Dose-Dependent Effects of Dichloropropanol on Liver Histology and Lipid Peroxidation in Rats

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Abstract: A rare outbreak of acute hepatic damage in workers exposed to dichloropropanols (DCPs) was reported recently. The purpose of the present study is to examine the effects of DCPs on various organs, the dose dependency and the pathogenetic potential of DCP hepatotoxicity. A single intraperitoneal injection was given to six groups of rats with 0.2 ml of 20% ethanol (control), or 1/8 x, 1/4 x, 1 x, and 2 x LD50 (0.11 ml/kg) of 1,3-dichloro, 2-propanol (DC2P) diluted in 20% ethanol. After blood samplings, all organs were subjected to histologic examinations with light and electron microscopes. Fresh liver tissues from further 4 control and 4 experimental rats sacrificed 6 hours after the injection of 20% ethanol and 1 x LD50 of DC2P were homogenized and subjected to evaluate lipid peroxidation, glutathione S-transferase activity and reduced glutathione contents in the liver. The rats administered with only ethanol or 1/8 and 1/4 LD50 of DC2P showed no serological or histopathological abnormalities. Marked elevation of serum glutamate pyruvate transaminase (SGPT) with a diffuse massive necrosis of the liver cells were noted in all rats of both the 1 x and 2 x LD50 groups, and irregular zonal necroses were found in 3 of 4 rats injected with 1/2 LD50. There were no serious toxic changes in other organs. Hepatic malondialdehyde level was significantly increased, associated with decreases of liver glutathione S-transferase activity and reduced glutathione content. It was concluded that the serious DC2P-toxicity was mainly found in the livers, dose dependently, and one of the causative mechanisms of this hepatotoxicity might be the lipid peroxidation.

Key words: Dichloropropanol, Hepatotoxicity, Lipid peroxidation, Glutathione, Rat

Introduction

Although the toxicity of dichloropropanols (DCPs) to experimental animals by ingestion or inhalation is cited in a few texts1,2), practical liver diseases with DCPs in humans were not available in the literature3). Our previous report3) documented human cases of fulminant hepatitis induced by 1,3-dichloro-2-propanol (DC2P), one of the isomers of DCPs, and also described that severe experimental hepatic damage was evoked in about half of rats with a single intraperitoneal injection of 1/2 x LD50 of DC2P, but not with the same volume of 2,3-dichloro-1-propanol (DC1P). The precise pathogenesis and dose dependency of the DC2P hepatotoxicity and its effect on other organs have not been resolved. In this paper, rats were administered with graded concentrations of DC2Ps, and autopsied to examine the dose dependent effects of DC2P on the liver and other organs. In addition, the effect of DC2P on lipid peroxidation, glutathione S-transferase and glutathione in the rat liver was examined.

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Materials and Methods

Experiment 1

Twenty two male Wistar rats of 8 weeks of age were administered a single intraperitoneal injection of 0.2 ml diluted DC2P in 20% ethanol at various degrees of concentration; 4 rats were injected with 2 fold, 6 with one fold, 4 with 1/2, 4 with 1/4, and 4 with 1/8 of oral-rat LD$_{50}$ amounts (0.11 ml/kg)$^{60}$, respectively (the intraperitoneal LD$_{50}$ was not available in the literature). Four control rats of the same age were injected with only 0.2 ml of 20% ethanol. All rats were purchased from an experimental company (Kyudo Animal Center) and kept in an air-conditioned room in our university with free access to food and water until use. The rats were sacrificed after blood samplings under an anesthesia by pentobarbitar. Whole bodies were inspected by the naked eye, and portions of the lungs, stomach, small and large intestines, liver, pancreas, kidneys, urinary bladder, testes, thyroid gland, spleen, thymus and lymph nodes were cut and fixed in 20% formalin for the histologic examination.

Further, small pieces of liver from each rat were fixed in 2.5% glutaraldehyde, postfixed in 2% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranium acetate and lead citrate, and observed under a transmission electron microscope, JEM 1200EX, with an accelerating voltage of 80 kV.

Experiment 2

Four control and 4 experimental rats were sacrificed 6 hours after the single injection of 20% ethanol and 1 x LD$_{50}$ of DC2P in 20% ethanol, respectively. After the blood samplings and perfusion with cooled saline, fresh liver tissues of the 8 rats were immediately homogenized in 7 ml of iced-cold 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl. The subcellular fractions were prepared by the method of Hogeboom$^{51}$, and were subjected to evaluate lipid peroxidation level estimated by malondialdehyde level$^{60}$, glutathione S-transferase activity and glutathione content in the liver. Reduced glutathione content was determined by fluorometric method$^{71}$, and glutathione S-transferase activity was estimated by the method following Habig et al$^{80}$. Small pieces of the livers were fixed in 20% formalin and examined histologically.

