Hepatic Fibrosis Produced in Rats by Repeated Intraperitoneal Injections of Swine Serum

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Pathological observations were made on male Sprague-Dawley rats which had received intraperitoneal injections of sterile swine serum twice a week for 12 weeks. Changes were found mainly in the liver, kidneys and spleen. In the liver, pseudolobules were formed in all animals after 8 or more weeks. Proliferation of collagen fibers was detected first around the central veins and in the portal tracts after 3 weeks, and then in the space of Disse. After 8 or more weeks, numerous fat-storing cells (Ito's cells) were found in the collagen septa subdividing the lobules. In the initial stage of fibrosis, infiltration of eosinophils and mast cells was observed around the central veins and in the portal tracts. There were no noticeable changes, however, in hepatocytes or sinusoidal lining cells throughout the experimental period. In the kidneys, fine electron-dense deposits were found in the mesangial area of glomeruli after 4 or more weeks. In the corresponding sites, specific fluorescence of rat IgG was detected by the immunofluorescence technique. In the spleen, swelling and increased lymphatic follicles with active germinal centers were noticed after 2 or more weeks.—Key words: Hepatic fibrosis, Fat-storing cell, Rat.


In hepatic fibrosis (or cirrhosis) of man and animals, mechanisms of fibrogenesis are not fully understood and the cells engaging in production of fibers are still controversial. It has been reported that hepatic fibrosis was induced in rats by repeated intraperitoneal injections of swine serum \([5, 7, 10]\). The present study was undertaken to elucidate the fibrogenic process in hepatic fibrosis with the aid of model fibrosis induced with swine serum. Another purpose of the present study was to clarify the initiating factor of the fibrogenesis.

MATERIALS AND METHODS

A total of 72 male Sprague-Dawley rats, six weeks of age, weighing averagely 210 g were used. Forty-two of these received intraperitoneal injections of 0.5 ml doses of sterile swine serum (Per-Freez Biologicals, Inc., Lot No. 41879) twice a week. After two, four, six, eight, 16 and 24 injections, six groups of seven animals were sacrificed in turn under ether anesthesia. The remaining 30 control animals were injected with saline and six groups of five animals were sacrificed in the same way and at the same intervals as in those injected with swine serum.

Immediately after sacrifice, the organs and tissues were fixed in a buffered neutral 10% formalin solution. Paraffin sections were stained with hematoxylin and eosin (HE). Periodic acid-Schiff reaction (PAS), Mallory-Heidenhein’s Azan stain, and toluidine blue stain (pH 7.0) were also used.

For electron microscopy, small blocks taken from the liver and kidneys were subjected to double fixation in 2.5% glutaraldehyde and 1% osmium tetroxide in a phosphate buffer solution. They were dehydrated in
graded alcohol and n-butyl glycidyl ether, and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed under an electron microscope, JEOL's model JEM-100C.

For immunofluorescence, the liver, kidneys and spleen collected from two animals from each group of animals injected with swine serum and those of controls at sacrifice. Then, these specimens were frozen in n-hexane immersed in dry ice-acetone and stored at −20°C. Cryostat sections were fixed in acetone at room temperature for 10 min. They were then treated with fluorescein-labelled rabbit anti-rat IgG and anti-swine albumin (both from Cappel Lab., Cochranville, Pa.) at 37°C for 60 min. After washing three times with PBS and mounting with glycerol in PBS (1:1), the sections were observed under a fluorescence microscope.

RESULTS

I. Macroscopic findings

Three of seven animals after eight injections of swine serum and all animals after 16 and 24 injections exhibited such hepatic changes as fading, irregularity of the surface, and increased hardness.

No noticeable changes were seen in any other organ, except splenomegaly observed after four or more injections of swine serum.

2. Light microscopic findings

Liver: No alteration was detected in any animal after two or four injections. In animals after six injections, infiltration of eosinophils and mast cells and increased spindle-shaped cells and fibrous elements were marked around the central veins (CVs) and in the portal tracts (PTs). The wall of interlobular arteries thickened and was deeply stained with eosin. There were no significant changes in the hepatocytes. In all the animals after eight injections, changes similar to the above-mentioned ones and proliferation of collagen fibers, radiating from CVs and PTs into the lobule, were seen everywhere in the specimens. In three of the seven animals, newly proliferated connective tissue fibers linked CVs to another CVs or to PTs and formed fibrous septa subdividing the lobules into

![Liver histology](image)

**Fig. 1.** Liver after 16 injections. Narrow collagen septa linking CV-CV or CV-PT are formed. Azan. ×70.

pseudolobules (Fig. 1). The septa were narrow, and included many spindle-shaped cells and a few eosinophils. Occasional acidophilic hepatocytic necrosis was seen in the vicinity of the septa. After 16 and 24 injections, collagenous septa were seen with increasing severity in all the animals. There were no noticeable changes in hepatocytes.

Other organs: In the spleen of animals after four or more injections, swelling and increased splenic lymphatic follicles were noticed. These follicles had active germinal centers containing many mitotic figures. No noticeable changes were observed in other organs including the kidneys and lymph nodes.

