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The genotoxic and oxidative damage potential of olanzapine in vitro

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Abstract

Olanzapine (OLZ) is an atypical antipsychotic drug and is commonly used for the treatment of schizophrenia and bipolar disorder (BD). However, recent reports indicated that this drug could exhibit cytotoxic effects on nervous and immune systems. To our knowledge, there is scarce data considering the genotoxic or oxidative damage potentials of OLZ on human lymphocyte culture system. Therefore, in this study, the genotoxic potential of OLZ (0 to 160 μM) have been evaluated in human whole blood cultures (WBCs) related to oxidative status. Sister-chromatid exchange (SCE) test was applied to estimate the DNA damage, and biochemical parameters (total antioxidant capacity [TAC] and total oxidative stress [TOS]) were examined to determine oxidative stress. Our results indicated that the tested antipsychotic drug did not induce SCEs in lymphocytes of treated cultures. However, the application of the highest OLZ concentration caused oxidative stress. It is concluded that the OLZ can be used safely, but it is necessary to consider the tissue damages that are likely to appear depending on the oxidative stress.

Keywords

Human lymphocytes, in vitro, olanzapine, sister-chromatid exchange, total antioxidant capacity, total oxidative stress

Introduction

Olanzapine (OLZ) is one of the widely used parenteral atypical antipsychotic drugs. It is an antagonist of the D_1 and D_2 dopamine, muscarinic cholinergic, and 5-HT_{2A} serotonin receptor (Heard et al., 2009). As the uses of antipsychotic medications increase, the number of overdoses continues to grow (Tan et al., 2009). And numerous investigations have provided evidence for clinically important metabolic adverse effects of antipsychotics.

The treatments with OLZ produced excessive weight gain, adiposity and secondary metabolic complications, like loss of glucose and insulin homeostasis, both in humans and in mice (Shertzer et al., 2010). OLZ overdose was associated with muscle toxicity (Waring et al., 2006), hepatotoxicity (Ozcanli et al., 2006), cardiotoxicity (Belhani et al., 2006) and fatal acute intoxication (Gerber and Cawthon, 2000; Merrick et al., 2001). The results of several clinical studies also revealed that OLZ induced hematological abnormalities such as leukopenia, granulocytopenia, neutropenia, thrombocytopenia and anemia (Cadario, 2000; Gajwani and Tesar, 2000; Mishra and Mohanty, 2010; Oyesanmi et al., 1999; Sayin and Cosar, 2006; Stergiou et al., 2005; Tu and Yang, 2002). Delieu et al. (2006, 2009) observed abnormal immature neutrophil leukocytes in schizophrenic patients treated with OLZ. But the probable cause of antipsychotic drug-mediated hematological adverse effects and related pathological consequences are still unknown. Thus, it was suggested that the assessment of antipsychotics on different animal models was essential (Mishra and Mohanty, 2010).

The long-term carcinogenesis assays performed on rats and mice exhibited positive (liver and mammary tumours) results (Physicians Desk Reference, 2005). The genotoxicity of OLZ has been investigated with a few genetic endpoints. It has been thought that OLZ

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may not cause mutagenicity, since non-mutagenic properties were obseved in bacterial and animal tests (Brambilla et al., 2007), although the genotoxicity of OLZ on humans both in vivo and in vitro remained uncertain. Besides, the safety of the treatment methods in which OLZ is used have caused much debate. Muench and Hamer (2010) pointed out that OLZ generally tends to cause more problems relating to metabolic syndrome, and it is necessary to take the results of the mutagenicity tests performed on human cells into account in the treatments in which this antipsycothic drug is used. Also, the early identification of biomarkers such as DNA damage and levels of anioxidants for special vulnerability to the effects of chemicals and detection of selected signs of precancerous damage in the body could provide the preventive measures and the saving of lives (Vojdani et al., 1997). Therefore, we aimed to assess the genotoxic (sister-chromatid exchange [SCE] assay) and oxidative effects (TAC and TOS levels) of OLZ on cultured human lymphocyte cells. The SCE assay is an ideal genotoxicity and cytotoxicity assay, since peripheral lymphocytes are easily accessible. Moreover, SCE is much more sensitive as a mutagenic biomarker than chromosome aberration and micronucleus. Some authors have also suggested that reactive oxygen species (ROS) may be implicated in the production of high basal SCE frequencies in chromosome-instability syndromes (Cinkilic et al., 2009; Lee et al., 1990; Therman and Susman, 1993).

