Biochemical and histological studies on H2-receptor antagonist ranitidine-induced hepatotoxicity in rats

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Received 3 February 2005: revised 13 June 2005

This study was designed to investigate the hepatotoxicity of ranitidine treatment in dose levels of 10, 30, and 50 mg/kg b.wt. for 3 weeks period in male rats. The results showed some adverse changes in rats treated with either 10 or 30 mg/kg. Treatment with dose of 50 mg/kg produced marked increase in the activity of both acid phosphatase in liver and aspartate aminotransferase in serum and liver, with a tendency for increase in serum alanine aminotransferase activity. Also, a significant decrease in the serum activity of both amylase and alkaline phosphatase was noted. Microscopic examination of livers of the same animals revealed absence of some hepatic cells, pyknotic nuclei, dilatation of blood sinusoids, binucleated cells, and infiltration of lymphocytes. These biochemical and histological changes indicate that ranitidine when given chronically in high dose could produce hepatotoxicity in rats.

Keywords: ACP, ALT, AST, Hepatotoxicity, Ranitidine, Rats, Serum

Ranitidine hydrochloride (Zantac) is a potent H2-receptors antagonist, and is used for the treatment of peptic ulcer diseases\(^1\). The drug has been shown to be safe at the therapeutic doses\(^2\). However, isolated cases of acute hepatitis due to therapy with ranitidine have been documented\(^3\). Liver injury induced by ranitidine was manifested in terms of moderate elevation of serum aminotransferases, modest hepatic infiltration by both lymphocytes and eosinophils, and slight focal hepatocellular necrosis\(^6\). The drug also caused liver cholestasis associated with increased plasma bilirubin and alkaline phosphatase (ALP)\(^8\). These adverse reactions were found to be temporary and subsided after cessation of therapy\(^8\). With increased use of ranitidine in recent years, small but significant incidence of hepatotoxicity is considered important and remains a subject of controversy\(^8,11,12\). Hence, further studies on ranitidine hepatotoxicity using animal model systems are necessary. In view of this, present study has been planned to examine the potential biochemical and histological abnormalities in the rat liver after chronic administration of the drug at dose levels of 10, 30, and 50 mg/kg/day.

Materials and Methods

Male albino rats, *Rattus rattus*, with mean body weight 190±5.22 g were used. The rats had free access to food and water. Ranitidine (Zantac) was obtained from Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt. The drug was administered daily by intramuscular injection (im) for 3 weeks at doses 10, 30 or 50 mg/kg/day. The control rats received no treatment.

At the end of the treatment, rats were anaesthetized by ether, sacrificed, and the blood samples were collected. Serum was isolated after centrifugation at 3000 rpm for 15 min. Portions of the livers were promptly removed and stored in the deep freezer together with the sera. The stored tissue brought to room temperature, weighed, and homogenized in known volume of distilled water. The tissue homogenates and sera were then used for biochemical analyses.

Activities of alanine aminotransferase (ALT)\(^13\), aspartate aminotransferase (AST)\(^13\), alkaline phosphatase (ALP)\(^14\), acid phosphatase (ACP)\(^14\), and
serum amylase (AM)\textsuperscript{15}, were estimated by colorimetric methods.

For histological studies, liver tissue samples were placed in Bouin’s fixative for 48 hr. After embedding in paraffin blocks, sections of 5 \(\mu\)m thickness were prepared, fixed on clean glass slides, and stained with haematoxylin and eosin (H and E)\textsuperscript{16}.

The data are given as mean \(\pm\) SE. Comparisons limited to two specific groups were evaluated using Student’s t test. \(P\) values equal to or below 0.05 were considered significant.

Results and Discussion

Treatment with high dose of ranitidine (50 mg/kg) for 3 weeks produced marked elevation in the serum AST activity with a tendency for increase in serum ALT activity (Table 1). Also, the hepatic activity of both AST and lysosomal hydrolytic enzyme ACP were increased significantly. These changes reflect the incidence of hepatotoxicity in the treated rats. Ranitidine may cause hepatocellular, cholestatic, or mixed hepatocellular-cholestatic injury in isolated cases\textsuperscript{7,8,11}, accompanied with elevation of plasma aminotransferases and ALP\textsuperscript{7,9}. However, in contradiction with published data, present findings showed marked decrease in serum ALP activity with no significant change in its hepatic level in rats treated with high dose of ranitidine (Table 1), probably due to extra-hepatic effects of ranitidine\textsuperscript{17-19}, and species specificity.

There was a decrease in the serum AM activity in rats treated with high dose of ranitidine (Table 1). However, H2-antagonists like ranitidine were unable to modify the basal plasma levels of AM via pancreatic secretion\textsuperscript{21-23}. Therefore, it seems likely that present decrease in serum AM activity may be related to drug-induced liver injury. Zilva and Pannall\textsuperscript{20} reported low plasma levels of AM in some cases of hepatitis.