3. Electron microscopic findings

Liver: In animals after six injections, increased collagen fibers and a few fibroblasts, eosinophils and mast cells were observed
around the CVs and in the PTs (Fig.2). The interlobular arterial walls were thickened with increased collagen fibers in the media and the internal elastic lamina lost its regular arrangement (Fig.2). These changes, particularly increased collagen fibers and fibroblasts around the CVs were severer after eight injections (Fig.3). The collagen septa extending from CVs into the lobule were often included some fibroblasts. At the same time, increased fat-storing cells (FSCs) and collagen fibers surrounding FSCs were marked along the space of Disse. FSCs containing abundant dilated rough endoplasmic reticula and several large fat droplets extended their long cytoplasmic processes along the space of Disse (Fig. 4). Occasional hepatocytic necrosis was seen everywhere in the lobule. After 16 and 24 injections, numerous FSCs were included in the collagen septa linking
distinct thickening of the glomerular basement membrane.

4. Immunofluorescent findings

Liver: No specific fluorescence of rat IgG or swine serum albumin was detected in the liver throughout the experimental period.

Kidneys: After four or more injections, granular or segmented specific fluorescence of rat IgG of various intensities was found in the mesangial area (Fig. 7).

Spleen: After four injections, lymphocytes with strong specific fluorescence of rat IgG were observed around the germinal center in the midzone of lymphatic follicles. These cells increased in number in response to the repetition of the injection.

DISCUSSION

The present histopathological observations revealed characteristic hepatic fibrosis in rats after successive intraperitoneal injections of swine serum.

The changes of the liver were characterized by formation of collagen septa resulting in subdivision of the hepatic lobule without foregoing hepatocytic disturbances. Proliferation of collagen fibers was detected first around CVs and in PTs, and then a little later in the space of Disse. Closely related to the increased collagen fibers, fibroblasts proliferated around CVs and in PTs. On the other hand, increased FSCs and collagen fibers surrounding FSCs were conspicuously observed in the lobule along the space of Disse.

These findings strongly suggest that the cells playing an essential role in hepatic fibrosis are fibroblasts and FSCs. Concomitant appearance of many FSCs with the formation of collagen septa may suggest the role of FSC in active fibrogenesis in hepatic fibrosis. Ito's FSC, or lipocyte [1, 4] or perisinusoidal cell [6,11], is considered to effect fibrogenesis [2, 3, 8, 9, 12]. Kent et al. [4] and McGee and Patrick [6] supported this view with their observations on hepatic fibrosis induced by carbon tetrachloride.
Generally, necrosis of hepatic parenchyma was well known as the initiating factor of hepatic fibrosis [8, 9]. In hepatic fibrosis induced by swine serum, however, necrosis of hepatocytes may be ignored as the initiating factor of fibrogenesis. Kimura et al. [5], who performed electron microscopy observations on the liver of rats treated by a procedure similar to that used in the present experiment, considered that alteration of the endothelial cells in the sinusoids may have some relationship with the activation of FSC. With respect to this point, no sign of endothelial involvement was observed in the present study.

Paronetto and Popper [7] observed deposition of the immune complex in PTs in advance to septal formation, whereas the present authors could never detect any specific immunofluorescence of rat IgG or swine serum albumin anywhere in the liver. On the other hand, specific immunofluorescence of rat IgG was observed in the mesangial area of the renal glomeruli and in the lymphocytes in the midzonal area of the splenic follicles in the present study.

There is no doubt about the causal relation between the successive intraperitoneal injections of swine serum and the hepatic fibrosis in the experimental system used in the present study. Some immunopathological event may participate in pathogenesis of the hepatic changes though the factors motivating the fibrotic process are still unknown. It is possible that infiltration of eosinophils and mast cells around CVs and in PTs may be concerned with activation of such interstitial cells as fibroblasts and FSCs.

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REFERENCES

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要約

プタ血清の反復投与によるラットの実験的肝線維症: 北村和之・八十八島・岩崎 仁・小嶋尚広*1・
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農学部）——無菌プタ血清を週2回、線返し腹腔内注射することによってラットに惹起される病理学的
変化を12週間にわたって観察した。変化は主として肝臓、腎臓および肺臓に認められた。肝臓では約3
週頃から中心静脈周囲および門脈域に結合繊維線維増生が認められ、さらに Disses 腫にも電顕的に観察さ
れた。8週以降では、全検索例の肝繊に偽小葉およびその線維性中隔内における多数の脂肪細胞が（伊
東細胞）が観察された。線維増生の初期には中心静脈周囲および門脈域に好塩球および肥満細胞の浸潤
が観察された。肝細胞あるいは肝細胞内細胞については検査期間を通じて変化を認めなかった。腎臓では光
顕的には変化は指摘されなかったが2週以降、電顕的にメサンギウム域に電子密度の高い物質の沈着が
観察され、蛍光抗体法ではその部位にラット IgG の沈着が認められた。腎臓では2週以降、腎中心活性化を
示すリンパ細胞の増大が目立った。