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Modifications of Hepatic Microvasculature in Early Period of Diabetic Rats

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Abstract. The liver is a central organ in control of glucose homeostasis. Diabetes mellitus is a chronic disorder of blood sugar metabolism. The association between two issues is well known at present. This study was investigated the effects of diabetes induced by streptozotocin (STZ) on the liver tissues and microvasculature of male Sprague-Dawley rat by using histological study and scanning electron microscopic study of vascular corrosion cast. Diabetes was induced by a single dose intraperitoneal injection of STZ 60 mg/kg, whereas the control group was injected with the citrate buffer. Four weeks after induction, the livers were excised and processed for histological and vascular corrosion cast studies. Markedly appearances of large portal triads, hypertrophy of hepatocyte, perisinusoidal fibrosis, and decrease in sinusoidal diameter were revealed. The alterations of hepatocytes and their vessels were exhibited in the early diabetes.

1. Introduction

The largest internal organs in the body is the liver that plays many vital functions, including infiltration of blood, formation of bile and coagulation factors, and detoxification. Moreover, one of many important roles of the liver is the maintenance of glucose homeostasis for a continued supply to organs that require a glucose energy source [1]. All those hepatic functions are regulated by liver blood flow into hepatic sinusoid that is high about 1,050 ml/min from the portal vein and an additional 300 ml/min form the hepatic artery [1]. Functional impairment of the liver frequently occurs in the patients with poorly controlled diabetes mellitus (DM), a metabolic disease characterized by hyperglycemia [2]. On the other hand, diabetes is often found in the patients with chronic liver disease, especially liver fibrosis [3]. The association of diabetes with liver disease has been mostly investigated. In many previous studies, the physical and biochemical alterations of the liver in the presence of diabetes are revealed. The diabetic animal model can be generated by various technique such as transgenic, viral, chemical inductions, and surgical removal of the pancreatic beta cells as well as even pancreatectomy [4]. The most well-known chemical induction, which has been used as a diabetogenic agent in laboratory animals, is streptozotocin (STZ). STZ is a nitrosourea that causes irreversible pancreatic beta cell destruction in rats, thereby providing a suitable animal model for research on type 1 diabetes mellitus [5, 7]. DM promotes liver fibrosis and inflammation, giving rise to hepatocellular failure [3]. However, the effect of diabetes on the hepatic microvessels, that are essential for maintain the liver physiologic functions, has not been studied. Therefore, the alterations of hepatic tissue and microvascular structures in short-term STZ-induce diabetic rats were investigated by using histological study and scanning electron microscopy (SEM) of hepatic vascular corrosion casts. This present study might provide the insight into liver pathology and microvascular complication in early pathological processes in diabetes which may offer primary medical prevention against liver disease.

2. Materials and Methods

A total of 14 male Sprague-Dawley rats, 5-8 week old, weighing 250-270 g obtained from National Laboratory Animal Center, Mahidol University, was constituted the animal model in this study. All animals were housed in the individual cage and was fed on a standard laboratory rat chow and water ad libitum. They were randomly assigned into two groups: control (n=6) and diabetes (n=8). Liver specimens were investigated by utilizing two approaches of light microscopy (LM) (n=6: control=3, DM=3) and SEM (n=8: control=3, DM=5), respectively.

Animal model of DM

Diabetes was induced by a single intraperitoneal injection of 60 mg/kg body weight STZ dissolved in 0.1 mol/L citrate buffer, pH 4.5 (Across Organic, Janssen Pharmaceutical, Belgium). Control rats were injected with vehicle alone in the same route. At 72 hours post induction and the end of experiment, whole blood glucose were measured by using OneTouch® Ultra® glucometer and glucose test strips (LifeScan, Inc. 2005, California, U.S.A.). Rats with more than 300 mg/dL of whole blood glucose were considered as the diabetes. All rats were kept for 4 weeks after induction to be the short-term period of diabetes.

Histological procedures

Under deep anesthesia, rats were perfused with 0.9% NaCl followed by Bouin's solution. The liver was then removed and postfixed in Bouin's solution overnight, afterwards the excised liver was processed into paraffin blocks and sectioned (5µm thick). The slides were stained with hematoxylin-eosin (H&E) to investigate the general morphology and Mallory's phospho-molybdic acid-hematoxylin for collagen fiber staining. The samples were observed under the microscope (Olympus BX41™, Japan) connected to a digital camera (Olympus DP 70™, Japan).

