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Impact of fluoxetine on liver damage in rats

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Abstract:
Fluoxetine (Flux) is a fluorine-containing drug that selectively inhibits serotonin reuptake. It is widely prescribed as a treatment for depression disorders. Hepatic side effects have been reported during Flux therapy. These reports led us to investigate the involvement of oxidative stress mechanisms in liver injury caused by Flux. It has been shown that exposure to fluoride (F^-) induces excessive production of free radicals and affects the antioxidant defense system. Based on this knowledge, we examined the F^- concentration in serum and urine during administration of Flux.
In our study, the effects of one month of Flux treatment on lipid and protein peroxidation, the concentration of uric acid in the liver and the activity of transaminases and transferases in the serum were investigated in rats. Eighteen adult male Wistar rats were divided into three equal groups of six animals each: (I) controls who drank tap water and received 1 ml of tap water intragastrically; (II) animals that received 8 mg Flux/kg bw/day intragastrically; and (III) animals that received 24 mg Flux/kg bw/day intragastrically. Flux treatment increased of the levels of carbonyl groups, thiobarbituric acid reactive species (TBARS) and the uric acid content in the liver. The activities of alanine transaminase (ALT), aspartate transaminase (AST) and glutathione-S transferase (GST) increased in the serum of the treated groups. The Flux levels in the plasma of the treated rats increased significantly in a dose-dependent manner. We observed no changes in the concentration of fluoride in either the serum or the urine of treated rats compared to the control group.
In conclusion, our study indicates that Flux induces liver damage and mediates free radical reactions. Our data also indicate that Flux does not release F^- during metabolism and does not affect physiological levels of F^- in the serum or urine.

Key words:
fluoxetine, oxidative stress parameters, ALAT, AST, GST, fluoride, male rats, liver

Introduction

Substantial pharmaceutical research over the past several years has focused on the use of fluorinated compounds. Fluorine-containing drugs are used as general anesthetics, anti-fungal drugs, antibiotics, anti-anxiety medications, steroids, anti-inflammatory agents, antimalarial drugs, cholesterol-lowering agents, chemotherapeutic agents for cancer treatment, anti-psychotics and antidepressants [43]. The very high electronegativity of fluorine can modify electron distribution in these molecules, affecting their absorption, distribution and metabolism. The incorporation of fluorine into a biologically active compound alters the electronic, lipophilic and steric parameters of the compound and can critically increase biological activity, chemical and metabolic stability, and bioavailability of the compound. Fluorine substitution in a drug molecule can influence not only pharmacokinetic and pharmacodynamic properties, but also the toxicology of the compound [39, 43]. The benefit of fluorinated drugs in human medicine is very well established; however, much less is known about the danger of these compounds to human health. One side effect of prolonged use of fluorinated drugs is liver injury [14, 15, 22, 31, 37].

Fluoxetine is a widely prescribed drug for treatment of neurological disorders, such as depression and anxiety [7, 26]. It is a potent and selective serotonin reuptake inhibitor with antidepressant properties – commonly sold as Prozac or Serafem. Chemically, fluoxetine is (±)-*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine. Fluoxetine can be considered a successful drug for treatment of several diseases, based on its favorable safety/efficacy ratio. However, bleeding, lung damage and cardiotoxicity have been reported during fluoxetine therapy [5–7, 19]. Several authors have reported that fluoxetine induces hepatotoxicity and affects the activity of liver enzymes, although the mechanism responsible for these changes remains unknown [14, 15, 22, 31].

This study was designed to investigate the possible role of oxidative stress on liver injury in male rats treated with fluoxetine. In biological systems, reactive oxygen species cause destructive and irreversible damage to cellular components, such as lipids and proteins [25, 27, 38].

It has been shown that some organic fluorine-containing compounds can metabolize into fluoride ion [39]. In a previous study, we found that F^- induces oxidative damage of tissues [30]. Therefore, the aim of this study was to determine the concentration of fluoride in the serum and urine of rats exposed to fluoxetine.