Materials and methods

Experimental design

The study was carried out by using blood samples from three healthy, non-smoking male donors, aged 25, 27 and 30 years. Questionnaires were obtained for each blood donor to evaluate exposure history and informed consent forms were signed by each donor. For all the volunteers, hematological and biochemical parameters were analysed and no disease was detected. Approximately, 20 mL of blood was collected, by venipuncture, into syringes containing sodium heparin as anticoagulant. Blood was taken the same day of the initiation of the experiment between 9.00 and 9.30 am to minimize possible confounding effects of dietary factors.

Human peripheral blood lymphocyte cultures wereset up according to a slight modification of the protocol described by Evans and O'Riordan (1975). The heparinized blood (0.5 mL) was cultured in 5 mL of culture medium (Chromosome Medium B, Biochrom®, Leonorenstr. 2-6.D-12247, Berlin) with 5 μg mL^{-1} of phytohemagglutinin (Biochrom®). Olanzapine (Cas No. 132539-06-1; $C_{17}H_20N_4S$) was obtained from Zyprexa, Eli Lilly and Company (Indianapolis, USA; Figure 1). The drug was dissolved in HCl. Concentrated solution (1 mM; pH adjusted to 6.5–7 with $NaHCO_3$) was stored at –80°C and working solutions were prepared daily. The concentration range (0, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 μM) was selected according to the work of Wiklund et al. (2010). The OLZ concentrations were applied to the cultures just before the incubation period for 72 hours (except for total antioxidant capacity [TAC] and total oxidative stress [TOS] analysis). Each individual lymphocyte culture without OLZ was studied as a control group.

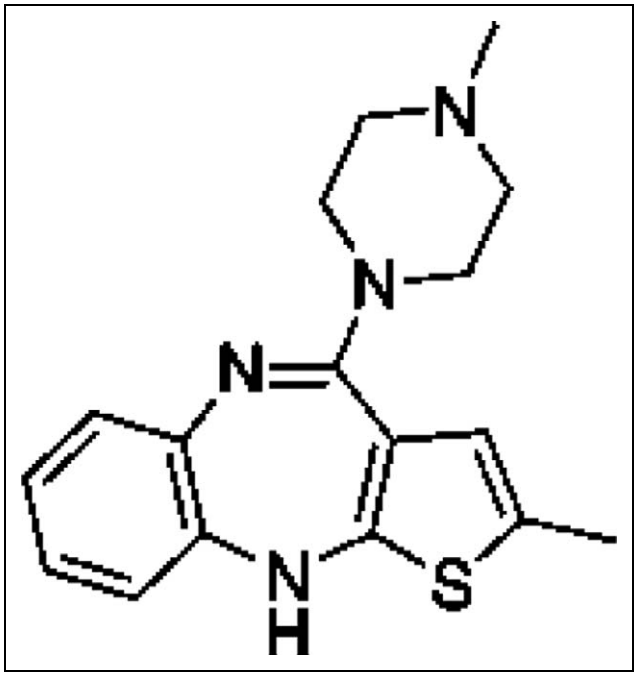

Figure 1. Chemical structure of olanzapine.

SCE assay

With the aim of providing successive visualization of SCEs, 5-bromo-2′-deoxyuridine (Sigma®, final concentration 20 μM) was added after culture initation. The cultures were incubated in complete darkness for 72 hours at 37°C. Exactly 70 hours and 30 min after beginning the incubations, colcemid (Sigma®) was added to the cultures to achieve a final concentration of 0.5 μg L^{-1}. After hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v),

Table 1. The frequencies of SCE values in human lymphocyte treated with olanzapine (OLZ) in cultures for 72 hours

Treatments	No. of samples	SCEs/cell ± S.D.
NC	3	6.48 ± 0.94
OLZ concentrations (as μM)		
PC (MMC)	3	12.45 ± 2.21[a]
1.25	3	6.73 ± 1.27
2.5	3	6.27 ± 1.08
5	3	6.49 ± 0.77
10	3	6.69 ± 0.72
20	3	6.42 ± 0.90
40	3	6.80 ± 0.93
80	3	6.83 ± 1.11
160	3	6.91 ± 1.36

SCE: sister chromatid exchanges; NC: negative control; PC: positive control; MMC: mitomycin C (2 μg/mL).
[a] $p < 0.05$.

centrifugation and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged, and then differentially stained for inspection of the SCE rate according to the fluorescence plus Giemsa (FPG) procedure (Perry and Wolff, 1974). For each treatment condition, 25 well-spreaded second division metaphases were scored, and the values obtained were calculated as SCEs per cell. All slides were coded prior to scoring. SCE scoring was carried out by only one person (B.TOGAR) using a light microscope (Prior) at ×1000 magnification under oil immersion.

TAC and TOS analysis

The automated TAC and TOS assays were carried out by commercially available kits (Rel Assay Diagnostics, Turkey) on plasma samples of OLZ-treated cultures for 1 hour (Erel, 2004, 2005).

Statistics

The statistical analysis of experimental values in the SCE, TAC and TOS analysis was performed by one-way analysis of variance (ANOVA) and Fisher's LSD test using the S.P.S.S. 13.0 software. And the level of 0.05 was regarded as indicative of statistical significance for all tests.

Results

Table 1 and Figure 2 show the dose-response characteristics of the cultures of human peripheral blood lymphocyte for SCE frequency SCE points,

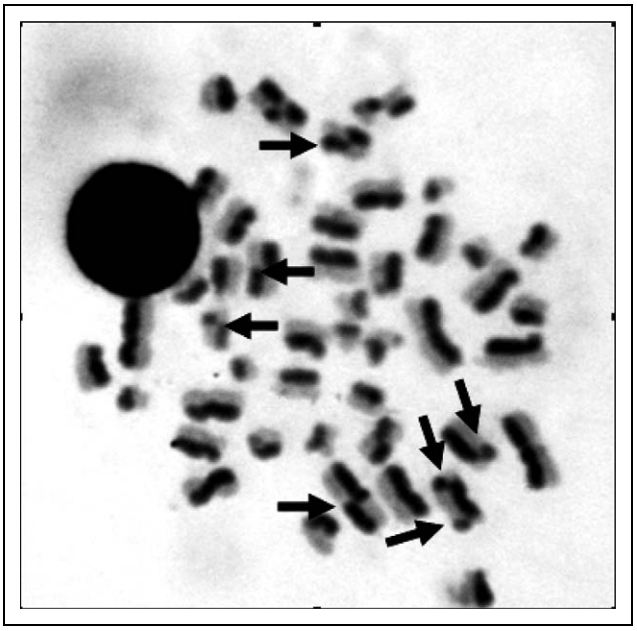

Figure 2. Arrows show sister chromatid exchanges points (a sample metaphase from 80 μM of OLZ-treated culture).

respectively. OLZ was not effective in increasing the SCE frequency. A dose relationship was also not observed. It was concluded that OLZ does not affect the genetic material in human lymphocytes at a wide range of concentrations in the SCE assay.

The effect of OLZ applications on antioxidant capacity and oxidative status in human erythrocytes was determined by TAC and TOS analysis, respectively. As shown in the results in Table 2, the TAC value decreased from 6.08 to 5.27 and 4.91 μmol/L with the addition of 80 μM and 160 μM OLZ, respectively. In addition, The TOS level was significantly elevated in 160 μM OLZ-treated cultures (39.36%) in comparison with the control group.

Discussion

In this study, the tested drug, OLZ, was shown to lack genotoxic activity in cultured human lymphocytes when assessed for induction of DNA damage in the SCE assay. Similar to our finding, there are a few reports on OLZ genotoxicity in non-human models. OLZ was not found mutagenic in the *Salmonella typhimurium* reverse mutation (Ames) test, mouse lymphoma L5178Y gene mutation test, the chromosomal aberration (CA) test in Chinese hamster ovary (CHO) cells and unscheduled DNA synthesis (UDS) test in rat hepatocytes (Physicians Desk Reference, 2005; Brambilla et al., 2007). In addition to these in

Table 2. The levels of TAC and TOS in human whole blood cultures treated with OLZ for 1 hour

Treatments	No. of Samples	TAC (mmol Trolox Equiv./L)	TOS (μmol H_2O_2 Equiv./L)
Control	3	6.08 ± 0.57	11.71 ± 2.76
OLZ concentrations (as μM)			
1.25	3	5.89 ± 0.76	11.02 ± 3.07
2.5	3	6.32 ± 0.87	10.87 ± 3.86
5	3	6.13 ± 0.72	10.44 ± 3.24
10	3	6.22 ± 0.61	12.24 ± 2.69
20	3	5.97 ± 0.83	11.86 ± 3.04
40	3	5.81 ± 0.76	12.38 ± 3.15
80	3	5.27 ± 0.44[a]	12.49 ± 2.76
160	3	4.91 ± 0.89[a]	16.32 ± 3.61[a]

[a] $p < 0.05$.

vitro results, it has been reported that OLZ did not induce SCE formations in bone marrow cells of Chinese hamsters or micronucleus formations in mice in vivo (Physicians Desk Reference, 2005; Brambilla et al., 2007).

It was found that DNA demethylation and SCE formations were connected through a perturbation of the cell machinery at the level of the replication fork, producing an increase of the error-prone ligation. Recent findings obtained using demethylating chemicals exhibited increase of SCE formations in mammalian cells like human lymphocytes and CHO cells (Perticone et al., 1987). In our current investigation, the SCE rates were found similar in OLZ-treated and control cultures. Thus, according to obtained results, we could suggest that lymphocyte DNA did not appear to be demethylated by OLZ. As a matter of fact, it was shown that the antipsychotics CLZ and sulpiride but not haloperidol (another antipsychotics) or OLZ activated brain DNA demethylation at their clinically relevant doses in mice (Dong et al., 2008). On the other hand, Lokshin et al. (1999) stated that CLZ and OLZ exhibited similar pharmacological profiles. Although the mechanism of CLZ toxicity is also still not clear, it is reported that the toxicity (agranulocytocis and hepatotoxicity) of CLZ resulted from the accumulation of reactive metabolites of this drug (Lu et al., 2008). And, the in vitro studies demonstrated that four different primary oxidative metabolites (NdM olanzapine, 7-OH olanzapine, 2-OH olanzapine, and N-O olanzapine) were formed from OLZ by human liver microsomes (Wrighton and Ring, 1999). However, to date, very scarce information is available on genotoxic and oxidative damage potentials or accumulation of these metabolites.

The present study also demonstrated that OLZ caused alterations (at higher doses) in both levels of TAC and TOS of human blood cells in vitro. The previous studies showed that OLZ was oxidized to a reactive nitrenium ion by HOCl, and by an MPO/H2O2/Cl_system. However, the OLZ reactive metabolite had a lower tendency to cause toxicity on human neutrophils and monocytes (Gardner et al., 1998). Patel et al. (2004) observed a significant increase in malonaldehyde (MDA) levels in pancreas of OLZ-treated Sprague Dawley rats. In addition, Aliyazicioglu et al. (2007) assessed the effects of treatment with lithium, alone or in combination with antipsychotic OLZ, on oxidant-antioxidant status in patients with bipolar disorder (BD) and found the antioxidant enzyme activities in lymphocytes were decreased after both types of treatment. Otherwise, the results of the study by Martins et al. (2008) indicated that OLZ did not induce oxidative damage in rat brain. The effects of OLZ on TAS and lipid peroxidation in schizophrenic patients were investigated by Al-chalabi et al. (2009). They have reported that OLZ treatment for 2 months caused significant increases of serum TAS levels and reductions of serum MDA levels in comparison to respective pretreatment values. Similarly, it was opined that OLZ had a significantly lower chance of producing free radical-induced damage during the therapy of schizophrenia (Sing et al., 2008).

In the present study, erythrocytes were also present in the incubation medium and erythrocytes are known to have glutathione peroxidase and glutathione-S-transferase (GST; Geyikoglu and Turkez, 2005; Ozturk and Gumuslu, 2004). On the other hand, glutathione is a major component of RBCs (Ray, 1984) that plays a central role in the antioxidant defenses of

cells (Meister, 1983). Again, GST has previously been thought to protect cells against cancer by detoxifying mutagenic xenobiotics (Fiander and Schneider, 2000). Bakare et al. (2009) examined the effect of OLZ on GST-M1 protein levels and GST enzyme activity in primary cultured rat cerebral cortical cells and found that chronic treatment with OLZ increased both GST-M1 protein levels and GST enzyme activity.

In summary, the results obtained in the current study have shown that the OLZ lacked genotoxic activity when tested in vitro SCE assay, and suggest that exposure to this antipsycothic drug under clinical conditions does not pose a genotoxic risk. But it will also be necessary to take into consideration the cell damages in overdose, which are likely to develop depending on the oxidative stress.

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