Results

Experiment 1

One of the 4 rats in the 2 x LD$_{50}$ group died 10 hours after the injection, and the other 3 rats in this group were killed at the same time because they were also severely wasted and dying. Two of six rats in the 1-fold LD$_{50}$ group died at 17 and 20 hours after the injections, respectively, and the other 20 rats of each group, including the controls, were sacrificed 24 hours after the injection. Titers of serum glutamate pyruvate transaminase (SGPT) of the Experiment 1 are summarized in Fig. 1. The titers of all 8 rats in both the 1/8 and 1/4 LD$_{50}$ groups were almost equal to those of the control group. One, one, and two rats of the 1/2 x LD$_{50}$ group showed normal, mild and moderate elevation of SGPT, respectively. All rats in both 1 x LD$_{50}$ and 2 x LD$_{50}$ groups, except for two in the 1 x LD$_{50}$ group with no available blood sample, showed a marked elevation of SGPT (from 2,370 to 11,355 IU/l). The SGPT titers of the 2 x LD$_{50}$ group, which were all assessed 10 hours after the DC2P injections, were not higher than that of 1 x LD$_{50}$ group, assessed 24 hours after the injections. The titers of serum glutamate oxaloacetate transaminase (SGOT) were higher than those of SGPT titers in all rats, but the pattern of SGOT titers was quite similar to that of SGPT titers (data not shown).

The histopathologic changes are summarized in Table 1, which discloses that serious organotoxicity was found in the liver. Massive centrilobular necrosis was prominent in all 10 rats in both 2 x and 1 x LD$_{50}$ groups (Fig. 2). At 10 hours after the injection (the 2 x LD$_{50}$ group), many neutrophilic polymorphs and a few mononuclear cells were recruited in the necrotic areas, followed by infiltration of a greater number of mononuclear cells at 24 hours than that at 10 hours.
In accordance with the biochemical data, all rats in both 1/8 and 1/4 LD₅₀ groups revealed no hepatocytic necrosis, though mild centrilobular congestion was noted in two of the 1/4 LD₅₀ group. Furthermore, there was no cholestatic profile even in the rats with severe hepatocytic necrosis in the both 1 x and 2 x LD₅₀ groups.

Focal degenerative changes of the renal tubular epithelium and multiple erosions of the gastrointestinal tract with hemorrhage were frequent extrahepatic lesions in the cases with severe hepatic damage. A mild interlobular edema of the pancreas and focal peritoneal inflammatory infiltrates were sometimes seen in both control and experimental rats. These pancreatic and peritoneal lesions might be induced by ethanol used for the solvent of DC2P and the control injections.

Ultrastructurally, there were no significant degenerative changes of the hepatocytes in all rats of both 1/8 x and 1/4 x LD₅₀ groups. In and around the severe necrotic areas of the 1/2 x, 1 x, 2 x LD₅₀ groups, marked endothelial disruptions and extravasation into the space of Disse, as well as hepatocytic necrosis, were noted (Fig. 3).

**Experiment 2**

Although average body weight of both control (213 ± 7.5 g) and experimental (216 ± 6.7 g) groups was almost equal, total liver weight of the experimental groups (10.6 ± 0.8 g) was heavier than that of control (7.3 ± 0.2 g; P<0.001).
A prolonged prothrombin time and marked decreases of blood neutrophils and platelets were common findings in the experimental groups (Fig. 4), and massive hepatocytic necrosis with neutrophil infiltration and hemorrhage were noted histologically in all experimental rats.

A summary of hepatic lipid peroxidation level, glutathione S-transferase activity and reduced glutathione content is shown in Table 2. Lipid peroxide level (malondialdehyde) of the experimental group was significantly increased, and glutathione S-transferase activity and reduced glutathione content were significantly decreased in the experimental groups.

**Discussion**

Epichlorohydrin is an industrial material widely used for composing epoxy resin, synthetic glycerol, a surfactant and a solvent for rubber, and is synthesized through a reaction of two isomers of dichloropropanol; that is, DC1P and DC2P. Although toxic effects on several animals and a possible mutagenicity of DC2P were described, a severe hepatotoxic lesion due to DC2P in humans has not been reported, as far as we know.

Our previous report described two lethal cases of fulminant hepatic injury following a DC2P exposure. These two cases were included in the 5 patients described by Iwasa et al. as a rare outbreak of acute hepatic damage in workers exposed to dichloropropanols. In their report, 12 workers were

![Fig. 3. Rupture of endothelial lining (*) with extravasation of erythrocytes.](image)

Note fragments of degenerated endothelial cells (arrows). Uranyl acetate and lead citrate × 3960. Bar: 2 μm.

![Fig. 4. Hematologic data 6 hours after the dichloropropanol injection.](image)

RBC: erythrocytes (× 10⁴/mm³), WBC: leukocytes (/mm³), Plt: platelets (× 10⁴/mm³), PT: prothrombin time (% against standard).