Materials and Methods

Animals and experimental design

The experiment was conducted in 18 male Wistar Han rats (6-weeks old, weighing ≈180 g) obtained from the Tri-city's Academic Animal Experimental Centre, Poland. The animals were maintained under standard laboratory conditions (temperature 20–22°C in a natural light-dark cycle, humidity 55–60%). All animals were fed a standard laboratory pellet diet prepared by Feeds Production Plant A. Morawski, Poland (ISO PN 9001 and IQ Net Certificate). The study design was approved by the Local Bioethical Commission for Animal Studies in Gdańsk. After 10 days of acclimation, animals were divided into three groups of six rats each:
1. Controls received 1.0 ml tap water intragastrically once a day *via* stomach tube.
2. Exposed animals received 8 mg Flux/kg bw/day (1.0 ml once a day *via* stomach tube).
3. Exposed animals received 24 mg Flux/kg bw/day (1.0 ml once a day *via* stomach tube).

The fluoxetine solution was prepared daily with chloride fluoxetine (99.9% Polpharma, Starogard Gdański, Poland). Water consumption was measured daily, and body weight was assayed once a week. Every week, 4 rats from each group were placed into metabolic cages and 24 h of urine samples were collected. After one month of treatment, the animals were sacrificed and blood and liver samples were collected. Blood samples were obtained by cardiac puncture and the blood was collected in tubes without anticoagulant; serum was separated by centrifugation at 3,000 rpm for 10 min at 4°C. Samples of serum were frozen at –20°C until analysis. Livers were quickly removed, washed in cooled 0.9% NaCl and homogenized in ice-cold buffer (100 mM KH_2PO_4-K_2HPO_4; pH 7.4 containing 1.15% KCl) using a T 10 basic Ultra-Turrax homogenizer, IKA-WERKE. The samples were centrifuged at 3000 rpm for 10 min at 0°C, and the supernatant was removed. The homogenates were stored at –80°C. ALAT, AST, GST activities, protein concentration in the serum and levels of carbonyl groups, TBARS and uric acid in the liver were assayed for all samples.

Analytical procedures

Plasma levels of fluoxetine were determined using liquid–liquid extraction and reversed-phase high-performance liquid chromatography with ultraviolet detection [35 with some modifications].

The concentration of F^- in the urine was determined potentiometrically using a fluoride ion – specific electrode (Orion) and an Ag/AgCl reference electrode after dilution with equal volumes of TISAB buffer [17]. The activities of ALT and AST were determined according to Bergmeyer et al. [9, 10], GST activity was assayed according to Anosike et al. [4] with some modifications, uric acid levels were measured according to Bulgar and Johns [12], the concentration of thiobarbituric acid reactive substances was assayed according to Rice-Evans et al. [40] and the concentration of carbonyl groups was determined according to Levine et al. [34]. Serum protein content was determined by the method of Lowry et al. [36] and, creatinine levels were determined by the method of Folin and Morris [21].

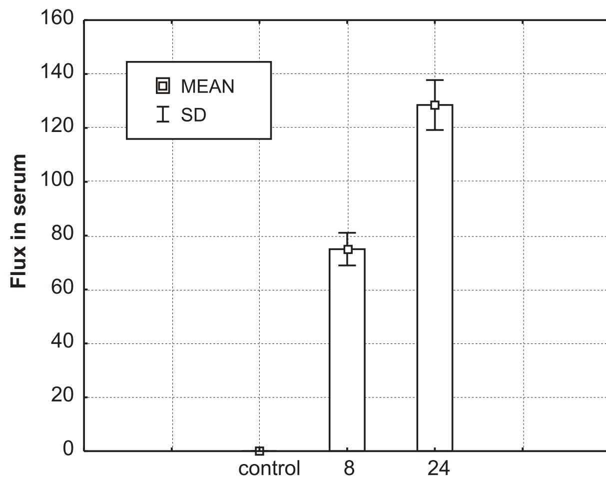

Fig. 1. Concentration of fluoxetine (Flux) in serum (ng/ml)

