Antinecroinflammatory Effects of Atorvastatin Against Carbon Tetra Chloride-induced Hepatotoxicity in Rats

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Abstract

Atorvastatin is widely used in the treatment of hepatic disease. The aim of present study is to determine the necroinflammatory activity of atorvastatin against CCl₄-induced hepatotoxicity in rats. Thirty adult Wistar male albino rats were collected and these rats divided randomly into 5 groups. Group I was as Control group and received intraperitoneal injection of saline (1mg.kg⁻¹), Group II received CCl (1 mg/kg, s.c), Group III received Atorvastatin (5 mg/kg, p.o) +CCl (1 mg/kg, i.p), Group IV received Atorvastatin (10 mg/kg, p.o) +CCl (1 mg/kg, i.p) and Group V received Atorvastatin (15 mg/kg, p.o) +CCl (1 mg/kg, i.p) for 28 consecutive days. At the 28 day blood samples from all rats of every group were collected and levels of SGPT, SGOT, ALP and Bilirubin by standard kits were assayed. The liver tissues were taken for histopathological examination. From histopathological study in group I no abnormal changes were observed and in group II, III and IV different abnormal changes with different degrees consist of fatty change, lymphocytic infiltration, necrosis, congestion and hemorrhage were distinguished. In end for Group V no fatty change were observed and was similar to normal hepatocyte. The animals treated with CCl₄ exhibited a significant (P<0.001) rise in SGOT, SGPT, ALP and Bilirubin levels when compared to the control group. The results of this study clearly demonstrated that the atorvastatin exhibited potent hepatoprotective activity against CCl₄-induced hepatic damage in rats. This may be due to their antioxidant and free radical scavenging properties. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the CCl₄-induced hepatocellular damage because these are cytoplasmic in location and are released into the circulation after cellular damage.

Key words: Atorvastatin, hepatotoxicity, necroinflammatory, CCl₄.

Introduction

Statins are inhibitors of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, in cholesterol biosynthesis. As such, they have been widely used in clinical practice as cholesterol lowering agents to reduce morbidity and mortality from coronary artery disease. There is evidence from clinical studies and in vitro experiments that statins have additional anti-inflammatory properties in atherosclerotic disease, which are unrelated to their lipid lowering activity. Clinical studies have previously suggested that statins might show a beneficial clinical effect in...
inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis. Furthermore, preliminary data obtained in models of pulmonary inflammation suggest that the effects manifest in rheumatoid patients can be achieved also in asthma [1].

Statins modify inflammatory pathways and lipid metabolism in vitro and in animal models of human disease. The liver performs many functions vital to the health of the organism. The liver transforms and excretes many drugs and toxins. These substances are frequently converted to inactive forms by reactions that occur in the hepatocytes. Historically, plants have been used in the folk medicine to treat various diseases. Experimental work on several plants has been carried out to evaluate their efficacy against chemically induced liver toxicity. Carbon tetrachloride (CCl₄) was the first toxin for which it was shown that the injury it produces is largely or entirely mediated by a free-radical mechanism. Its main toxic effects are shown on the liver. Toxic levels administered to animals produce fatty accumulation in the liver due to a blockage in the synthesis of the lipoproteins that carry triglycerides away from this organ. It is believed that CCl₄ is metabolized by the P₄₅₀ system to give the trichloromethyl radical, a carbon-centred radical. Several P₄₅₀ are involved including CYP2E1, the 'ethanol-inducible' cytochrome P₄₅₀. Hence, CCl₄ - induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver[2]. Recent reports suggest that atorvastatin may have beneficial effects in patients with nonalcoholic steatohepatitis associated with the metabolic syndrome, suggesting a potential usefulness for this drug in the treatment of chronic liver diseases [3]. In addition, lovastatin and simvastatin inhibit cell growth of cultured HSC4[4]. The combinatory use of pitavastatin and candesartan, an ANG II receptor, type 1 (AT1) blocker, inhibits liver fibrogenesis in carbon tetrachloride (CCl₄)-treated rats (28). Nevertheless, simvastatin, used without an ANG II type 1-receptor blocker, does not seem to affect liver fibrogenesis in vivo [3]. Hepatotoxicity is thought to involve two phases [5]. First, CCl₄ is metabolized by cytochrome P₄₅₀ in hepatocytes, giving rise to highly reactive trichloromethyl radicals. Second, inflammatory responses caused by CCl₄ play an important role. In the latter process, some hepatic cells, including Kupffer cells (KCs), hepatic stellate cells (HSCs) and sinusoidal endothelial cells (SECs) are activated to secrete cytokines which mediate the liver fibrogenesis in vivo [6]. The aim of present study was to investigate the necroinflammatory activity of atorvastatin against CCl₄ -induced hepatotoxicity in rats.

Materials and methods

Animals:

30 Adult Wistar male albino rats weighing between 150 and 200 g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water ad libitum. The animals were housed in polypropylene cages. Ethical committee in accordance with animal experimentation and care has approved all animal procedures [2].

Drugs and Chemicals:

Atorvastatin (Pfizer, Madrid, spain) Carbon tetrachloride - SD Fine Chemicals, Mumbai All drugs and chemicals were purchased commercially and were of analytical grade.

Experimental Design:

Induction of experimental hepatotoxicity:

Hepatotoxicity was induced by injecting CCl₄ subcutaneously at a dose of 2 ml/kg body weight for 28 consecutive days [2,5].

Evaluation of hepatoprotective activity:

Animals were divided randomly into five groups, consisting of six animals each. The rat dose was calculated on the basis of the surface area ratio [6].

GroupI Control (Normal saline 10 ml/kg, p.o) (n=6) Group II CCl₄ (1 ml/kg, s.c) (n=6) GroupIII Atorvastatin (5 mg/kg, p.o) +CCl₄ (1 ml/kg, i.p) (n=6) GroupIV Atorvastatin (10 ml/kg, p.o) +CCl₄ (1 ml/kg, i.p) (n=6) Group V Atorvastatin (15 ml/kg, p.o) +CCl₄ (1 ml/kg, i.p) (n=6) All the groups were treated for 28 consecutive days [2,5]. At the end of this period, animals were kept overnight fasting and were sacrificed. Blood samples were withdrawn, serum separated and estimated for biochemical parameters. Liver tissues were removed for the determination of histopathological examinations.
Measurement of Biochemical Parameters:

Blood samples were collected from retro-orbital plexus under ether anesthesia and the serum was used for the assay of marker enzymes namely SGPT, SGOT, ALP and bilirubin. The enzyme levels were assayed using standard kits obtained from Ranbaxy Diagnostics Ltd., New Delhi, India [21].

Histopathological Examination:

A portion of liver tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Hemotoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of ×40.

Statistical Analysis:

Values were represented as mean±SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test using statistical package for social sciences (SPSS) version 10. P<0.05 was considered significant [1,19].

Results and discussion

In histopathological studies, no abnormalities observed in normal control rats. Rats receiving CCL₄ for 28 consecutive days developed a high degree of steatosis with severe cytoplasmic vacuolation and wide spread hepatocellular necrosis with nuclear pyknosis and karyolysis. Accumulation of Kupffer's cells with active phagocytosis was prominent in the central portions of the hepatic lobules. In portal areas, there was severe lymphocytic infiltration with periportal hepatocellular degeneration and necrosis. In sections of CCL₄ + atorvastatin (5 mg/kg) treated livers there was pronounced fatty changes in the centers of the hepatic lobules and lymphocellular infiltrations in the periportal fields. In histopathological studies of CCL₄ + atorvastatin (10 mg/kg) treated livers, there were variable amounts of fatty change of hepatocytes and lymphocytic in central portions of the lobules and, to a lesser extent mononuclear infiltrations in the portal areas. Microscopically, there was no significant hepatocellular damage except small areas of focal degeneration in the livers of CCL₄ + atorvastatin (15 mg/kg) treated rats. The lobular architecture of these livers was near to normal. The changes of every group in following figures have been shown.

