Scanning Electron Microscopical Study of Intrahepatic Blood Vessels during Development of Experimental Cirrhosis in Rats

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Summary: The distribution and morphological changes of intrahepatic blood vessels in CC14-induced cirrhotic rat liver were studied. The terminal hepatic arterioles (THA) and terminal portal venules (TPV) showed a marked hemangiomatous or racemose proliferation during the early and middle stage of cirrhosis. The connective tissue around the hyperplastic nodules was rich in small vessels. The hepatic veins (HV) were decreased in number and slightly flattened and tortuous. The number of hepatic and portal venules decreased in the advanced stage. Their branches were arranged in rings and loops. Many anastomoses had formed in the middle and advanced stages of cirrhosis. The hepatic sinusoids showed stenosis and obliteration throughout the experiment. The results indicated that in early stage THA and TPV were increased and hepatic veins were all reduced and showed marked morphological changes. The blood supply of regenerative nodules was derived mainly from PV and, to a lesser extent, from HA.

Key words: SEM, intrahepatic blood vascular casts, experimental cirrhosis

A number of investigators have studied the alterations of intrahepatic vascular pattern in cirrhosis of liver by injecting different dyes into the blood vessels, serial sectioning, radioautography and vascular casting. Attention was paid mainly to the nature and morphology of changed vessels. However, their studies were limited by lower resolving power of light microscopy or by technical difficulty to prepare casts. Since scanning electron microscopy (SEM) found wide application in the field of medicine, normal intrahepatic vascular distribution pattern has been studied thoroughly. In the present investigation, the vascular casting, freeze-cracking and SEM were used to study the distribution and three-dimensional changes of intrahepatic blood vessels in CCl4-induced cirrhosis in rats.

MATERIALS AND METHODS

Induction of cirrhosis

96 (female 32) Wistar rats weighing 180-300 g were fed with simple corn flour mixed with 0.5% cholesterol and 20% swine fat in the first two weeks, and 25% - 30% alcohol was given as the only drink. The rats were given 0.2 ml carbon tetrachloride per 100 g body weight twice weekly for 4 - 10 weeks. They were weighed before each injection and the dose was calculated. The rats were sacrificed respectively on the 30th day (group 1, 12 animals injected 7 times), the 60th day (group 2, 12 animals injected 12 times), and the 90th day (group 3, 8 animals injected 18 times). The rest of the rats died during the experiment, 6
normal rats served as controls, 2 of which were sacrificed each time with the same interval of time as in the experimental groups.

**Perfusion of the liver**

The abdominal and thoracic cavities of the rats were opened so that the liver and heart were exposed after intraperitoneal injection of 0.5% pentobarbital sodium solution. The livers were irrigated via aorta cannula with Ringer's solution containing 0.5% heparin to remove blood, followed by 1.25% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) for 10 min to fix tissue at a constant pressure of 70–80 mmHg. The right lobe of the liver was removed for other samples. Afterwards the casting mixture of 5–10% acrylonitrile-butadiene-styrene polymer (ABS) acetone solution mixed with red or blue oil painting colour was perfused slowly through aorta and/or portal cannula. The perfusion was discontinued, when the casting mixture flowed freely from the draining vessel. After hepatic artery (HA), hepatic vein (HV) and portal vein (PV) were ligated, livers were taken out and put in running water for 48 h to harden the mixture.

**Casting sample preparation**

The liver that had become rigid was macerated in 38% hydrochloric acid for 2–3 days. Afterwards it was washed gently in running water for 24 h to remove decayed hepatic tissue, and then air-dried. Nine casts in group 1, eight in group 2, seven in group 3 were obtained. The casts were trimmed under stereomicroscope, and suitable blocks for SEM were resected, mounted on specimen stubs and coated with gold in a vacuum-evaporator. Samples were observed at an accelerating voltage of 10 kv in Hitachi S-520 SEM.

**Freeze cracking**

The freeze cracking was carried out according to Suzuki's method with slight modification. The cracked samples were critical point-dried and coated with gold in a vacuum-evaporator and examined with S-520 SEM. The livers of the control animals were treated and observed in the same manner as in the experimental groups.

**RESULTS**

**Hepatic artery**

In normal rat hepatic casts each portal vein radical was accompanied by a hepatic artery branch. Through observation on the experimental animal hepatic casts by SEM, it was found that HA and arterioles except for terminal hepatic arterioles were not increased nor enlarged. In group 1, the capillary peribiliary plexus distributed along PV was about 8 µm, while its afferent and efferent vessels were 15 µm in diameter. The perportal capillaries showed a marked hemangioma-like proliferation and budding. These buds had nearly an equal diameter, some varied in length, whereas others were just budding (fig.1). Many proliferated capillaries were observed in the connective tissue of periportal and interlobular spaces in freeze-cracking samples (fig.2). In group 2, some larger arteries and portal veins were seen to stretch out many capillaries with irregular ramifications and lumina of various size, of which some anastomosed with adjacent peribiliary plexus, and others sprouted to form the second or third ramification, showing racemose angiomatous proliferation (fig.3). The connective tissues of the periportal spaces were still richly supplied with capillaries, which were less in number than those in the freeze-cracking specimens in group 1. In group 3 casts, the arteriole radicals were found to be tortuous and irregular (fig.4) and some small vessels to drain directly into the hepatic sinusoids of the regenerative nodules.

**Hepatic vein and portal vein**

HV was reduced gradually with the development of cirrhosis. The terminal hepatic venules and preterminal hepatic venules could hardly be seen, whereas the terminal portal venules (TPV)