Effect of Long-Term Disulfiram Administration on Rat Liver

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Abstract. No hepatotoxicity was demonstrated after 90 days in rats treated with two dose levels of disulfiram. A reversible inhibition of microsomal p-nitroanisole demethylase activity was found. In phenobarbital-treated rats disulfiram 100 mg/kg did not alter the induction response as indicated by the cytochrome P-450 content, but inhibited the p-nitroanisole activity to control levels. As no sign of histological liver damage was found, we conclude that the rare cases of disulfiram hepatotoxicity in man may be due to an allergic reaction.

When disulfiram (DS) was introduced as a supportive treatment for alcoholism 30 years ago only few animal studies and clinical investigations were required. Recent observations of fatal liver damage due to DS (4, 7, 11) prompted us to evaluate the possible hepatotoxic effect of long-term administration of DS in rats. Moreover, we studied the effect of DS on the phenobarbital-induced rat liver.

Material and Methods

33 female Wistar rats, weighing 190–202 g were divided in three groups called DS-10, DS-100, and CON. Groups DS-10 and DS-100 received 10 and 100 mg DS/kg body weight, respectively, while the CON group received the vehicle alone. DS was given as a suspension in 0.46% carboxymethylcellulose. The volume given was 1 ml, independently of the dose of DS. Control animals were treated with an equal volume of vehicle. DS was given by gavage twice weekly for 90 days. In a separate study 14 rats were given DS, 100 mg/kg, daily for 8 days by gavage together with either phenobarbital, 50 mg/kg i.p. (DS-100 PB) or 1 ml of isotonic saline i.p. (DS-100 SA). Rats were fed ad libitum with standard laboratory chow and had free access to water. They were housed in cages which were periodically moved, so that each group had the same environmental factors throughout the experiment. The body weight was monitored weekly. At the time of sacrifice one rat from group DS-100 had a body weight significantly below the average of the same group, and was therefore excluded from the final examinations. At the end of the experiment surgery was performed at 24, 72 and 96 h after the last administration of the drug. In the group of DS-100 PB and DS-100 SA rats, surgery
was performed after 24 h. After 18 h of starvation the rats were operated on under ether anaesthesia, between 9:00 and 12:00 a.m. The livers were inspected, removed and weighed.

A specimen of each liver was fixated in 4% formaldehyde for light microscopy examinations. The remnant liver was weighed and washed four times in 20 ml ice-cold 1.15% KCl solution and homogenized.

The preparation of liver microsomes (1) and the determination of microsomal protein (5), cytochrome P-450 (12), p-nitroanisole demethylation (12), and total glutathione content (15) were performed by the methods indicated.

Light microscopy examinations of liver tissue were done blindly on hematoxylin-eosin and van Gieson stained specimens. A Mann-Whitney test was used in the statistical evaluation of the results.

Results

All rats in the DS-treated groups survived. 1 rat from the control group died on the second day after the first administration of the vehicle.

No significant intergroup differences were found with respect to liver weight, contents of protein and cytochrome P-450 (table I) in the rats treated during 13 weeks. The liver weight, cytochrome P-450, and p-nitroanisole demethylation values were significantly higher in the phenobarbital-treated rats receiving DS during 8 days than in the saline-treated rats (p < 0.002) (table I). Values of p-nitroanisole demethylase of the three groups subdivided according to the different times of sacrifice after the last administration of DS are given in table II.

A pronounced decrease of p-nitroanisole demethylase activity was found 24 h after DS administration (p < 0.001), whereas no significant difference was found at later intervals. The cytochrome P-450 levels, however, were not influenced by the time interval from DS administration to sacrifice. The group of DS- and phenobarbital-treated rats (DS-100 PB) had levels of p-nitroanisole activity identical to the control rats in the long-term study (CON), whereas the short-term and long-term treated DS rats had a similar depression of their p-nitroanisole activity.

Table I. Body weight, liver weight and biochemical results of female Wistar rats treated twice weekly during 13 weeks with DS-10, DS-100 and CON, and daily during 8 days with DS-100 PB and DS-100 SA (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 10)</th>
<th>DS-10 (n = 11)</th>
<th>DS-100 (n = 11)</th>
<th>DS-100 PB (n = 7)</th>
<th>DS-100 SA (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>261.1 ± 4.3</td>
<td>257.7 ± 4.3</td>
<td>236.9 ± 3.5</td>
<td>231.5 ± 3.8</td>
<td>221.6 ± 2.2</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>7.3 ± 0.3</td>
<td>7.2 ± 0.2</td>
<td>7.0 ± 0.2</td>
<td>9.5 ± 0.3*</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Protein (homogenate) mg/g liver</td>
<td>225.6 ± 7.2</td>
<td>225.3 ± 8.8</td>
<td>207.3 ± 7.9</td>
<td>193 ± 3.9</td>
<td>201 ± 7.5</td>
</tr>
<tr>
<td>Cytochrome P-450 nmol/g liver</td>
<td>15.7 ± 0.8</td>
<td>15.7 ± 0.8</td>
<td>14.4 ± 1.1</td>
<td>20.0 ± 0.9*</td>
<td>15.5 ± 0.9</td>
</tr>
<tr>
<td>Cytochrome P-450, nmol/mg microsomal protein</td>
<td>0.64 ± 0.01</td>
<td>0.61 ± 0.02</td>
<td>0.59 ± 0.02</td>
<td>0.81 ± 0.03*</td>
<td>0.66 ± 0.01</td>
</tr>
</tbody>
</table>