Vascular corrosion cast technique and SEM

By using vascular corrosion cast technique; Batson's no. 17 plastic mixture (Polyscience Inc., U.S.A.) was injected into the ascending aorta after perfusion with 0.9% NaCl. After plastic mixture polymerization, the liver was removed, corroded in 40% KOH and washed in the water to remove the remaining tissues. The specimens were critical point dried, mounted on a SEM stub and coated with gold palladium. The liver samples were viewed and photographed under a SEM (JEOL JSM 25S, Japan).

3. Results

To compare the fundamental structures of the normal liver to those of the diabetic one, the liver sections stained with H&E were performed. The livers of control rats had normal structures (Fig. 1A). The liver was composed of lobules (Fig. 1), which were roughly hexagonal in shape with a central vein (CV) in the middle and portal triads (PT) at the corner, in the figure PT and CV in the corner (Fig. 1B). The hepatocytes organized into one cell thick of hepatic cords or plates running radially from the central vein. They were separated by hepatic sinusoid that converged toward the central vein.

The liver of diabetic rat showed the dilatation of three components in the portal triads; the interlobular branches of portal vein, hepatic artery, and bile duct (Fig. 1B).

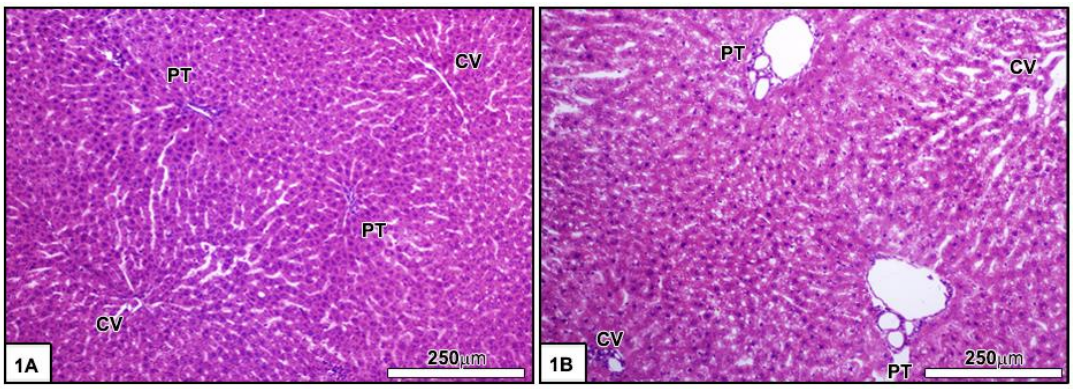

Fig. 1. Light micrographs of liver in control (1A) and diabetic rats (1B). Code: PT= portal triads, CV= central vein.

In the Mallory's phospho-molybdic acid-hematoxylin staining, with the lower magnification, the control rats (Fig. 2A), the collagen fibers mainly concentrated around the portal triad (PT). In the diabetic rats (Fig. 2B), a marked increase in blue colored strands of collagen fibers (black arrowheads) was mainly found in the perisinusiodal space. The hepatocytes (H) regularized into hepatic cords radiating from portal triads (Figs. 2A-B). Moreover, there were enlarged portal triads (PT) in diabetes (Fig. 2B), when compared to those in the control rats (Fig. 2A). With the higher magnification, there were obviously collagen fibers (black arrowheads) accumulated in the entire length of sinusoidal lumen (S) in diabetes (Fig. 2D). In the contrary, the collagen fibers were found very faint along sinusoidal lumen (S) in the control rats (Fig. 2C). Hypertrophy of hepatocytes (H) (Fig. 2D) were examined in the diabetic liver.

