LOCALIZATION OF TYPE IV PROCOLLAGEN mRNA AND PROLYL 4-HYDROXYLASE mRNA on CARBON TETRACHLORIDE ADMINISTERED TO RAT LIVER BY IN SITU HYBRIDIZATION

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Carbon tetrachloride administrated liver is a very characteristic experimental model for studying liver fibrosis. It has been reported that type IV collagen is localized in the space of Disse and sinusoid during the cirrhotic process. It has been suggested that mesenchymal cell synthesizes type IV collagen as the result of an immunohistochemical study. This study was undertaken to clarify the localization of type IV procollagen mRNA and prolyl 4-hydroxylase mRNA by in situ hybridization on carbon tetrachloride administrated rat liver.

MATERIALS AND METHODS: 50%CC14 in olive oil (2ml/kg body weight) was administered to male wistar rats by intraperitoneal injection (twice a week) for 10 weeks. The control rats were not treated. Five rats were killed in each group after 1, 2, 4, 6, 8 and 10 weeks of treatment. For histological observation, the liver specimens were fixed with 10% formalin, embedded in paraffin, sectioned and stained with H&E and Azan stains. For in situ hybridization, α1(IV)procollagen cDNA (7S region), and prolyl 4-hydroxylase β-subunit cDNA were labeled with digoxigenin-dUTP by the random primer method. The sections were rehydrated with PBS buffer
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and incubated with proteinase K (10 μg/ml) and were hybridized with hybridization buffer containing 300-500 ng/ml labeled cDNA overnight at 40°C. The sections were washed and incubated with anti- digoxigenin antibody conjugated to alkaline phosphatase, and the sections were observed by light microscopy.

RESULTS: Type IV procollagen mRNA and prolyl 4-hydroxylase mRNA were not detected until four weeks, however both mRNA were observed in the mesenchymal cells of the portal area and the zone around the central veins at six weeks.

After eight weeks, type IV procollagen mRNA was observed not only in mesenchymal cells but also in some hepatocytes. (Fig 1)

Fig.1. Type IV procollagen mRNA is detected in mesenchymal cells (a, x100) and hepatocytes (b, x100) after eight weeks.

REFERENCES: