Effect of the Selective Serotonin Reuptake Inhibitor Fluoxetine on Carbon Tetrachloride Induced Hepatic Damage in Rats

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Abstract: The effect of the selective serotonin reuptake inhibitor fluoxetine on the development of liver injury induced in rats with carbon tetrachloride (CCL₄), was investigated and compared to that of silymarin. Hepatotoxicity was induced by CCL₄ orally (0.28 ml. kg⁻¹ followed by 0.14 ml. kg⁻¹ after one week). Fluoxetine at three dose levels (5, 10 or 20 mg kg⁻¹) or silymarin (25 mg kg⁻¹) were administered orally daily for 14 days, starting at time of administration of CCL₄. The effect of fluoxetine was evaluated both on biochemical markers as well as histologically. The daily administration of fluoxetine for 14 days conferred significant protection against the hepatotoxic actions of CCL₄ in rats. It decreased the increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and also prevented the development of hepatic necrosis caused by CCL₄ as determined 14 days after drug administration. The effect of fluoxetine was dose-dependent one. Serum ALT levels decreased by 22.8, 46.1 and 62.5%, AST by 29.5, 49.6, 56.3% and ALP by 14.3, 22.9, 50.3% vs control values, respectively. In comparison, the elevated serum ALT, AST and ALP levels decreased to 38.3, 40.8 and 31.7% of controls, respectively by 25 mg kg⁻¹ of silymarin. Histopathologic examination of the livers of rats treated with CCL₄ + fluoxetine showed less necrosis and fibrosis compared with the CCL₄-control group. The study demonstrates that fluoxetine treatment in the model of CCL₄-induced liver injury results in less liver damage. It is suggested, therefore, that fluoxetine is likely to be safe in the therapy of depressive symptoms in chronic liver disease or complicating regimens employing interferon-alpha in the treatment of chronic hepatitis C infection.

Key words: Fluoxetine, silymarin, carbon tetrachloride, liver injury, rat

Introduction

The newer antidepressants such as the selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors (SSRIs) have become the most frequently prescribed drugs today for the pharmacotherapy of depression. Amongst the SSRIs, fluoxetine (Prozac), is most commonly used as first line therapy (Anderson, 2000). The therapeutic efficacy of the SSRIs antidepressants depends on an enhancement of 5-HT neurotransmission in key brain regions (Blier and de Montigny, 1994). Blockade of the neuronal 5-HT reuptake mechanism (plasma membrane transporter) increases the extracellular concentration of 5-HT, which ultimately leads to the beneficial increase in 5-HT neurotransmission.

The neurotransmitter serotonin is located both in brain and gut. In the gut, serotonin controls tone and motility due to small muscle contraction. In the brain and spinal cord, serotonin acts as an important neurotransmitter involved in a variety of physiologic and behavioral functions ranging from

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control of sleep and wakefulness, feeding, thermoregulation, cardiovascular function, emesis, sexual behavior, spinal regulation of locomotor function, emotional and psychotic behavior and drug-induced hallucinatory state (Gibbonson, 2000).

The issue of serotonin and liver disease is of particular interest from several points of view. First, the SSRIs are widely prescribed agents and their effect on the liver in the healthy and especially in patients with chronic hepatitis is clearly of clinical significance. In rare instances, fluoxetine caused acute hepatitis (Cai et al., 1999) or cholestasis (Johnston and Wheeler, 1997). Experimental evidence indicated that neuromodulators act in the central nervous system to regulate bile secretion, hepatic blood flow and modulate hepatic injury through the different autonomic nervous pathways. Thus, it has been shown that the intracisternal administration of thyrotropin-releasing hormone enhanced hepatic blood flow (Tamori et al., 1998) and protected against CCl4-induced acute liver injury in rats (Sato et al., 2003), while intracisternal injection of corticotropic releasing factor exacerbated hepatic injury induced by CCl4 in rats (Shiro et al., 1999). The concentrations of the serotonin precursor, tryptophan and its metabolite, 5-HIAA were increased up to 4-fold in brains of rats with various degrees of portal-systemic shunting and were significantly correlated with the degree of shunting and with arterial ammonia levels (Lozeva et al., 2004). In addition, a possible link between serotonin and hepatic regeneration was demonstrated, where platelet-derived serotonin was shown to be involved in the initiation of liver regeneration after heptectomy (Lesurtel et al., 2006), while serotonin receptor 2 blockade with Ketanserin arrested liver regeneration after partial heptectomy (Papadimas et al., 2006).

