MICROCIRCULATORY RESPONSES TO ESTRADIOL BENZOATE IN CHRONIC LIVER DAMAGE INDUCED BY CARBON TETRACHLORIDE IN THE RAT

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Summary

Microcirculatory responses to estradiol benzoate (ES) under condition of chronic liver damage induced by long-term administration of carbon tetrachloride (CCl₄) was investigated in the rat. One hundred and five male rats were divided into the following groups receiving 0.1 mg/100 g body weight ES injected intraperitoneally 5 times per week: (1) controls, exposed to CCl₄ alone; (2) rats treated with ES from the fourth week of CCl₄ exposure; (3) animals treated with ES from the 11th week of CCl₄ exposure. In rats receiving CCl₄ alone, liver cirrhosis was induced by 10 consecutive weeks of exposure. Microangiograms of the liver demonstrated conspicuous rarefaction of the vascular tree. On the other hand, animals treated with ES had neither atrophic liver nor rarefaction of the intrahepatic vascular tree. ES produced also intrahepatic neovascular proliferation in the cirrhotic liver. After long-term CCl₄ administration, ES treated rats had extremely enlarged nodules with tumor-stain like findings, giving rise to a structure differing from hepatocellular carcinoma which latter generally displays a broom-swept appearance. It is concluded that in providing potent angiogenesis in the liver, ES protects the liver against microcirculatory derangement and parenchymal damage induced by CCl₄.

Key Words: Estradiol benzoate, Microangiogram of liver, Carbon tetrachloride-induced liver injury, Angiogenesis.

Introduction

Liver cells are known to be vulnerable to oxygen deprivation. In considering the role of microcirculation in regulating the blood flow, the intrahepatic microcirculatory system may be greatly responsible for liver tissue oxygenation, and its condition can be related to the aggravation as well as prevention of hepatic injuries. On the other hand, disturbed hormone metabolism, especially estrogen, has been found in varying degrees in patients with liver cirrhosis. Although it has been suggested that exogenous estrogen exerts a proliferative effect
on hepatic blood vessels\textsuperscript{1}), the role of this compound in the damaged liver is still a matter of debate. The purpose of this study was to evaluate the efficacy of synthetic estrogen in preventing the development of hepatic failure and to investigate microangiographic changes in the hepatic vasculature after liver damage with or without the administration of estrogen.

**Materials and Methods**

Male Wistar rats weighing approximately 170g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan. The animals were maintained on a basal diet (NMF) purchased from Oriental Yeast Co. Ltd., Tokyo, Japan. Estradiol benzoate (ES), a synthetic estrogen (Teikoku-Zoki Pharmaceutical Co. Ltd., Tokyo), was injected intraperitoneally in the experimental groups at a dose of 0.1 mg/100 g body weight five times weekly. 50\% Mika Conc in 5\% gelatine solution was used as contrast material. For the induction of cirrhosis, carbon tetrachloride (CCl\textsubscript{4}), mixed with an equal amount of olive oil, was administered subcutaneously in all groups, including controls, at a dose of 0.2 ml/100 g body weight twice weekly. Laparotomy was performed under ip pentobarbital sodium anesthesia and contrast material was injected into the abdominal aorta through a polyethylene tube with gentle thumb pressure on the syringe until the contrast material filled the liver surface. After total hepatectomy the liver was fixed in ice-cold 10\% formalin for gelling. Microangiography was performed using a soft X-ray apparatus (Softex CBM type, Softex Co. Ltd., Japan) on the whole livers as well as on 2–3 mm slices. The findings were compared with the histologic findings. For histologic examinations, liver specimens were fixed in 10\% formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and with reticulin. One hundred and five animals were divided into the following groups: (I) controls (n=35), treated with CCl\textsubscript{4} alone; (II) rats treated with ES from the fourth week of CCl\textsubscript{4} exposure (n=35); (III) animals treated with ES from the 11th week of CCl\textsubscript{4} exposure (n=35).

**Results**

Group I: CCl\textsubscript{4} administration induced hepatic fibrosis within a short period of time, and all animals developed cirrhosis by the 10th week. Microangiograms revealed changes in intrahepatic vascular trees after 7 weeks exposure to CCl\textsubscript{4}, and rarefaction of the peripheral vascular trees and irregular contour of relatively large vessels by 10 weeks exposure (Fig. 1). Fig. 2 shows the microangiogram obtained at 13 weeks after commencement of injection of CCl\textsubscript{4}, demonstrating extreme rarefaction of the vascular trees. Histology disclosed regeneration nodules of varying sizes with marked fatty changes (Fig. 3). Rats of this group progressed to hepatic failure and all animals died within 15 weeks.

Group II: The livers almost all animals of this group had a smooth surface and normal consistency at 13 weeks of exposure to CCl\textsubscript{4}.

![Fig. 1. Microangiographic findings of rat liver exposed to CCl\textsubscript{4} for 10 consecutive weeks (Group I): extreme rarefaction of the vascular trees and irregular contour of relatively large vessels are demonstrated. (Scale shown in all figures of this text indicates millimeters.)](image-url)