

## **Chronic Liver Injury in Rats by Carbon Tetrachloride Inhalation**

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Chronic liver injury is commonly induced in rats by repeated carbon tetrachloride ( $\text{CCl}_4$ ) injection (McLean et al. 1969). There have been several investigations of  $\text{CCl}_4$  hepatotoxicity by its inhalation (David et al. 1981), although only a few reports on chronic liver injury is available. The present study was thus carried out to determine whether or not the inhalation method can replace the ordinary injection method for inducing chronic liver injury.

### **MATERIALS AND METHODS**

Male Sprague-Dawley rats (Charles River Japan, Inc., Tokyo), weighing 200 to 250 g each, were used at 24-25°C throughout this study. A wooden box with a glass front (42 X 53 X 30 cm) was fitted with an electric fan to distribute  $\text{CCl}_4$  vapor equally throughout the box. Compressed air via a flow meter was bubbled into through two bottles; the first was filled with water and the second with  $\text{CCl}_4$  solution (99.9% purity).  $\text{CCl}_4$  concentrations were determined by a Kitagawa's gas detector (Kohmyo Rikagaku Co., Ltd., Osaka). Ten to fifteen rats fasted overnight were placed in the chamber, and  $\text{CCl}_4$  vapor was blown in at a flow rate of 2 l/min until rats were anesthetized (Lazarew 1929). Hyperactivity of rats was induced 6 to 11 min after  $\text{CCl}_4$  exposure and thereafter activity was decreased. Rats became comatose within 15 min of the exposure, when  $\text{CCl}_4$  concentration in the chamber (in the breathing air) was approximately 180 ppm.  $\text{CCl}_4$  vapor was immediately released from the box into the atmosphere through a bottle of olive oil;  $\text{CCl}_4$  concentrations were less than 10 ppm, and harmless to man. Treated animals were left in the chamber, and air alone was blown into the box until they completely recovered. The  $\text{CCl}_4$  amount inhaled during the treatment was approximately 50-100 mg per rat. Arterial blood  $\text{PCO}_2$  and  $\text{PO}_2$  were within normal levels even in

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anesthetized rats. The induction time to narcosis and the recovery time from anesthesia were not different during the repeated exposure.  $\text{CCl}_4$  exposure at a flow rate of 2 l/min was performed twice a week for 8 weeks. For testing acute hepatic injury in this preliminary study, fasted rats were exposed to a single inhalation of  $\text{CCl}_4$  vapor at two different flow rates (2 and 4 l/min) as described above and sacrificed 24 hr later. Serum alanine aminotransferase (ALT) activities were determined by a GPT UV test (Wako Pure Chemical Co., Osaka).

## RESULTS AND DISCUSSION

A single inhalation of  $\text{CCl}_4$  vapor caused a marked increase in both serum ALT activity (2 l/min;  $621 \pm 133$  IU and 4 l/min;  $2089 \pm 968$  IU,  $p < 0.05$ . Control;  $32 \pm 10$  IU. Mean  $\pm$  SD from 10 to 15 rats) and percent liver weight [(liver weight/body weight)  $\times 100$ . 2 l/min;  $4.2 \pm 0.4\%$  and 4 l/min;  $4.3 \pm 0.1\%$ . Control;  $3.4 \pm 0.3\%$ ]. Hepatotoxic effect of  $\text{CCl}_4$  vapor was also confirmed by histological examination of the liver. These results indicate that the extent of liver injury is strongly influenced by increasing the flow rate of  $\text{CCl}_4$  vapor, i.e.,  $\text{CCl}_4$  concentrations in the chamber.

In  $\text{CCl}_4$ -repeatedly inhaled treated rats, the gain of body weights at the end of 8 weeks' treatment (130–150% of the initial body weight) were not significantly different from those in  $\text{CCl}_4$ -injected rats; 0.1 ml of 50%  $\text{CCl}_4$  solution in olive oil per 100 g body weight was injected subcutaneously twice a week for 8 weeks, as previously performed in our laboratory (Hayashi 1983). Much lower incidence of death during the experiment was observed in  $\text{CCl}_4$ -inhaled rats (25% on average of 65 rats) than injected rats (46% on average of 46 rats). Repeated  $\text{CCl}_4$  exposure for 8 weeks successfully produced chronic liver injury with nodular liver surface and extensive fibrosis (Fig. 1). The similar findings were also observed in the 8th week of  $\text{CCl}_4$  injection.

$\text{CCl}_4$  inhalation method, especially at 2 l/min flow rate under these conditions described above, is effective and convenient for producing chronic liver injury in a large number of rats at the same time. Repeated  $\text{CCl}_4$  exposure for longer than 8 weeks might produce liver cirrhosis as produced in 12 weeks by injection (Hayashi 1983).

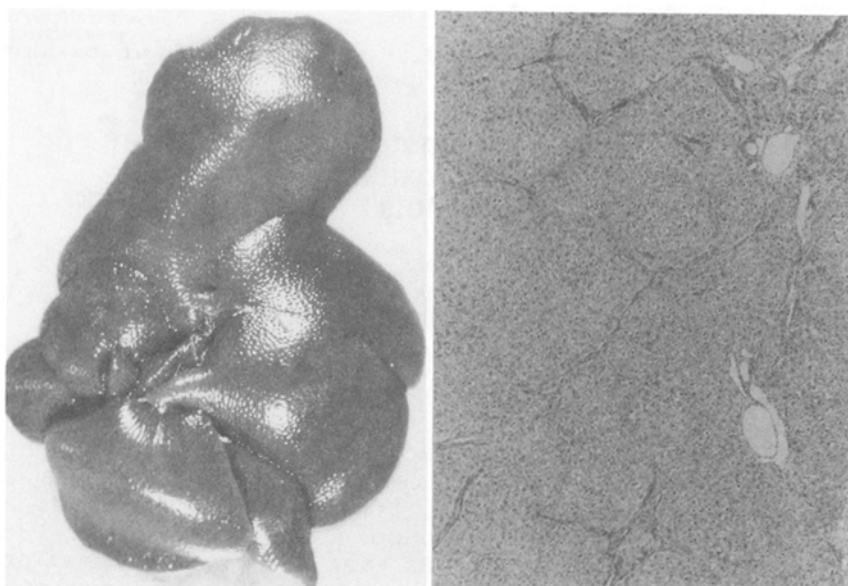


Figure 1. Macroscopic and microscopic findings of the liver in  $\text{CCl}_4$ -repeatedly inhaled rats. Liver surface is finely nodular, and extensive fibrosis can be seen (H-E stain).

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