Biochemical abnormalities induced by high dose of ranitidine were further confirmed by microscopic examination of liver tissue (Fig. 1). The results showed evidence of hepatocellular injury which was characterized by erosion of endothelium cells of blood vessel, absence of some hepatic cells, appearance of pyknotic nuclei, presence of karyolysis, dilatation of blood sinusoids, and increased number of binucleated cells. Also, inflammatory reactions around the blood vessels as reflected by the presence of infiltration of lymphocytes and increased activation of Kupffer cells were observed. These histopathological changes are in agreement with published data on human liver showing intralobular infiltration of lymphocytes, aggregation of eosinophils, and slight focal hepatocellular necrosis on ranitidine therapy\textsuperscript{6,8}. Also, administration of ranitidine in rats after two-thirds hepatectomy resulted in substantial delay of liver cell proliferation, profound liver steatosis, and marked dilatation of blood sinusoidal spaces\textsuperscript{12}.

Treatment with ranitidine at dose of 30 mg/kg/day produced insignificant biochemical changes, except for markedly increased ACP and decreased ALT activity in the liver. Histological examination of liver sections of this animal group exhibited mild inflammatory reaction and dilatation of hepatic sinusoids. In rats treated with ranitidine 10 mg/kg/day, no remarkable changes were noted in the analyzed parameters, except an inhibition in ACP and an increase in ALT activity in the liver. Microscopic findings showed almost complete absence of inflammatory reactions and slight histological changes, such as mild dilatation of blood sinusoids and few numbers of pyknotic nuclei (data of 10 and 30 mg dose not shown).

Table 1—Changes in the activity of serum and liver enzymes in rats treated with ranitidine (50 mg/kg) for 3 weeks

<table>
<thead>
<tr>
<th></th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>ACP</th>
<th>AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Serum (U/L)</td>
<td>11.93</td>
<td>13.98</td>
<td>68.60</td>
<td>100.78</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td>(\pm 1.14)</td>
<td>(\pm 1.48)</td>
<td>(\pm 6.46)</td>
<td>(\pm 10.60)</td>
<td>(\pm 20.37)</td>
</tr>
<tr>
<td>Liver (U/g)</td>
<td>6.02</td>
<td>6.15</td>
<td>2.58</td>
<td>4.18</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>(\pm 0.43)</td>
<td>(\pm 0.64)</td>
<td>(\pm 0.40)</td>
<td>(\pm 0.52)</td>
<td>(\pm 0.18)</td>
</tr>
</tbody>
</table>

\(a = P<0.05\), \(b = P<0.025\), \(c = P<0.01\)

AST: asparate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; ACP: acid phosphatase; AM: amylase
Fig. 1a-d—Photomicrographs of sections of rat liver treated with 50 mg ranitidine/kg body wt. for 3 weeks showing dilatation of blood sinuoids (ds), pyknotic nuclei (pk), lymphocytic infiltration (li), binucleated cells (bc), pyknotic nuclei (pk), karyolysis (ky), erosion of the endothelial layer of the blood vessels (ev), and increased number of Kupffer cells (k) [H E x400].

Present results and other published data demonstrated dose dependent hepatotoxic effects of ranitidine in chronic animal studies. However, ranitidine-induced hepatotoxicity in human is controversial. Published data displayed either no significant effects on hepatic functions or serious hepatotoxicity in patients treated with therapeutic doses of ranitidine. Therefore, it seems likely that at level of therapeutic doses treatment there are interindividual variations regarding tolerance of ranitidine, which may, in part, be due to the drug-induced hypersensitive reactions. In general, hepatotoxicity due to ranitidine treatment may be related to factors such as dose used, route and period of administration, species sensitivity, and drug-induced hypersensitive reactions.

Published data on the mechanistic pathways of ranitidine hepatotoxicity appeared inconclusive. It has been proposed that the metabolism of amino-alkyl furan ring structure of ranitidine may lead directly to hepatic oxidative damage. In contrast, some authors demonstrated that ranitidine, unlike other furans, does not have the potential for causing direct hepatocellular injury by oxidative damage. However, ranitidine hepatotoxicity has been explained on basis of the ability of the drug or one of its metabolites to generate an immunological reaction. In accordance, present results showed an inflammatory reaction as reflected by infiltration of lymphocytes in rats treated with either 50 or 30 mg/kg of ranitidine.

In conclusion, present biochemical and histological study provides an additional evidence that chronic administration of ranitidine in rats could produce hepatic adverse effects in a dose dependent manner.

References
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