Portal infusion of the SSRI fluvoxamine enhanced net hepatic glucose uptake during a hyperinsulinemic hyperglycemic clamp in dogs (Moore et al., 2004). Clinically, there is data to suggest that modulation of serotonin receptors is involved in the pathogenesis of depression observed in chronic hepatitis patients receiving interferon-alpha (IFN-alpha). In this context, IFN-alpha down regulated glucocorticoid receptor (GR) and serotonin receptor 1A (5-HT3A) levels in cell lines and these recovered after withdrawal of IFN-alpha or addition of desipramine or fluoxetine (Cai et al., 2005). It was also suggested that selective alterations of the serotonin system could be implicated in the pathogenesis of hepatic encephalopathy, a serious neuropsychiatric complication of chronic liver disease (Lozeva et al., 2004). Furthermore, the chronic fatigue syndrome associated with chronic hepatitis C was reduced by the 5-HT3 receptor antagonist ondansetron (Pich et al., 2005).

In the light of the above, the present study was designed to investigate the effect of fluoxetine, a prototype SSRIs on hepatic injury induced by CCl4, using rat as the experimental animal. The effect of the agent was compared to that of silymarin, a standardized extract, derived from the milk thistle plant, containing about 60% polyphenol silibinin and is used as a hepatoprotective agent, because of its antioxidant and membrane stabilizing properties (Flora et al., 1998; Saller et al., 2001). Liver damage was assessed by determining serum enzyme activities as well as by hepatic histopathology.

Materials and Methods

Animals

Sprague-Dawley rats of both sex, weighing 120-130 g were used throughout the experiments and fed with standard laboratory chow and water ad libitum.

CCl4-induced Hepatic Injury

Hepatic injury was induced by treating rats by gavage with CCl4-olive oil (1:1, 0.28 mL/100 g body weight) followed by 0.14 mL/100 g body weight one week later. Starting on the time of the first dose of CCl4 administration, rats (n = 6/group) also received either silymarin (25 mg kg⁻¹), fluoxetine (at three dose levels of 5, 10 and 20 mg kg⁻¹). Control rats were treated with olive oil (0.28 mL/100 g body weight followed by 0.14 mL/100 g body weight one week later) (n = 6). Two more groups (n = 6 each) received either saline or the higher dose of fluoxetine (20 mg kg⁻¹) but no
CCL4. Drugs were administered once daily by the oral route. The animals were killed on day 15 after the first dose of CCL4 or olive oil administration. Rats had free access to food and drinking water during the study.

**Biochemical Assessment**

At the end of the experiments, blood samples were obtained from the retro-orbital vein plexus, under ether anaesthesia. ALT and AST activities in serum were measured according to Reitman-Frankel colorimetric transaminase procedure (Crowley, 1967), whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg (1971), using commercially available kits (BioMérieux, France).

**Histological Studies**

At the end of the treatment period, rats were killed, livers were excised and fixed in 10% formalin saline. Sections were prepared and stained with hematoxylin and eosin stain (H and E) for histological examination.

**Statistical Analysis**

All results are expressed as means±SE. Comparison of the values before and after CCL4 was made by paired Student's t-test. Multiple group comparisons were performed by ANOVA followed by Duncan test. p<0.05 was considered statistically significant.

**Drugs and Chemicals**

Carbon tetrachloride (BDH Chemicals, England), fluoxetine (Amoun Pharmaceutical Co., Cairo, A.R.E.) and silymarin (Sedico Pharmaceutical Co., Cairo, A.R.E.) were used.

**Results**

**Biochemical Changes**

In rats treated with CCL4-olive oil the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in plasma were markedly raised by 212.2, 177.4 and 158.3% indicating the severity of hepatic injury, congestion and cholestasis caused by CCL4 (Table 1). Fluoxetine given to saline-treated (normal rats) had no significant effects on plasma levels of ALT, AST or ALP enzymes. On the other hand, fluoxetine given to CCL4-treated rats resulted in a marked and significant decrease of elevated ALT, AST and ALP levels. The effect of the drug was dose-dependent. Fluoxetine at 5, 10 or 20 mg kg⁻¹ significantly decreased the raised plasma ALT by 22.8, 46.1, 62.5%, AST by 29.5, 49.6, 56.3%, ALP by 14.3, 22.9, 50.3%, respectively compared with the CCL4 control group. In comparison, the elevated serum ALT, AST and ALP levels decreased to 38.3, 40.8 and 31.7% of controls, respectively by 25 mg kg⁻¹ of silymarin (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L⁻¹)</th>
<th>AST (U/L⁻¹)</th>
<th>ALP (U/L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>67.5±1.9</td>
<td>77.9±2.2</td>
<td>52.7±1.4</td>
</tr>
<tr>
<td>+ Fluoxetine 20 mg kg⁻¹</td>
<td>67.4±3.0</td>
<td>75.1±1.7</td>
<td>55.6±1.1</td>
</tr>
<tr>
<td>CCL4 control</td>
<td>210.8±7.9</td>
<td>216.1±4.3</td>
<td>136.1±2.6</td>
</tr>
<tr>
<td>+ Fluoxetine 5 mg kg⁻¹</td>
<td>162.7±3.2</td>
<td>152.4±4.5</td>
<td>116.6±3.3</td>
</tr>
<tr>
<td>+ Fluoxetine 10 mg kg⁻¹</td>
<td>113.6±2.8</td>
<td>109.0±2.3</td>
<td>105.0±3.0</td>
</tr>
<tr>
<td>+ Fluoxetine 20 mg kg⁻¹</td>
<td>79.0±1.8</td>
<td>94.3±3.8</td>
<td>67.7±5.6</td>
</tr>
<tr>
<td>+ Silymarin 25 mg kg⁻¹</td>
<td>130.0±5.6</td>
<td>126.0±6.7</td>
<td>53.0±5.7</td>
</tr>
</tbody>
</table>

Results are means±SE. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan’s multiple range test. *p<0.05 compared with the CCL4 control group. †p<0.05 compared with fluoxetine 5 mg kg⁻¹-treated group. Rats treated with fluoxetine 20 mg kg⁻¹ or silymarin showed significantly less ALP values compared with the fluoxetine 10 mg kg⁻¹-treated group. Rats treated with fluoxetine 10 or 20 mg kg⁻¹ showed significantly lower plasma ALT and AST level than those given silymarin. Rats treated with fluoxetine 20 mg kg⁻¹ showed significantly lower plasma ALT and AST values compared with the group treated with fluoxetine 10 mg kg⁻¹.
Fig. 1: A photomicrograph of a section of liver tissue of a control rat showing the central vein and the hepatocytes arranged in cords radiating from the central vein in an anastomosing manner to form a spongework or labyrinth. These cords are separated from each other by blood sinusoids, which are nearly equal in size (Hx. and E. X 60)

Fig. 2: A magnified photomicrograph of a liver section of a control rat showing the normal appearance of hepatocytes arranged in a series of branching and anastomosing perforated plates. The hepatocytes appear polyhedral in shape with clearly defined cell membranes. Their acidophilic cytoplasm takes a lace-like or granular appearance with clumps of basophilic material. The nuclei are large rounded or ovoid in shape. They are of the vesicular type with well-defined one or two nucleoli (Hx. and E. X 150)

Histopathological Changes

The liver of saline control rats revealed the characteristic hepatic architecture (Fig. 1 and 2). The liver of rats subjected to CCl₄ showed centrilobular necrosis, ballooning and fatty degeneration of hepatocytes around the central vein. Severe dilatation of central and portal veins were observed (Fig. 3 and 4). Fluoxetine administered to CCl₄-treated rats, resulted in a dose-dependent protective effect. Partial protection was evident histologically on the administration of low dose of fluoxetine (5 mg kg⁻¹). Severe dilatation and congestion of blood vessels and small patchy areas of necrosis were still present (Fig. 5 and 6). Examination of liver sections from rats treated with CCl₄ and fluoxetine at 10 mg kg⁻¹ showed normal sized central veins and small necrotic patches (Fig. 7 and 8). Fluoxetine administered at 20 mg kg⁻¹ resulted in more or less normal hepatic architecture (Fig. 9 and 10). On the
Fig 3  A photomicrograph of a section of liver tissue of a rat given carbon tetrachloride showing severe dilatation of central venous, dilatation and congestion of portal veins in the portal areas with dilatation of the other contents of this area (hepatic artery and bile duct). The normal architecture of the liver tissue is not yet preserved (Hx and E X 60).

Fig 4  A magnified photomicrograph of the previous section showing a portal area containing dilated vessels. The portal vein in this area is severely dilated and congested with blood. From this area a fibro-vascular membrane extends containing newly formed blood vessels. The hepatic cells show severe damage and degeneration (Hx and E X 150).

Fig 5  A photomicrograph of a section of liver tissue of a rat given carbon tetrachloride and fluoxetine at 5 mg kg⁻¹, showing severe dilatation and congestion of a blood vessel, small patchy areas of necrosis and widening in some blood sinusoids. Some of the hepatocytes plates are twisted in an abnormal direction (Hx and E X 60).
Fig. 6. A magnified photomicrograph of the previous section showing signs of edema in between the hepatocytes with patches of necrosis. A part of the dilated blood vessel is seen with the congested blood (red blood cells) in its lumen. The hepatocytes restore their normal appearance (H&E X 150).

