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Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: A histopathological study

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ABSTRACT

Chronic exposure to stress contributes to the etiology of mood disorders, and the liver as a target organ of antidepressant and antipsychotic drug metabolism is vulnerable to drug-induced toxicity. We investigated the effects of chronic administration of fluoxetine (15 mg/kg/day) or clozapine (20 mg/kg/day) on liver injury via the measurement of liver enzymes, oxidative stress and histopathology in rats exposed to chronic social isolation (21 days), an animal model of depression, and controls. The activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the liver content of carbonyl groups, malonyldialdehyde (MDA), reduced glutathione (GSH), cytosolic glutathione S-transferase (GST) and nitric oxide (NO) metabolites were determined. We also characterized nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2) and CuZn-superoxide dismutase (CuZnSOD) protein expression as well as histopathological changes. Increased serum ALT activity in chronically-isolated and control animals treated with both drugs was found while increased AST activity was observed only in fluoxetine-treated rats (chronically-isolated and controls). Increased carbonyl content, MDA, GST activity and decreased GSH levels in drug-treated controls/chronically-isolated animals suggest a link between drugs and hepatic oxidative stress. Increased NO levels associated with NF-κB activation and the concomitant increased COX-2 expression together with compromised CuZnSOD expression in clozapine-treated chronically-isolated rats likely reinforce oxidative stress, observed by increased lipid peroxidation and GSH depletion. In contrast, fluoxetine reduced NO levels in chronically-isolated rats. Isolation induced oxidative stress but histological changes were similar to those observed in vehicle-treated controls. Chronic administration of fluoxetine in both chronically-isolated and control animals resulted in more or less normal hepatic architecture, while clozapine in both groups resulted in liver injury. These data suggest that clozapine appears to have a higher potential to induce liver toxicity than fluoxetine.

1. Introduction

Chronic psychosocial stress contributes to the pathogenesis of mood and anxiety disorders (Strekalova et al., 2005), which may be caused by serotonin, norepinephrine or dopamine deficiency in the brain (Feighner, 1999; Trujillo, 1996). Fluoxetine, an antidepressive drug, inhibits the reuptake of serotonin by the serotonin reuptake transporter, thus enhancing and prolonging serotonin signaling, while the atypical antipsychotic clozapine (Meltzer, 1995) has affinity for both serotonergic and dopaminergic receptors (Bymaster et al., 1996). As the liver is the primary site of drug metabolism, the effect of drugs on the integrity of the liver is important. Fluoxetine and clozapine are extensively metabolized in the liver by the isoenzymes of the mixed-function oxidase cytohrome P450 system, primarily CYP1A2 and CYP2D6 isozymes,

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respectively (Dumortier et al., 2002; Inkielewicz-Stepniak, 2011). Fluoxetine undergoes extensive biotransformation to the active metabolite norfluoxetine (Hiemke and Hartter, 2000), which is pharmacologically comparable to fluoxetine (Wong et al., 1995), while clozapine is metabolized to its active metabolite, norclozapine (desmethylclozapine) (Prior et al., 1999). Intraperitoneally-administered drugs, together with their metabolites, rapidly reach peak levels in the liver (Ferno et al., 2009; Parli and Hicks, 1974). And although these treatments generate acceptable outcomes in mood and anxiety disorders, they may result in hepatic toxicity and undesirable side effects.

It has been shown that drugs may cause liver injury via oxidative/nitrosative stress (Bautista and Spitzer, 1990). Souza et al. (1994) reported that fluoxetine (as well as norfluoxetine) effects energy metabolism in rat liver mitochondria and is potentially toxic in high doses. Its administration in mice has been reported to cause changes such as steatosis (fatty change) and hepatocyte enlargement (Bendele et al., 1992). Studies with clozapine have demonstrated oxidative stress and oxidative cell injury via increased levels of membrane lipid peroxidation and total protein oxidation in the brain and other organs (Barakauskas et al., 2010; Polydoro et al., 2004). Furthermore, hepatotoxicity may be mediated by toxic intermediates of drug metabolism (Castiella and Arenas, 1994).

Biochemical changes in the tissue can be determined by biomarkers of oxidative stress. Thus, lipid peroxidation indicates oxidative damage via an increase in malonyldialdehyde (MDA) that contributes to significant toxicity (Devbhuti et al., 2009), while carbonyl group content is an indicator of protein oxidation (Dalle-Donne et al., 2003; Halim et al., 2004). Alterations in reduced glutathione (GSH) and glutathione S-transferase (GST), which participate in the conjugation of toxic electrophiles with GSH, indicate deleterious oxidative changes (Stadtman, 1992). In addition, oxidative stress may affect protein expression and the activity of antioxidant enzymes such as CuZn-superoxide dismutase (CuZnSOD) (Zlatković and Filipović, 2011). One factor that may link oxidative stress and liver injury is the transcription factor nuclear factor-κB (NF-κB). NF-κB can stimulate the expression of a variety of genes, such as cyclooxygenase-2 (COX-2), detected in Kupffer cells that are believed to be important factor in liver injury (Nieto et al., 2000).

