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A rabbit model for liver fibrosis

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Summary
This experiment was carried out to investigate the role of cells participating in fibrosis induced by bile-duct ligation in rabbits. Histologically, bile stasis, degeneration and focal necrosis of hepatocytes, bile ductular proliferation, and an increase of the connective tissue were seen in periportal regions. Immunohistochemically, it was found that the majority of cells observed in the fibrosis regions were positive cells (spindle cells) for alpha-smooth muscle actin (ASMA). It is suggested that the spindle cells, probably transforming from Ito cells or myofibroblasts, play an important role in the pathogenesis of hepatic fibrosis.

Introduction
Bile duct ligation has frequently been used as a model for the production of hepatic cirrhosis in the dog *(Bosch et al., 1983)* and in rats *(Dufour et al., 1994)*. Previous investigations have demonstrated that common bile duct ligation causes the progressive bile stasis, focal necrosis, bile ductucular proliferations and periductucular and periportal inflammation in rats *(Bolt et al., 1981)*. Extracellular matrices in liver fibrosis are known to be produced by myofibroblasts that are transformed from fat-storing cells. The development of the fibrotic process is thought to be mediated by various fibrogenic mediators *(Akiyoshi and Terade, 1988)*. However, to our knowledge, there is not much information about this subject (bile-duct ligation-induced liver fibrosis) in rabbits. The purpose of this paper is to investigate cells participating in fibrosis induced by bile-duct ligation in rabbits.

Material and Method
Twenty adult, male, New Zealand rabbits, weighing between 1.5-2 kg were used in these experiments. There were two groups; control and treatment (n=10). The rabbits in the treatment group were premedicated with 0.5 ml/kg atropine intramuscularly (i.m.) (Atropine sulphate, 1 mg/kg, Vetas) and 5 minutes later 0.5 ml/kg rompun i.m. (Xylazin hydrochloride, 23-32 mg/ml, Bayer). For general anaesthesia 1.5 ml/kg ketalar were applied i.m. (ketamin hydrochloride, 50mg/ml., Eczacibası). After preoperative preparations the abdomen was entered through a midline cranial abdominal laparotomy. The common bile duct was identified at the edge of the hepatoduodenal ligament and double ligated as close to the duodenum as possible with non-absorbable suture material. Residual bile was aspirated from the gall bladder. The abdomen was then irrigated with warm lactated Ringer's solution before closure. Subcutaneous tissue and skin were closed routinely and an antibiotic administered for 7 days. The wounds were checked and cleaned daily until suture removal after 7 days. Two weeks after operation, livers of rabbits in each group (control and treatment) were removed under general anaesthesia. All animals received humane care according to criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Histopathological Examination:
Liver tissue specimens were fixed in 10 % neutral buffered formalin. Tissues were embedded in paraffin and sections were stained with hematoxylin and eosin (HE). Additional sections were stained with van Gieson and Masson trichrome. Immunohistochemical staining for alpha-smooth muscle actin (Zymed Laboratories, South San Francisco, CA), cytokeratin (Dako Labs., Santa Barbara, CA), CD68 (Dako), Alpha-fetoprotein (AFP) (Zymed) was performed with the avidin-biotin-peroxidase complex (ABC) procedure (HSU et al., 1981), using commercially available IP kits (Shandon, CadenzaTags peroxidase Kit with AEC, No: 407300).

Histopathological Findings:
In the ligated rabbits, liver histology showed progressive bile stasis in the bile ductular lumen and cholestasis in the cytoplasm of hepatocytes, degeneration and focal necrosis of hepatocytes, bile ductular proliferation, and periductular and periportal inflammation with mononuclear cell infiltrations, mostly spindle cells. An increase of the connective tissue was seen significantly in these regions. Radiation of bile ductular proliferation and fibrosis from portal tracts into the parenchyma were frequently seen in many regions (Fig 1).
However, no well-defined nodules indicative of cirrhosis were seen. There was no significant alteration of fibroblasts in the portal tracts. Immunohistochemically, positive cells for alpha-smooth muscle actin (ASMA) was particularly detected in the fibrosis regions (Fig. 2). Spindle cells in the fibrosis regions showing positive for alpha-smooth muscle actin (ASMA). Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain X 280. (Fig. 3). AFP was moderately present only in the cytoplasm of hepatocytes. CD68 immunohistochemistry staining showed a few portal macrophage infiltrations. There was cholestasis with an increase in serum bilirubin. The data of serum bilirubin were presented elsewhere.