Tab. 1. Water and feed consumption

Group of animals	Water consumption ml/24 h	Feed consumption g/24 h
Controls	36.9 ± 4.2	28.6 ± 1.4.826
Flux 8 mg/kg bw	31.9 ± 2.3	26.2 ± 2.9
Flux 24 mg/kg bw	35.1 ± 9.1	24.2[b] ± 2.4

[b] Student's *t*-test, significance $p < 0.01$ compared to control. Values are the mean ± SD of 6 rats per group

Tab. 2. Body weight of rats (g)

Group of animals	Exposure time in weeks				
	0	1	2	3	4
Controls	191.5 ± 6.27	218 ± 6.89	239 ± 9.81	269 ± 8.56	294 ± 6.54
Flux 8 mg/kg bw		221 ± 7.61	248 ± 8.56	268 ± 4.69	283 ± 8.89
Flux 24 mg/kg bw		222 ± 4.99	246 ± 7.45	257 ± 8.43	278[a] ± 9.14

[a] Student's *t*-test, significance $p < 0.05$ in comparison to control. Values are the mean ± SD of 6 rats per group

Tab. 3. Fluoride levels in serum (μg/ml) and urine (μg/mg creatinine)

	Control	Flux 8 mg/kg bw	Flux 24 mg/kg bw
Fluoride in serum	0.059 ± 0.009	0.067 ± 0.006	0.064 ± 0.012
Fluoride in urine	2.43 ± 0.032	2.56 ± 0.028	2.61 ± 0.041

Values are the mean ± SD of 6 (serum) and 4 (urine) rats per group

Tab. 4. Effects of fluoxetine (Flux) administration on plasma biochemical profile in rats

	Control	Flux 8 mg/kg bw	Flux 24 mg/kg bw
ALT (U/l)	10.3 ± 2.42	15.3[a] ± 3.78	29.0[c] ± 3.75
AST(U/l)	15.8 ± 4.66	22.8[a] ± 5.87	38.3[c] ± 7.17
GST (U/l)	14.5 ± 4.09	17.7 ± 3.14	23.5[b] ± 3.27
Protein (g/l)	55.8 ± 7.47	60.1 ± 8.57	66.7[a] ± 5.47

[a] Student's *t*-test, significance $p < 0.05$ in comparison to control, [b] Student's *t*-test, significance $p < 0.01$ in comparison to control, [c] Student's *t*-test, significance $p < 0.001$ in comparison to control. Values are the mean ± SD of 6 rats per group

Tab. 5. Oxidative stress parameters in the liver of rats

	Control	Flux 8 mg/kg bw	Flux 24 mg/kg bw
TBARS (nM/g protein)	10.3 ± 2.42	15.3^a ± 3.78	29.0^c ± 3.75
Carbonyl group (μM/g protein)	15.8 ± 4.66	19.8 ± 8.87	38.3^c ± 7.17
Uric acid (μM/g protein)	14.5 ± 4.09	17.7 ± 3.14	23.5^b ± 3.27

Significance: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, in comparison to control (Student's *t*-test). Values are the mean ± SD of 6 rats per group

Statistical analysis

All statistical calculations were carried out with the STATISTICA 9.0 computer program. Results are expressed as the means ± standard deviation (SD). Variance analysis was done with the de Morgan statistics for paired and Snedecor's F-distribution for unpaired data. Paired and unpaired Student's *t*-tests were used to determine statistical significance of differences between groups. The p-values are presented with the data.

Results

The water and feed consumed by the three groups of rats are presented in Table 1. We observed decreased feed consumption in the group treated with 24 mg Flux/kg bw/day.

The body weight of the rats used in the experiment is indicated in Table 2. Treatment with 24 mg Flux/kg bw/day resulted in a significant decrease in body mass.

Data on the serum concentration of fluoxetine are presented in Figure 1. As expected, exposure to fluoxetine resulted in a significant increase in serum levels of fluoxetine in a dose-dependent manner.

Fluoride concentrations measured in serum and urine are shown in Table 3. We did not observe changes in the level of F^- in the serum or urine of treated rats.