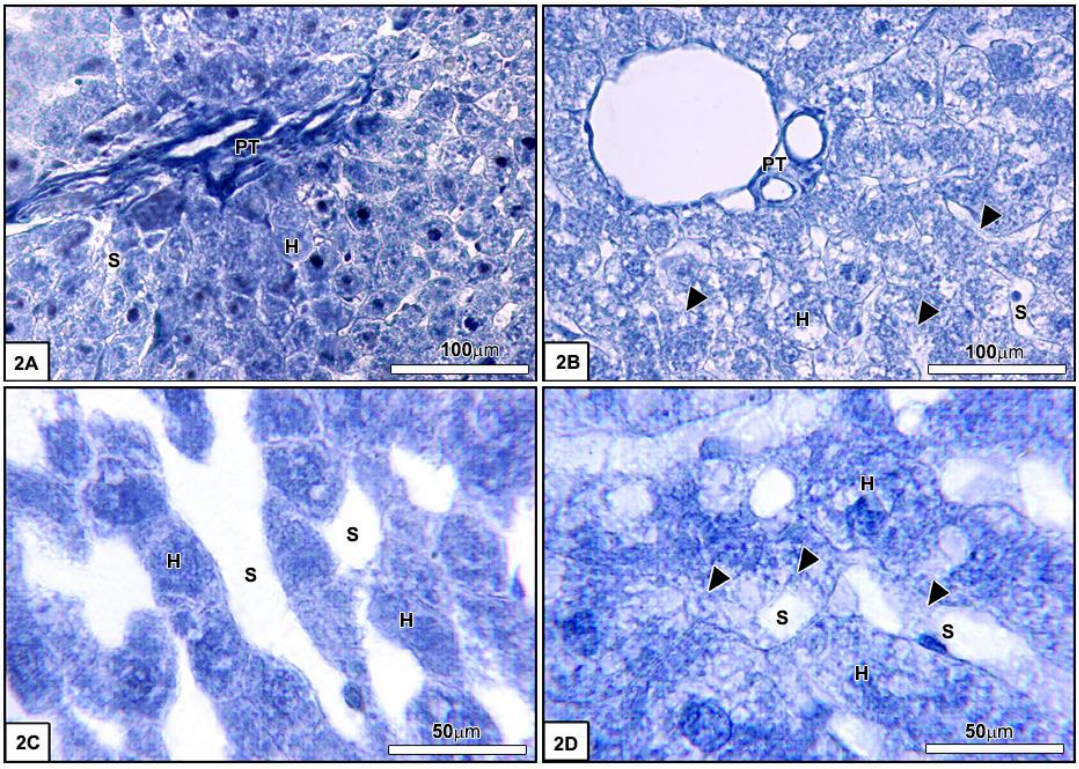

Fig. 2. Light micrographs of liver in control (2A, 2C) and diabetic rats (2B, 2D). Code: PT= portal triad, S= sinusoidal lumen, H= hepatocyte, black arrowheads= collagen fibers.

In the SEM study on corrosion casts of rat liver vasculatures, the sinusoidal vessels were a plexiform networks of anastomosing sinusoidal capillaries. The formation of these vessels was a uniform regular mesh similar to the sinusoidal capillaries pattern in the histological appearance. Each meshwork surrounded a tubular cavity, a central vein (CV) (Fig. 3). With low magnification images, the dense interconnecting networks in casts of the normal liver (Fig. 3A) were shown, by contrast, more loosen polygonal meshes occurred in the diabetic liver (Fig. 3B). Some area in diabetic liver showed the defect in the vascular network (a black star) (Fig. 3B). All sinusoidal capillaries drained into the central vein (CV) (Figs. 3A-B).With high magnification image, the tortuous anastomotic sinusoids in the liver were shown (Figs. 3C-D). There was a striking decrease in diameter in the diabetic stage (Fig. 3D) when compared to those with the control rats (Fig. 3C). It was indicated that the destructions of liver tissue and microvasculature in early diabetic duration.