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<th>Lipid peroxide level in total liver homogenate (nmol/Malondialdehyde/h/100 mg protein)</th>
<th>Glutathione S-transferase activity (nmol product/min/mg protein)</th>
<th>Reduced glutathione content (μg/g liver)</th>
<th>Reduced glutathione content (μg/liver)</th>
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<tr>
<td>Control (n=4)</td>
<td>86.0 ± 14.5</td>
<td>1909.5 ± 415.5</td>
<td>1238.8 ± 107.9</td>
<td>9321.3 ± 683.3</td>
</tr>
<tr>
<td>Exposed (n=4)</td>
<td>227.2 ± 36.3*</td>
<td>449.3 ± 45.4*</td>
<td>710.5 ± 39.3*</td>
<td>7546.5 ± 882.6*</td>
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Significantly different from control: *P<0.01, **P<0.05.
engaged in the cleaning of a saponification tank for the synthesis of dichlorohydrin in a factory. Acute hepatic injuries developed in five workers, two of which were lethal, but 7 workers were saved from any hepatic symptom and biochemical abnormality. Our successive experiments also clarified that DC2P but not DC1P induced submassive hepatic necrosis in about half of the rats administered with a single intraperitoneal injection of a half volume of LD50. However, there is no precise study concerning the dose-dependency of DC2P hepatotoxicity or the histopathological effects of the DC2P exposure on other organs.

The severity of the hepatotoxicity and the dose of DC2P were fairly well correlated in the present study, as shown by both biochemical and histopathologic results, and the critical concentration of DC2P which induces hepatic necrosis in rats was between 1/4 and 1/2 LD50. The reason why the SGPT titers in the 2 × LD50 group were lower than those in the 1 × LD50 group might be attributable to the difference of time when the sampling was done after the DC2P injection. As shown in our previous study, the highest SGPT titers appeared at 24 to 48 hours after the DC2P injections. However, in this study, the 1 × LD50 group was sacrificed at 24 hours, while the 2 × LD50 group was sacrificed after only 10 hours, because the rats died of high toxicity.

In the case of carbon tetrachloride poisoning, severe liver injuries are usually accompanied by preceding neural and gastrointestinal symptoms and a subsequent deterioration of the renal function. Similar symptoms of the upper gastrointestinal tracts, such as nausea and vomiting and anal oliguria with azotemia were noted in the lethal cases of DC2P poisoning. Multiple gastrointestinal erosions with hemorrhage and mild renal tubulopathy were also occasional findings in the rats with severe hepatocytic necrosis in the present study. However, these extrahepatic lesions were not so serious and possibly subsequent to the severe hepatic failure. Thus, the main target organ of the DC2P toxicity was thought to be the liver.

The mechanism of DC2P-induced hepatic necrosis is not clear, and there is very limited information concerning the metabolism of DCPs. DC2P is a chlorinated chemical like carbon tetrachloride, which is the well-documented chlorinated chemical inducing hepatocytic necrosis. An active metabolite, that is a free radical, is widely accepted to be responsible for carbon tetrachloride-induced hepatocytic necrosis. The cytotoxicity caused by carbon tetrachloride is more severe in the centrilobular areas than in the perportal areas, which is similar to the finding caused by DC2P administration. These facts suggest that the toxic action of DC2P might be similar to carbon tetrachloride, as noted in Sax's handbook, though the detection of such free radicals was not attempted in the present study.

In this study, we have observed increased levels of hepatic lipid peroxidation (increased malondialdehyde) in rats treated with DC2P. One of the dominant mechanisms of carbon tetrachloride poisoning is lipid peroxidation, which is a common mechanism for many toxic agents. Increased hepatic malondialdehyde level (lipid peroxidation) was known in liver injuries with carbon tetrachloride, styrene and styrene oxide, and ethylene oxide. In addition, a significant decrease in hepatic glutathione content and glutathione S-transferase activity was evident in our experiment. Intracellular glutathione plays an important role in maintaining a reduced environment and in protecting tissue proteins and lipids from oxidants such as hydrogen peroxide. In accordance with this role, hepatic lipid peroxidation increased after glutathione depletion, both in vivo and in isolated hepatocytes. Glutathione S-transferase is a group of enzymes capable of conjugating glutathione with structurally diverse electrophilic compounds. Some of these enzymes are also known to exhibit glutathione peroxidase activity and reduce lipid hydroperoxides. These results suggest that the increased hepatic lipid peroxidation is at least in part due to the depletion of glutathione content and the impairment of the protective action of glutathione S-transferase.

On the other hand, some studies have shown that lipid peroxidation generates a variety of toxic aldehyde products, including 4-hydroxyalkenals. It has been reported that conjugation of 4-hydroxyalkenals with glutathione is catalyzed by glutathione S-transferases. Thus, the observed decrease in glutathione S-transferase activity could have two important consequences: one being impaired detoxification of toxic products of lipid peroxidation, and the other being lipid hydroperoxidation.

It was concluded that the serious DC2P-toxicity was dose dependent and mainly found in the livers, and the lipid peroxidation might be one of the causative mechanisms of this hepatotoxicity.

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References