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Immunohistochemical Study of Epoxide Hydrolase Induced by Trichloroethylene in Rat Liver

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Epoxide hydrolase catalyzes the hydrolation of potentially toxic, electrophilic epoxides that are often generated during cytochrome P-450 catalyzed monooxigenation, forming the corresponding trans-dihydrodiols (Baron et al. 1979). It is well-known that trichloroethylene is metabolized by cytochrome P-450 containing mixed-function oxidase systems to trichloroethylene oxide, which decomposes to other metabolites (Miller and Guengerich, 1982,1983, Kawamoto et al. 1987). As trichloroethylene is an epoxide, epoxide hydrolase is suspected to catalyze the hydrolation of trichloroethylene oxide. No reports have appeared about the relationship between trichloroethylene and epoxide hydrolase. In this report, we studied the effect of trichloroethylene on epoxide hydrolase immunohistochemically.

MATERIALS AND METHODS

Male Wistar rats weighing about 220g were used. Groups of rats were treated with either phenobarbital (80mg/kg/day, intraperitoneally, in serine) for 4 days, 3-methylcholanthrene (20mg/kg/day, intraperitoneally, in olive oil) for 4 days, or trichloroethylene (1.0g/kg/day, intraperitoneally, in olive oil) for 5 days. Control rats were given appropriate volumes of vehicle. After the last treatment, the rats were starved for 24h and killed by decapitation. A portion of the median lobe was removed from each liver and was prepared for immunohistochemical analysis.

The immunohistochemical localization of epoxide hydrolase in liver was carried out by the unlabeled antibody peroxidase-antiperoxdase technique (Baron et al., 1980) with minor modification. The presence of epoxide hydrolase within hepatocytes was visualized at the light microscopic level as a brown stain after completion of unlabeled antibody peroxidase-antiperoxidase staining. Whole normal rabbit serum and whole rabbit anti-epoxide hydrolase serum were obtained as described previously (Ogino et al., 1982,1983). Swine antiserum to rabbit immunoglobulins and soluble peroxidase-rabbit antiperoxidase complex were obtained from DAKO Corporation.

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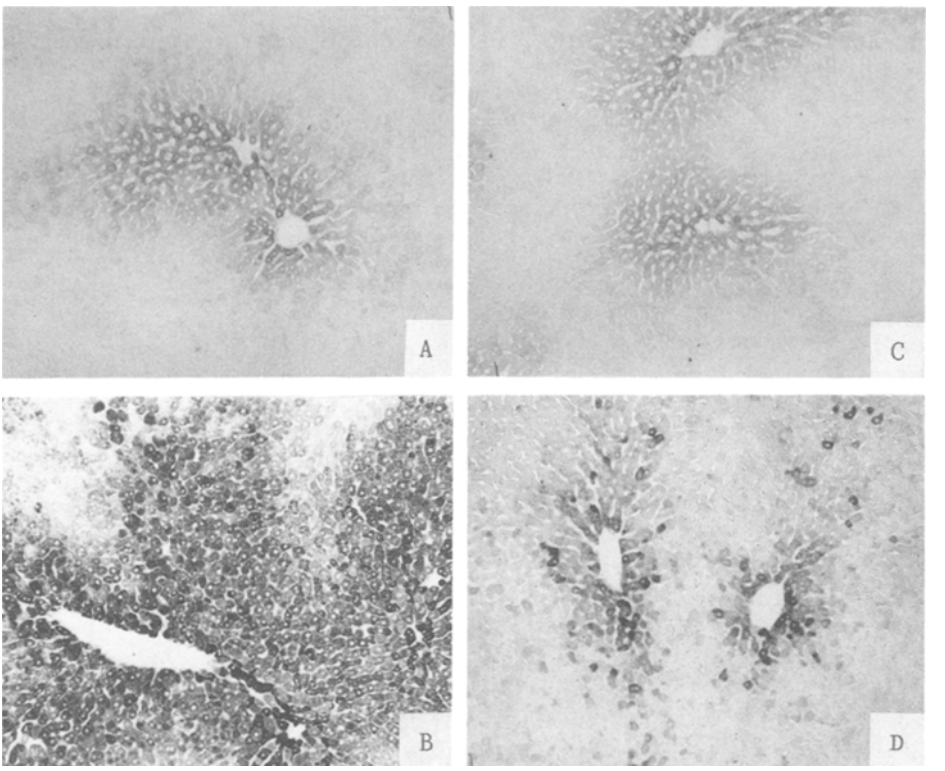

Figure 1. Immunohistochemical localization of epoxide hydrolase within the rat liver. A, section from the liver of a rat pretreated with olive oil for 5 days. B, section from the liver of a rat pretreated with phenobarbital for 4 days. C, section from the liver of a rat preteated with 3-methylcholanthrene for 4 days. D, section from the liver of a rat pretreated with trichloroethylene for 5 days.

RESULTS AND DISCUSSION

Epoxide hydrolase was detected within parenchymal cells throughout the livers of phenobarbital, 3-methylcholanthrene, trichloroethylene and vehicle pretreated rats. Immunohistochemical staining for epoxide hydrolase was not found within cells associated with the hepatic vasculature, Kupffer cells, or sinusoidal cells. Hepatocyte nuclei in livers of both control and xenobiotic-pretreated rats were not appreciably stained for epoxide hydrolase. These findings are consistent with pevious observations made by Kawabata et al. (1983).

Cells within the centrilobular regions were stained more intensely than those within the midzonal and peripheral regions of the lobules of olive oil treated rats (Fig. A). After 4 days of phenobarbital treatment, the antibody binding (Fig. B) was greater in the centrilobular and midzonal regions than that in livers of vehicle-treated rats. In contrast, 3-methylcholanthrene did not alter the immunohistochemical staining (Fig. C). These findings

also agreed well with those by Kawabata et al. (1983).
In the livers of rats treated with trichloroethylene for 5 days, some hepatocytes with intense staining for epoxide hydrolase were scattered not only in the centrilobular region but also in midzonal and peripheral regions (Fig. D). Occasionally, some hepatocytes in the cetrilobular region were intensely stained in the livers of rats treated with 3-methylcholanthrene or vehicle, but such hepatocytes were not observed in the midzonal and peripheral regions.
Epoxide hydrolase in each hepatocyte was not induced uniformally by trichloroethylene. In the liver tissue of rats treated with phenobarbital, the induction of epoxide hydrolase is not also uniform. The light and darker staining in hepatocytes are recognized. It is not clear what roles the hepatocytes with dark staining play in the metabolism of xenobiotics. It is also unknown whether the activities of another enzymes are induced in these hepatocytes.

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