Fig. 1: A, Representative section from Liver of normal control rat showing normal hepatic cells with nuclei and cytoplasm (H&E, ×400). B, Photomicrograph of the liver of carbon tetrachloride treated rats showing fatty infiltration with wide spread hepatocellular lytic necrosis and hemorrhage (H&E, ×400). C, Photomicrograph of the liver of carbon tetrachloride treated rats showing lymphatic infiltrations in portal areas (H&E, ×400). D, Photomicrograph of the liver of carbon tetrachloride + atorvastatin (5mg/kg) treated rat showing broad areas of fatty change and necrosis in central portion of the hepatic lobule and kupper's cell accumulation (H&E, ×400).
Fig. 2: Microscopic appearance of the liver of tetrachloride + atorvastatin (5 mg/kg) treated rats showing congestion and inflamed portal tracts (H&E, ×400). B, Microscopic appearance of the liver of carbon tetrachloride + atorvastatin (10 mg/kg) treated rat showing congestion in hepatocytes from central portion of the lobule (H&E, ×400). C, Microscopic appearance of the liver of carbon tetrachloride + atorvastatin (15 mg/kg) treated rat showing no fatty changes, except random foci of hydropic degeneration (H&E, ×400). D, Histologic parameters of liver in periportal field from carbon tetrachloride + atorvastatin (15 mg/kg) treated rat is near normal and no prominent vacuolation is seen in this area (H&E, ×400).

Biochemical Parameters:

The animals treated with CCl₄ exhibited a significant ($P<0.001$) rise in SGOT, SGPT, ALP and bilirubin levels when compared to the control group (Table 1).

Discussion:

Administration of atorvastatin resulted in prevention of hepatic injury in a dose dependent manner. Especially Administration at the dose of 15 mg/kg almost normalized the histological architecture of the liver resembling, showing its potent hepatoprotective effects. The present study investigates the effects of a statin (atorvastatin) on the necroinflammatory actions in the liver. In the assessment of liver damage by CCl₄, the determination of enzyme levels was largely used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the CCl₄-induced hepatocellular damage [2,7,8,9,10]. The reduction of CCl₄-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver [2,9,10]. The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad (Figure 1). The liver section of CCl₄-treated rats showed fatty infiltration, widespread hepatocellular lytic necrosis and hemorrhage with lymphocyte infiltrations in portal areas (Figure 2-3). This could be due to the atorvastatin of highly reactive free radicals because of oxidative stress caused by CCl₄. Simultaneous administration of atorvastatin along with CCl₄ prevented these effects (Figure 4-8). Thus, histopathological studies revealed that concurrent administration of CCl₄ with the atorvastatin exhibited protection of liver cells, which further confirmed the above results. The results of this study clearly demonstrated that the atorvastatin exhibited potent hepatoprotective activity against CCl₄-induced hepatic damage in rats. This may be due to their antioxidant and free radical scavenging properties. Statins reduced the expression of proinflammatory...
Table 1: Effect of atorvastatin on serum biochemical parameters against CCl4-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.492 ± 0.5820</td>
<td>27.750 ± 0.5064</td>
<td>16.597 ± 0.4301</td>
<td>0.4433 ± 0.01022</td>
</tr>
<tr>
<td>CCl4</td>
<td>95.465 ± 0.462</td>
<td>78.412 ± 0.6377</td>
<td>51.055 ± 0.4419</td>
<td>2.222 ± 0.1085</td>
</tr>
<tr>
<td>Atorvastatin (5mg/kg, p.o) + CCl4</td>
<td>97.983 ± 0.8165</td>
<td>76.568 ± 0.6377</td>
<td>51.177 ± 0.3114</td>
<td>30.957 ± 0.3507</td>
</tr>
<tr>
<td>Atorvastatin (10mg/kg, p.o) + CCl4</td>
<td>65.985 ± 0.3611</td>
<td>51.117 ± 0.3114</td>
<td>30.957 ± 0.3507</td>
<td>0.9133 ± 0.02848</td>
</tr>
<tr>
<td>Atorvastatin (15mg/kg, p.o) + CCl4</td>
<td>26.686 ± 0.4688</td>
<td>21.138 ± 0.3145</td>
<td>0.5381 ± 0.2197</td>
<td>0.4717 ± 0.004773</td>
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</tbody>
</table>

Values are expressed as mean±SEM; n=6 each group; P<0.001 vs. CCl4-treated group (ANOVA followed by Dunnett's test).

Fig. 9: Level of ALP concentration in serum from control and treatment groups (n=6) in acute CCl4 liver injury of Wistar male albino rats. Serum concentration of ALP was measured at 28 days after the single subcutaneously injection of CCl4 and treated with atorvastatin in treatment group. All results refer to mean±SEM. ***P<0.001 compared with that of the control group.

Fig. 10: Level of SGOT concentration in serum from control and treatment groups (n=6) in acute CCl4 liver injury of Wistar male albino rats. Serum concentration of SGOT was measured at 28 days after the single subcutaneously injection of CCl4 and treated with atorvastatin in treatment group. All results refer to mean±SEM***P<0.001 compared with that of the control group atorvastatin (10mg/kg, p.o) + CCl4 and atorvastatin (15mg/kg, p.o) + CCl4 and *P>0.05.

Fig. 11: Level of SGPT concentration in serum from control and treatment groups (n=6) in acute CCl4 liver injury of Wistar male albino rats. Serum concentration of SGPT was measured at 28 days after the single subcutaneously injection of CCl4 and treated with atorvastatin in treatment group. All results refer to mean±SEM***P<0.001 compared with that of the control group atorvastatin (10mg/kg, p.o) + CCl4 and atorvastatin (15mg/kg, p.o) + CCl4 and *P>0.05.
cytokines, which promote recruitment of inflammatory cells [2,11,12]. Our results strongly suggest that statins exert anti-necroinflammatory effects in the liver. Also we suggested that atorvastatin attenuates the pathogenic events induced by ANG II in the liver, including oxidative stress, inflammatory events, and expression of profibrogenic genes. Statins reduced the expression of proinflammatory cytokines, which promote recruitment of inflammatory cells [13,2]. Moreover, atorvastatin reduced oxidative stress in the liver, which is an important event leading to hepatic inflammation [14]. Finally, we recently demonstrated that statins decrease endothelial dysfunctions in rats with experimental cirrhosis, which is a pathogenic event linked to local inflammation and fibrogenesis [15]. Atorvastatin reduces the inflammatory actions (IL-8 secretion and ICAM-1 expression) stimulated by ANG II [16,17]. This effect was associated with a reduction in ANG II-induced NF-κB activation [18]. Studies shows that atorvastatin administration totally prevented all signs of histological damage, and reverted the increased liver expression of some pro-inflammatory factors (MT-I, PAI-1), reducing plasma ALP concentrations to below control levels. This strong anti-inflammatory activity of atorvastatin could be attributed to two factors: the well-known inhibition of NFκB activation by atorvastatin and the reduced liver metabolism of fructose, resulting from atorvastatin-mediated decreased expression of fructokinase, thus alleviating hepatic metabolic stress and the production of pro-inflammatory products. Atorvastatin administration also partially prevented the increased expression of these inflammatory markers [19]. Atorvastatin and rosuvastatin possess dose-dependent antioxidant, analgesic, and anti-inflammatory activities [20]. Further studies should evaluate this hypothesis.

References