* p < 0.02.
Table II. \( p \)-Nitroanisole demethylase activity (nmol/mg liver protein/10 min) of female Wistar rats treated twice weekly during 13 weeks with DS-10, DS-100 and CON, and daily during 8 days with DS-100 PB and DS-100 SA (mean and range)

<table>
<thead>
<tr>
<th>Hours from last drug administration to sacrifice</th>
<th>CON</th>
<th>DS-10</th>
<th>DS-100</th>
<th>DS-100 PB</th>
<th>DS-100 SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>9.66 (n = 4)</td>
<td>4.23 (n = 4)</td>
<td>1.73 (n = 4)</td>
<td>8.91 (n = 7)</td>
<td>2.69 (n = 7)</td>
</tr>
<tr>
<td>72</td>
<td>8.14 (n = 4)</td>
<td>9.63 (n = 4)</td>
<td>7.64 (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>10.26 (n = 2)</td>
<td>10.92 (n = 3)</td>
<td>7.0 (n = 3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The liver content of glutathione was similar in phenobarbital-treated (DS-100 PB) and control rats (2.0 ± 0.09 vs. 2.0 ± 0.05 mg/g liver). Macroscopic appearance and light microscopy findings of the livers were normal in all animals.

Discussion

Our study indicates that DS is not a predictable hepatotoxin. The group of rats treated with 10 mg DS/kg body weight twice weekly received a dose comparable to that employed in the clinic. After 3 months of treatment no hepatic damage was observed either in this group or in the group receiving a ten times larger dose. *Child and Crump* (3) performed a long-term study on dogs administering DS, 100 mg/kg/day, and did not find any sign of hepatotoxicity. During the last 30 years, DS had been extensively used in the treatment of alcoholism. Nevertheless, only rare cases of hepatotoxicity have been reported, which also indicate that DS may be classified as an unpredictable hepatotoxin. Among the reported cases, however, only 2 patients had allergic phenomena (11).

DS is reduced to diethylidithiocarbamate by glutathione and probably also by free SH groups of proteins (14). It is a potent inhibitor of many enzymes probably by blocking essential SH groups on the enzyme by mixed disulphide formation (14). It has been demonstrated that microsomal drug-metabolizing enzyme activity is inhibited by DS (8, 13, 16, 17), and a similar inhibition of the enzymes of the D-glucuronic pathway was recently found (10). In our study DS administration did not affect the glutathione content of rat liver.

After a single dose of DS significant decreases in cytochrome P-450 activities and microsomal ethylmorphine N-demethylase activity in rat liver were found (13). However, contin-
ued administration of DS during a 12-day period to rats indicated that these changes may be reversible (17). Our study demonstrates that the cytochrome P-450 content is neither changed after 8 days nor 13 weeks of DS administration. However, p-nitroanisole demethylase activity is reversibly depressed. The restitution to normal values occurs within 24 h after the last administration of DS.

Carbon disulphide, which is a metabolite of DS, has been shown to cause a depression of drug-metabolizing enzymes, and pretreatment with phenobarbital produces liver necrosis in the rat (2, 9). Moreover, diethyldithiocarbamate, the principal metabolite of DS is also capable of producing hepatotoxicity in fasting phenobarbital-treated rats (9). Our investigation in phenobarbital-induced rats has not supported the hypothesis that the hepatotoxic effect of DS in man is mediated by a reactive metabolite formed in the microsomes, e.g. carbon disulphide (9). No liver necrosis was demonstrated in the induced rats treated with DS, and we find a normal induction response as measured by the cytochrome P-450 content. Recently, a destruction of cytochrome P-450 was demonstrated after carbon disulphide administration (6).

In conclusion, hepatotoxicity of DS was demonstrated neither in the induced rat liver nor in rats treated during nearly 13 weeks. The depression of microsomal drug metabolism persists after 3 months of DS treatment, but is a reversible phenomenon.

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References


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