Discussion
It is known that the sinusoid in normal liver is Ito composed of endothelial cells, Kupffer cells, Ito cells, pit cells, and extracellular matrix component *(Scheuer and Lefkowitch, 1994)*. Immunoelectron microscopy showed that ASMA-positive perisinusoidal cells were Ito cells; their ultrastructural features corresponded to those of myofibroblastic cells, and the other sinusoidal lining cells were negative for ASMA *(Enzan et al., 1996)*. Activation and transformation of lipocytes (Ito cells, stellate cells) into alpha-actin-positive myofibroblast-like cells is an essential step in the initiation of liver fibrosis *(Demirci et al., 1996)*. Ito cells in necrotic areas show myofibroblastic transformation and play a central role in the postnecrotic liver fibrosis, but portal fibroblasts play no significant part in this type of fibrosis *(Enzan et al., 1995)*. In the experiment presented here, fibrosis, consisting of broad bands of collagen fibres, spindle cells, and few macrophages, was frequently linked with the portal areas.

Fig. 1.

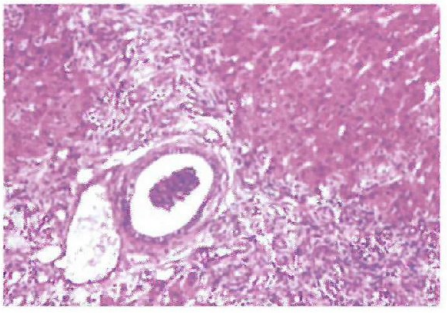


Fig. 2

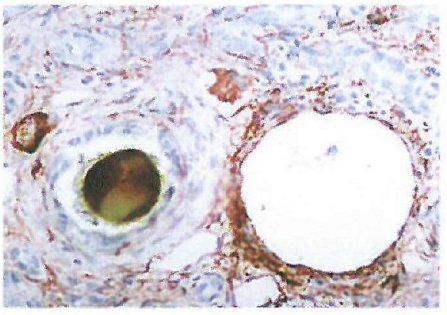
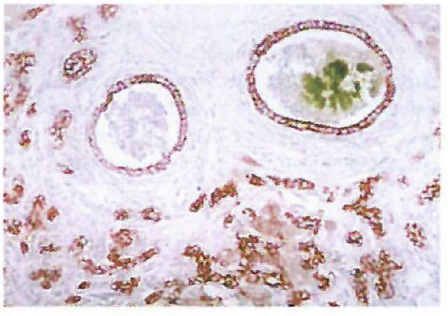


Fig. 3



Because the spindle cells were positive for alpha-smooth muscle actin, they might be Ito cells or myofibroblasts. These results suggest that the spindle cells may play an important role in the pathogenesis of hepatic fibrosis, which might arise from the portal area and perisinusoidal cells. The bile canalicular proliferations and hyperplastic bile duct epithelial cells reactions might be due to increased biliary pressure, the initiating factor in bile duct cell division. Serum elevation of AFP occurs in a large percentage of patients with liver cell carcinoma, and this feature can also be seen in the patients with rare hepatitis *(Barwick, 1996)* and during regeneration of liver tissue following experimental extirpation of liver tissue *(Hau et al. 1996)*. Brunello et al. (1993) stated that the alpha-fetoprotein increased in 43.58% of the patients with hepatocellular carcinoma and in 6.25% of controls with chronic liver diseases. In this study, AFP was in the cytoplasm of hepatocytes in paraffin-embedded liver tissue sections, as well.

The results presented here are similar to those previously reported in rats *(Desmouliere et al., 1999)* and dogs *(Nyland & Fisher, 1990; Shiga et al., 1996)* in terms of clinical and morphological appearances. In conclusion, it can be suggested that common bile duct ligation of the rabbits might be used as a model for the production of hepatic fibrosis, in addition to models in rats and dogs.

Refernces

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