroxidation (measured as TBARS level) was changed. The analysis of TBARS levels after the incubation of plasma with the drugs (QUE, OLA and HAL) showed significant differences in comparison to the control values. The two-way ANOVA test showed that the differences in TBARS levels significantly depended on the studied drug (drugs at lower concentrations: $p = 5.3 \times 10^{-4}$, and at higher concentrations: $p = 2.2 \times 10^{-5}$), but did not depend on the time of incubation ($p > 0.05$) and interaction ($p > 0.05$).

In the post hoc analysis (NIR test), some significant differences in the TBARS levels between QUE and HAL were found (fig. 1). After 24 h of incubation of plasma with QUE or HAL (at lower and higher concentrations), the differences in TBARS levels between drugs ($p = 5.9 \times 10^{-4}$, $p = 2.2 \times 10^{-5}$, respectively) were significant as well as after 1 h of incubation ($p = 0.04$, $p = 0.02$, respectively). OLA (1-hour incubation), contrary to HAL and QUE, did not cause significant changes in the level of plasma TBARS. After 24-hour incubation of plasma with OLA (at higher concentrations), an increased level of TBARS was observed ($p = 0.01$) (fig. 1).

Fig. 1. The comparison of QUE, OLA and HAL effects on plasma lipid peroxidation (in vitro; expressed as TBARS levels). Data were calculated as differences in comparison with data of controls (without drugs). The asterisk indicates significance (NIR test). Drug concentrations: HAL, 4 and 20 ng/ml; QUE, 175 and 275 ng/ml; OLA, 20 and 40 ng/ml.

Fig. 2. The effects of drugs (incubation 24 h) on lipid peroxidation of human plasma expressed as TBARS level. HAL: 4 and 20 ng/ml; QUE: 175 and 275 ng/ml; OLA: 20 and 40 ng/ml; control samples (without drugs).

QUE after 1 and 24 h of incubation caused a significant decrease (about 8 and 15%, respectively) in plasma lipid peroxidation (expressed as TBARS) in comparison with control ($p < 0.02$) (fig. 2). OLA caused no changes of TBARS levels ($p > 0.05$). Contrary to QUE and OLA, HAL, especially at the higher concentration (20 ng/ml) and after 24 h, caused a significant increase (about 14%, $p = 0.036$) in TBARS in plasma (fig. 2).

Discussion

Oxidative stress is a state when there is an imbalance between ROS generation and antioxidant defenses in favor of the former. There are multiple pathological consequences of increased ROS production. ROS induce oxidative stress and damage to all types of biomolecules including lipids and are involved in various pathophysi-

ological processes and disorders. Oxidative stress and the oxidative changes in different biomolecules may be involved in the pathology of schizophrenia [14]. The abnormalities and peroxidation of membrane phospholipids and polyunsaturated fatty acids caused by oxidative stress play an important role in etiopathogenic mechanisms of schizophrenia [15–21]. The changes of membrane dysfunction caused by lipid peroxidation seem to be secondary to ROS generation and may contribute to specific aspects of schizophrenic symptomatology and complications of its treatment [22, 23]. Our earlier results have shown that the level of TBARS was significantly increased in plasma of patients with schizophrenia [21, 24], whereas the activities of antioxidant defense enzymes were diminished [24]. We have also found oxidative stress, measured as isoprostane level (8-iso-PGF_{2α}), in the urine of schizophrenia patients [24]. The increase in lipid peroxidation products in plasma of first-episode patients with schizophrenia (no antipsychotic drugs) has also been described [20, 25]. Therapy with antipsychotics may affect lipid metabolism. Oxidative injury in schizophrenia can be caused by pathophysiological processes of the disease and probably also by treatment with antipsychotics [26–28]. An increase in lipid peroxidation was observed in platelets [21] and in plasma [24, 28, 29] measured by TBARS level during treatment with antipsychotics. Dietrich-Muszalska [28] demonstrated an increased plasma lipid peroxidation after incubation of human plasma of healthy subjects with HAL (in vitro). Pai et al. [30] and Lohr et al. [2] in the 1990s found that oxidative stress contributed to the toxicity of HAL, which activated a sequence of cellular processes leading to cell death and the production of ROS that was an integral part of that cascade. An experimental study on animals showed an elevated level of lipid peroxidation and peroxidative neuronal injury caused by HAL [31]. The toxicity of HAL leads to changes in mitochondrial membrane potential, generation of ROS, decrease in the concentration of glutathione and increase in intracellular Ca²⁺ both in cortical and hippocampal neurons [31]. Increased lipid peroxidation in cerebrospinal fluid and plasma of TD patients was observed, and antipsychotics-mediated oxidative injury was suggested in the development of TD [2, 5, 29, 32]. The risk of appearance of EPS during the treatment with SGAs is significantly smaller, especially in patients treated with QUE and OLA, and depends on the doses of these drugs [8, 10, 33]. SGAs such as QUE and OLA at lower doses might not significantly influence the increase in oxidative stress, measured by the level of lipid peroxidation. Kropp et al. [34] showed that the concentration of

malondialdehyde after 3 weeks of pharmacotherapy was significantly higher in the group treated with HAL than in that treated with SGAs (clozapine, risperidone, QUE, and amisulpride). Dakhale et al. [35] found a tendency to a decrease in the lipid peroxidation level after treatment with SGAs. Comparative studies showed that SGAs caused significantly less oxidative damage than HAL [35, 36]. Our study indicates that HAL action was significantly different from QUE and OLA, since it produced a significantly higher level of TBARS. The effects of HAL observed in vitro may occur in vivo, i.e. in HAL-treated patients. Schizophrenic patients are usually treated with antipsychotics for a very long time and pharmacodynamic properties during long-term treatment are unknown. The present study showed a significant difference between actions of these drugs (in vitro). QUE at higher doses has a significant antioxidant effect. Similar data were demonstrated by Xu et al. [37]. They showed that QUE protects cultured cells against oxidative stress-related cytotoxicities induced by amyloid-β by blocking hydroxyl radical generation induced by amyloid. QUE by eliminating hydroxyl radical attenuates oxidative stress thus protecting brain cells against oxidative-stress-related damage and improving cognitive function in patients with schizophrenia [37]. The antioxidant mechanism of QUE may contribute to the low incidence of EPS and movement disorders caused by this drug in patients with schizophrenia, and may be effective in treating TD caused by other antipsychotics [38].

So far, we can compare our data to the results of 1 clinical study; only Kropp et al. [34] demonstrated in vivo antioxidant effect of QUE. The results of our study in vitro show that first-generation antipsychotics and SGAs at doses recommended for the acute episode of schizophrenia treatment have different effects on plasma lipid peroxidation. QUE, contrary to HAL, possesses antioxidant properties. The mechanism of pro- and antioxidant action of tested antipsychotics on lipids in plasma is not known and requires further studies.

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