Fig. 7. A photomicrograph of a section of liver tissue of a rat given carbon tetrachloride and fluoxetine at 10 mg/kg, showing central veins of normal size, while blood vessels in portal tracts are slightly dilated and congested. Small areas of necrosis are still present. Blood sinusoids specially those near the central veins are slightly dilated (H&E X 60).

Fig. 8. A magnified photomicrograph of the previous section showing a central vein of normal size and with intact endothelium. Radiating from this vein are plates of hepatocytes, most of them have the normal appearance of cytoplasm and nucleus. Only few cells specially those near the central vein suffer from fatty degeneration (H&E X 150).
Fig. 9 A photomicrograph of a section of liver tissue of a rat given carbon tetrachloride and fluoroacetate at 20 mg/kg, showing central veins of normal size and portal areas with dilated and congested vessels. No lymphocytic infiltration is detected, while very thin bands of fibrous tissue is seen extending from the portal area (H&E, ×60).

Fig. 10 A magnified photomicrograph of the previous section showing plates of normal hepatocytes radiating from a central vein in plates separated by blood sinuses. Slight widening is remarkable especially in those blood sinuses close to the central vein (H&E, ×150).

Fig. 11 A photomicrograph of a section of liver tissue of a rat given fluoroacetate at 20 mg/kg + saline (No CCl₄), showing completely normal liver tissue except for slightly dilated vessels in a portal tract (H&E, ×60)
other hand, no pathological changes could be noticed in liver sections from rats treated with the higher dose of fluoxetine alone (No CCl₄) (Fig. 10 and 11). Figure 12 shows photomicrograph of normal liver tissue.

Discussion

The present study provides evidence that in the CCl₄ model of hepatic toxicity, systemic administration of fluoxetine, a prototype SSRI, used in pharmacotherapy of depressive disorders, exerts hepatic protective effects. The repeated daily administration of the drug for 14 days to CCl₄-treated rats was associated with marked and significant decrease in leakage of hepatocellular enzymes (ALT, AST) and alkaline phosphatase into plasma and the histological degree of hepatocyte necrosis was attenuated. The effect of fluoxetine was dose related in doses ranging from 5 to 20 mg kg⁻¹.

The SSRIs have their primary mechanism the reuptake blockade of serotonin at synaptic terminals, resulting in an elevation of extracellular 5-HT concentrations in limbic regions of the brain that can act on various critical postsynaptic 5-HT receptors (Fuller, 1994; Goodnick and Goldstein, 1998). At doses of 10 mg kg⁻¹, the acute administration of fluoxetine or sertraline by i.p or s.c. routes, has been shown to induce an increase in extracellular 5-HT in terminal projection areas such as cortex, striatum, or diencephalon, which ultimately leads to the beneficial increase in 5-HT neurotransmission (Fuller, 1994; Pit耶yro and Blier, 1999). Chronic administration of SSRIs and long-term serotonin reuptake blockade by these drugs induces a desensitization of presynaptic somatodendritic 5-HT₁A autoreceptors of 5-HT neurons (that reduce both the rate of discharge of 5-HT neurons and the synthesis of 5-HT), thereby allowing their firing rate to recover in the presence of these drugs (Bel and Artigas, 1993; Pit耶yro and Blier, 1996). This allows for 5-HT neurotransmission to increase in the forebrain territories of 5-HT innervation, in which 5-HT₁A heteroreceptors are not subject to desensitization and may thus contribute to the mediation of the therapeutic effects (Blier and de Montigny, 1994).