The concentration of protein and the activities of AST, ALT and GST are presented in Table 4. We observed statistically significant decreases in the protein levels in the serum of rats exposed to 24 mg Flux/kg bw/day. We also demonstrated that the activities of ALT and AST in the serum of the Flux treated animals were significantly increased compared to the control group. GST activity increased in the serum of rats exposed to the higher concentration of Flux.

The concentrations of uric acid, carbonyl groups and TBARS measured in the experiment are shown in Table 5.

We observed an elevated index of lipid peroxidation in the liver tissues from both groups of fluoxetine-treated rats compared to the controls. Further, the levels of carbonyl groups and uric acid increased significantly in the liver of rats exposed to 24 mg Flux/kg bw/day.

Discussion

This study was designed to investigate the effect of one month of exposure to fluoxetine on oxidative stress markers in the liver of rats. To achieve this goal, we determined the level of TBARS (as an indicator of lipid peroxidation), the concentration of carbonyl groups (as an indicator of protein peroxidation) and the level of uric acid (which protects the cell from oxidative stress) in animals exposed to fluoxetine. Many studies have focused on the adverse effects of fluoxetine exposure on the liver in both patients and animals models [14, 15, 22, 42]. Elevated levels of aminotransferases [7, 10] and acute hepatitis have been observed among patients treated with fluoxetine in clinical trials [22, 31, 37]. In addition, animal studies have shown hepatocellular changes in mice exposed to fluoxetine [8]. However, the literature lacks information about the influence of fluoxetine on markers of free radical damage and the antioxidant defense in the liver. Fluoxetine is extensively metabolized in the liver by cytochrome P450 into norfluoxetine and a number of other metabolites [35, 41]. Souza et al. showed that fluoxetine has multiple effects on

the energy metabolism of rat liver mitochondria and is potentially toxic in high doses [42].

In experimental models used to measure oxidative stress parameters, rats were treated with 20 mg/kg body mass of fluoxetine [45, 46] for more than 14 days. In accordance with the data in the literature, the aim of our study was to determine the toxic effect of fluoxetine on the liver using similar (24 mg/kg bw/day) and lower (6 mg Flux/kg bw/day) doses of the drug.

It has been previously shown that fluoxetine reduces food intake and thus body weight in rats during sub-chronic and chronic treatment regimens, an effect apparently mediated by fluoxetine's impact on the serotonin (5-HT) signaling pathways [16]. Our experiment demonstrates that exposure to 24 mg Flux/kg bw/day for four weeks reduced food intake and body mass. Additionally, Gamaro et al. [24] observed significantly reduced food consumption and dramatic loss of body weight in female rats treated with 8 mg/kg (*ip*) of fluoxetine for 60 days. Dolfing et al. [18] observed body weight reductions in women and men treated with fluoxetine at a dose of 60 mg per day over 1-, 3-, 6- and 12-month periods.

As expected, our study demonstrates that exposure to fluoxetine results in a significant increase in fluoxetine levels in the serum in a dose-dependent manner [7, 41]. Some fluorinated compounds, both aliphatic and aromatic (methoxyflurane, enflurane, sevoflurane, 4-fluoro-L-phenylalanine, 3,5-difluoro-4-hydroxybenzoic acid), are metabolized to fluoride ions [39]. However, we did not observe any changes in the concentration of F^- in serum or urine of the treated rats, likely because fluoxetine contains a very stable carbon-fluorine bond in the trifluoromethyl (CF_3-) group [39]. Fluoxetine has no influence on the level of physiological fluoride in serum or in urine. However, it has been shown that 4-trifluoromethylphenol one of the metabolites of fluoxetine, increases fluoride ion levels in a time and concentration-dependent manner [29, 44].

Total protein concentration in serum is a common measurements performed in clinical laboratory diagnosis. Changes in the amounts of plasma proteins may result from alterations in protein synthesis or catabolism or from protein losses. In addition, hypoproteinemia can be caused by decreased protein synthesis and appears in liver disease [20, 33].