However, the roles of fluoxetine and clozapine on liver injury via liver enzymes, oxidative stress and histopathology of rat liver cells have yet to be determined. In the present study, we investigated the effects of chronic (21 days) administration of these drugs on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, liver content of carbonyl group, MDA, GSH, cytosolic GST activity and NO metabolites, as well as cytosolic NF-κB, COX-2 and CuZnSOD protein expression, in addition to histological analysis, following chronic (21 days) isolation (IS), an animal model of depression, and controls. We observed significantly increased oxidative stress in the liver of chronically-isolated rats as compared to vehicle-treated controls. Interestingly, the administration of clozapine and fluoxetine drugs in both chronically-isolated and control animals resulted in a potentially harmful increase in markers of oxidative stress and a compromised antioxidative system. Chronic IS had no significant effect on hepatic histopathological changes, and only the chronic administration of clozapine induced liver injury.

2. Materials and methods

2.1. Drugs

Flunisan tablets (containing 20 mg of fluoxetine-hydrochloride) and Leponex tablets (containing 25 mg of clozapine) were purchased from Hemofarm "Zorka Pharma" Šabac, Serbia and Novartis Pharmaceuticals UK, respectively. Fluoxetine-hydrochloride and clozapine reference standards were obtained from the same company from which the tablets were purchased. To prepare the fluoxetine solution for treatment, the Flunisan tablets were crushed and the content was dissolved in distilled, sterile water with the aid of ultrasound, and filtered through Whatman No. 42 filter paper. Clozapine solution was prepared daily by dissolving 140 mg of Leponex tablets in 0.6 ml of 1 N HCl with gentle heating, then diluting the solution with distilled water to 20 mg/ml. Solutions were neutralized with 1 N NaOH to a pH of 5.1 (Halim et al., 2004). Concentrations of fluoxetin-hydrochloride and clozapine solutions were determined using Ultra Performance Liquid Chromatography (UPLC) analysis (Kovacevic et al., 2006).

2.2. Animals and drug treatments

Adult male Wistar rats, 2.5 months old, weighting 300–350 g at the onset of the experiment served as subjects. Rats were maintained under standard conditions in a temperature-controlled environment (21–23 °C) on a 12 h/12 h light/dark cycle, with food (commercial rat pellets) and water available *ad libitum*. All experimental procedures were carried out in accordance with the Ethical Committee for the Use of Laboratory Animals of the Institute of Nuclear Sciences, "Vinča," which follows the guidelines of the registered "Serbian Society for the Use of Animals in Research and Education." Prior to stress exposure, the animals were housed in groups of four per cage and randomly divided into six groups. Control groups consisted of four animals per cage, while rats underwent chronic social IS stress were housed individually for 21 days, during which animals had normal auditory and olfactory experiences, but were deprived of any visual or tactile contacts with other animals. Fluoxetine-hydrochloride and clozapine were administered daily by intraperitoneal (i.p.) injections of 15 mg/kg and 20 mg/kg, respectively, during the 21 days in both control (Control + Fluox and Control + Cloz groups) and chronically-isolated (IS + Fluox and IS + Cloz groups) rats. The doses of fluoxetine (15 mg/kg/day) and closapine (20 mg/kg/day) were selected on the basis of the previous study in our laboratory, in which both drugs prevented chronic stress-induced depressive- and anxiety-like behaviors in rats (manuscript in preparation). Moreover, used dose of fluoxetine, in the literature data, has shown ability to achieve therapeutic plasma levels within the dose range for the treatment of depression (Czeh et al., 2005), while used dose of clozapine was in accordance with its receptor occupancy (Halim et al., 2004). The serum concentrations of fluoxetine or clozapine were determined by a Liquid Chromatography–Mass Spectrometry (LC–MS) (Djordjevic et al., 2005) and Liquid Chromatography–Tandem Mass Spectrometry (LC–MS–MS) method (Song et al., 2009; Waters, 2008), respectively. For the 15 mg/kg/day of fluoxetine in fluoxetine-treated controls, serum concentrations were 280 ± 50 ng/ml, while in chronically-isolated animals they were in the range of 203 ± 28 ng/ml, similar to those reported in human patients treated with therapeutically effective doses of 20–80 mg/day Prozac (100–700 ng/ml) (Dulawa et al., 2004). There was no significant difference between the Control + Fluox and IS + Fluox groups, that is in agreement with the findings of Czéh et al. (2007). Serum levels for the 20 mg/kg/day of clozapine, was in the range of 103 ± 18 ng/ml in clozapine-treated controls and 123 ± 18 ng/ml in chronically-isolated animals, that were comparable to therapeutic levels (100–700 ng/ml) (Sadock and Sadock, 2008). Vehicle-treated (Control and IS) groups received daily i.p. injections of normal saline (0.9% NaCl).

2.3. Serum hepatospecific markers

Blood samples were collected directly from the heart by cardiac puncture. Serum was obtained by centrifugation at 1500*g* for 10 min at 4 °C. Serum ALT and AST activity were measured by