Alterations in brain neuroplastic can affect hepatobiliary function. In this context, it was demonstrated that the intracisternal administration of thyrotropin-releasing hormone stimulates hepatic DNA synthesis (Yone da et al., 1997), enhances hepatic blood flow (Tanimori et al., 1998) and protects against CCl₄-induced acute liver injury through the vagal-cholinergic pathways in rats (Sato et al., 2003). In contrast, intracisternal injection of CRF exacerbated the development of CCl₄-induced acute
liver injury. Such an effect was mediated through the sympathetic-noradrenergic nervous systems. In this way, physiological stressors and enhancement of the sympathetic nervous activity exacerbate experimental liver injury (Iwai and Simazu, 1988; Fukudo et al., 1989; Hsu, 1992). This may occur partly through increasing CRF mRNA expression and CRF immunoreactivity in the hypothalamus and amygdala, which are important sites for the sympathetic nervous system (Kalin et al., 1994; Haas et al., 1998). Stimulation of the hypothalamus and activation of sympathetic nerves has been shown to aggravate experimental liver injury (Hsu, 1992, 1995). Lesions of the lateral or the ventromedial hypothalamus facilitate hepatic regeneration after partial heptectomy. Furthermore, the hypothalamus mediates hepatic apoptosis through the autonomic nervous system (Kiba, 2002). There are also data to suggest that alterations of serotonergic neurotransmission in brain areas could have important implications on hepatic function. Studies implicated the serotonin system in the pathogenesis of hepatic encephalopathy, a serious neuropsychiatric complication of acute and chronic liver disease (Herneth et al., 1998; Michalak et al., 2001; Holt et al., 2002; Lozeva et al., 2004). Studies revealed perturbations in the central serotonergic neurotransmission where both a decreased and an increase in 5-HT1A receptor binding in different serotonergic projection areas of the brain in portal-caval shunted rats (Apelqvist et al., 1998). A significant loss of serotonin transporter binding sites was observed in brain areas of rats with severe encephalopathy resulting from acute liver failure caused by hepatic devascularization (Michalak et al., 2001). High concentrations of quinolinic acid and other tryptophan metabolites in the CNS were found in dogs with portosystemic shunts and hepatic encephalopathy (Holt et al., 2002). Serotonergic turnover is exquisitely and selectively sensitive to the degree of porto-systemic shunting and hyperammonemia in rats (Lozeva et al., 2004). There is also evidence that altered central serotonergic neurotransmission contributes to fatigue complicating chronic hepatitis C and was reduced by the 5-HT3 receptor antagonist ondansetron (Jones, 2001; Piche et al., 2005).

Serotonin (5-hydroxytryptamine, 5-HT) is also a neuroendocrine component of the gastrointestinal tract. Approximately 95% of serotonin is located in the enterochromaffin cells of the gastrointestinal tract compared to 2% in platelets and 2% in the CNS (Glatzel et al., 2002; Gershon, 2003). Serotonin acts as a paracrine factor influencing hepatocyte proliferation. Serotonin induced DNA synthesis in primary cultures of rat hepatocytes, which is likely to be mediated through the serotonin S2 receptors of hepatocytes (Balasubramanian and Paulose, 1998). In mice, thrombocytopenia, or impaired platelet activity or lack of tryptophan hydroxylase 1, the rate-limiting enzyme for the synthesis of peripheral serotonin resulted in the failure to initiate cellular proliferation in the liver after heptectomy (Lesur et al., 2006). Cholangiocytes express the serotonin 1A and 1B receptors. Endogenous 5-HT released from platelet may contribute to liver tissue hypoperfusion, following hepatic ischemia-reperfusion (Murata et al., 2003).

In several studies, fluoxetine has been shown to exert potent anti-inflammatory effects (Bianchi et al., 1994, 1995; Abdel-Salam et al., 2003, 2004). The mechanism by which fluoxetine alleviates inflammation is not clear, though inhibition of substance P or involvement of the pituitary adrenal axis have been suggested (Bianchi et al., 1994, 1995). Fluoxetine increased the production of nitric oxide in BV2 murine microglial cells (Hu et al., 2006). In several studies, fluoxetine displayed immunomodulatory effects, suppressing the production of IFN-gamma and TNF-alpha and decreasing the interferon-gamma (IFN-gamma)/interleukin-10 (IL-10) production ratio, which is of critical importance for the determination of the capacity of immunocytes to inhibit or activate monocyte/lymphocytic functions (Kubera et al., 2001; Mues et al., 2005). Fluoxetine decreased the serum level of interleukin-beta 1 in hemodialysis patients (Lee et al., 2004). Fluoxetine also inhibited the corticosterone-induced gene transcription and the LPS-stimulated interleukin-6 (IL-6) production in mouse fibroblast cells (Budziszewska et al., 2005). These anti-inflammatory or immunomodulatory properties of fluoxetine, may be involved at least partly for the hepatic protection observed in the present study.
In summary, the present study demonstrates that in a model of CCl₄-induced hepatic damage, fluoxetine administered daily afforded significant hepatic protection. The exact mechanism of action by which fluoxetine lessens hepatocellular injury in the present study is not clear. It is suggested that enhancement of central serotonergic neurotransmission, release of peripheral serotonin or inhibition of the inflammatory response by fluoxetine might underlie the hepatic protective effects of the drug.

References