In this study, hepatic dysfunction was confirmed by a significant increase in AST, ALT and GST activities following fluoxetine treatment, which is consistent with previously documented findings [2, 20]. Transaminases (ALT and AST) are enzymes that are often indicative of toxic liver injury. An increase in blood transaminase activity is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes [20]. Plasma enzyme activities increase following damage to very few cell membranes. Increases in ALT activity appear to indicate hepatic diseases and are more specific for hepatic injuries compared to AST levels, due to the cellular location of this enzyme. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm where its concentration is higher than that of AST [20].

The glutathione *S*-transferases (GST) are a family of detoxification enzymes found in the cytosol of most cells. Some studies have indicated the importance of GST not only in detoxification of metabolites but also in regulation of stress [2, 4]. GST activity is a precise index of early stage liver damage in rats. The enzyme has a low molecular weight and can easily pass through liver cell membranes, thus appearing in the blood [2]. Some authors have described the cases of fluoxetine-induced hepatitis [15, 22, 31, 37].

Treatment of rats with fluoxetine for 30 days (which is the time course of therapeutic action of the antidepressant in humans) resulted in a significant increase in TBARS, carbonyl groups and uric acid concentrations in liver tissue.

Uric acid levels are lower in most mammals than in humans because of the presence of uricase, a hepatic enzyme that degrades uric acid into allantoin. Uric acid, a product of purine metabolism in humans, is used as a diagnostic parameter for hypertension, coronary artery disease, cardiovascular disease and diabetes [28, 32]. Afzali et al. [3] observed that higher levels of uric acid are associated with elevated serum ALT activity in hepatic injury. Uric acid is a strong scavenger of oxidative stress molecules or radicals [25]. Despite this, several studies have demonstrated that uric acid can also act as a pro-oxidant by generating free radicals [1, 28, 32]. The increase in uric acid concentration in liver of rats exposed to 24 mg Flux/kg bw/day suggests the presence of liver damage due to free radical generation and impaired antioxidant defense systems.

Carbonyl groups are the final products of protein oxidation [38]. Their levels in tissues and plasma serve as a relatively stable marker of oxidative damage. Reactive carbonyl compounds and residual carbonyl groups of modified proteins react covalently

with matrix tissue proteins and alter their structure and function. Oxidative modification of proteins has been suggested as a marker for oxidative damage and a causal factor in oxidative injury [13, 38]. Accumulation of oxidized proteins and their damaging actions during aging and in pathologies, such as diabetes, atherosclerosis, renal tumors, muscular dystrophy, and bronchopulmonary and neurodegenerative diseases have been well documented [11]. Our data indicate that fluoxetine-enhanced oxidation of proteins alters their structure and function and can result in liver oxidative damage.

In this study, lipid peroxidation, as measured by TBARS, an index of malondialdehyde production, was found to be elevated in the liver tissues of both groups of fluoxetine-treated rats compared to the control group. Furthermore, extensive tissue damage *via* free-radical-mediated lipid peroxidation can result in membrane disorganization and subsequent decreases in membrane fluidity. Measurement of TBARS provides a useful measure of membrane lipid peroxidation and may provide a direct assessment of the progression of liver injury at the cellular level [27].

Thompson et al. [44] have shown that one of fluoxetine's metabolites, 4-trifluoromethylphenol decreases intracellular glutathione concentration in liver slices. However, Zafir and Banu [46] observed no changes in antioxidant defense components and oxidative stress markers in the liver of non-stressed rats exposed to 20 mg Flux/kg for 21 days. They ascertained that treatment with fluoxetine ameliorates only stress-induced oxidative damage. Gałecki et al. [23] reported that the disturbance between pro- and anti-oxidative processes did not improve after three months of fluoxetine treatment in patients with depressive episodes.

In conclusion, increased activities of ALT, AST and, GST suggest severe hepatic injury resulting from the administration of fluoxetine. The increase of carbonyl groups, TBARS and uric acid concentration in liver of treated rats provides evidence for the pathogenic role of fluoxetine in inducing oxidative liver injury.

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