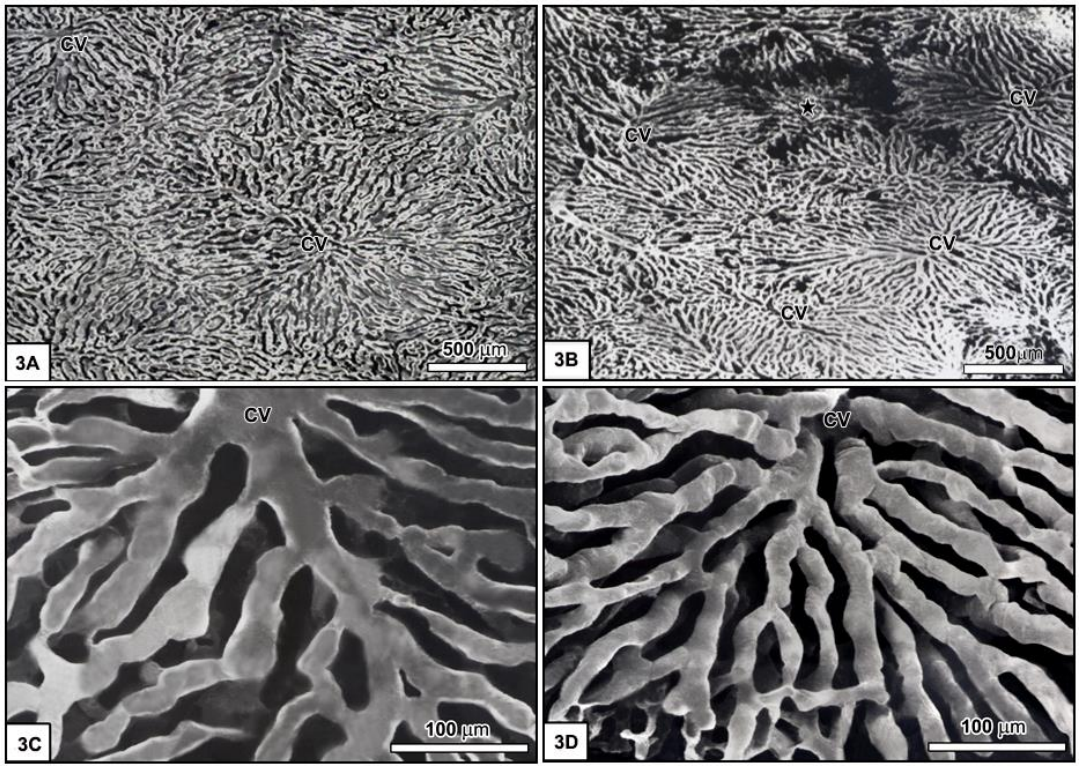

Fig. 3. SEM micrographs of liver vascular casts of control (3A, 3C) and diabetic rats (3B, 3D). Code: CV= central vein, a black star= vascular defect.

4. Discussion

The effects of diabetes on the surrounding liver tissues and hepatic microvasculature were important to examine, because these may yield the information on the liver damage related with dysfunction during the development of diabetes. In this study, DM induced a hypertrophy (increase in the cytoplasmic area) of hepatocytes and non-parenchymal cells. It is widely known that a deficiency in insulin during diabetes prevents the blood glucose to the cells. Then, the cells are unable to utilize the glucose and lack in sufficient energy. The metabolic overload occurs in the cells to get their energy, therefore, the swelling of the hepatocytes in the diabetic rats was observed, corroborated data by Remedio et al. [6] and Kume et al. [7]. They found that high increase in number of mitochondria in the hepatocytes of both alloxan-treated rats and STZ-induced diabetic mice, and it is indicated that there is a high demand of ATP in the diabetic cells. Moreover, the swollen mitochondria as a compensation of impaired energy production has been reported in the hepatocytes of diabetic rats [8].

An increase in numbers of the intracellular organelles and their sizes may be related to hypertrophy of the liver cell. In this observation, the sizes of structures in the portal area in the DM are larger than those in the control groups. All compositions in the portal area, interlobular branches of portal vein, hepatic artery and bile duct, dilated. Recent study has been reported that diabetes is a risk factor for hepatic perisinusoidal fibrosis via active hepatic stellate cells [9, 10]. Moreover, vasoactive dilator production such as nitric oxide (NO) is reduced in the diabetic condition [11]. Decreased NO leads to constrict sinusoidal capillaries. Either hepatic fibrosis or vasoconstriction of sinusoidal capillaries contributes to increase intrahepatic resistance as portal hypertension [12]. It was suggested that dilated vessels at portal area may associate with portal hypertension to provide sufficient nutrient and oxygen to the liver. In addition, an elevation of bile acid secretion and high biliary flow have been reported in diabetes, which supported bile duct dilatation in this study [5]. It is certain that the chronic diabetic state leads to hepatic fibrosis and forms cirrhosis later [2]. In the present study, an increased collagen fiber deposition along the perisinusoidal space was found at the beginning of diabetic progression and the late stage of diabetes reported by our previous report [10]. By using transmission electron microscopy, the numerous collagen fibers in the perisinusoidal space or space of Disse in diabetic liver were identified [10]. In the possible mechanism, hyperglycemia contributes to an increased formation of advance glycation end products (AGE), which is a result of spontaneous reactions of free sugars with several proteins [3]. AGE interacts with the receptors in the hepatic stellate cells, leading to the inappropriate expression of profibrogenic cytokine that finally results in liver fibrosis [9, 13]. The extensive deposition of perisinusoidal collagen from the active stellate cells throughout the liver compressed the sinusoidal space, then, the reduction in sinusoidal diameter as evidence in SEM study on vascular corrosion cast of diabetic liver, was shown in this study. The same situation was also observed by other study [14]. Another cause of sinusoidal narrowing is the activation of protein kinase C (PKC) pathway induced by hyperglycemia. PKC activation reduces the vasodilator compound, particularly NO, which is a potent vasodilator, however it increases the vasoconstrictive factors, such as the vascular endothelial growth factor, resulting in narrowing of the vascular lumen [15].

5. Conclusion

The evidences of liver pathology and its vascular structural abnormalities induced by early diabetes were hypertrophic hepatocytes, dilatation of portal triads, extensive deposition of sinusoidal collagen, and sinusoidal narrowing. This new data should be explained that the pathology of diabetic liver tissues and their microvascular frameworks providing basic knowledge from the animal model. So that, this finding contributes an important data for clinical research and application in the future.

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