Short Communication

CYTOMEGALIC HEPATOCYTES AND BILE DUCT HYPERPLASIA IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

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Abstract: Following the induction of diabetes by streptozotocin (SZ) (50 mg/kg/day for 5 consecutive days), cytomegalic hepatocytes (0/7 at 4 weeks after the last SZ-injection (4WAI), 2/7 at 8WAI and 3/7 at 12WAI) and bile duct hyperplasia (0/7 at 4WAI, 2/7 at 8WAI and 2/7 at 12WAI) developed in some mice. Cytomegalic hepatocytes showed a prominent increase in number of mitochondria and were frequently accompanied with invagination of cytoplasm into nuclei. Prominent bile duct hyperplasia was found in 4 out of 5 mice bearing cytomegalic hepatocytes. Hyperplastic bile ducts were lined with epithelia of various types and accompanied no apparent connective tissue proliferation around them. (J Toxicol Pathol 7: 261–265, 1994)

Key words: Streptozotocin, Mice, Cytomegalic hepatocytes, Bile duct hyperplasia

Streptozotocin (SZ), which has a prominent toxic action on islet beta cells, is now frequently used to induce complications of diabetes in laboratory animals. It is also known that SZ has acute toxic effects on many organs and leads to delayed-type toxicity in some organs. Therefore, full attention should be paid to systemic histopathological alterations when SZ-treated animals are used for studies on complications of diabetes. Recently, we observed some interesting hepatic alterations in SZ-treated mice which were offered to the study on experimental diabetic glomerulopathy. This paper describes the histopathology of the hepatic alterations.

Forty-two 8-week-old ICR: CD-1 male mice (Charles River Japan Inc., Kanagawa) weighing 38.7±2.0 g were used. The animals were housed in an isolator caging system (Niki Shoji Co., Tokyo) in an animal room under controlled conditions (temperature: 23±2°C, humidity: 55±5%) and fed MF pellets (Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum. A half of the animals were intraperitoneally injected with 50 mg/kg/day of SZ, which was dissolved in 0.1 M citrate buffer (pH 4.5) just before daily use, for 5 consecutive days, and 7 each of them were killed by heart puncture under ether anesthesia at 4, 8 and 12 weeks after the last SZ-injection (4, 8 and 12WAI), respectively. The remaining animals were injected with citrate buffer alone and killed in the same way.

At autopsy, immediately after sampling blood for blood glucose contents, the liver was taken from each animal, weighed and fixed in 10% neutral buffered formalin. Four μm-paraffin sections were stained with hematoxylin and eosin (HE). Some of them were also stained by periodic acid-Schiff (PAS) or Masson's trichrome staining.

Small pieces were taken from the 10% neutral
buffered formalin-fixed liver, in which cytomegalic hepatocytes were observed light microscopically. They were refixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in 1.0% osmium tetroxide in the same buffer and embedded in epoxy resin (Quetol 812, Nissin EM Co., Ltd., Tokyo). Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a JEOL-1200EX electron microscope (JEOL Co., Ltd., Tokyo).

Significant depression of bodyweight gain and marked and persistent elevation of blood glucose level (over 500 mg/dl) were recorded throughout the experimental period. The liver to bodyweight ratio increased with the lapse of time and reached the level of 1.6 times larger than controls at 12WAI.

Throughout the experimental period, the most common histopathological change in the liver was hypertrophy of hepatocytes due to an increase of intracytoplasmic acidophilic granules (Fig. 1). Hepatocytes generally had little glycogen granules and sometimes showed nuclear enlargement with intranuclear inclusions. These hepatocytes bear some histological similarities to so-called oncocytic cells as previously pointed out by Almocida et al.\textsuperscript{11} in mice which were given a single dose of SZ (200 mg/kg) and killed 4WAI.

As shown in Table 1, cytomegalic hepatocytes were observed in several animals at 8 and 12WAI, and some of them accompanied a marked bile duct hyperplasia. At 8WAI, being common to 2 cases (Table 1), cytomegalic hepatocytes diffused almost all over the hepatic lobules. In some hepatic lobules of these 2 cases, bile duct hyperplasia was simultaneously found occupying a large area of the

![Liver of a control (A) and an SZ-injected mouse (B) at 4WAI. Hypertrophy of hepatocytes of an SZ-injected mouse due to an increase of intracytoplasmic acidophilic granules. HE ×125.](image)

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<th>Table 1. Incidence of Cytomegalic Hepatocytes and Bile Duct Hyperplasia in SZ-Induced Diabetic Mice</th>
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<td>Cytomegalic hepatocyte</td>
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\textit{WAI: Weeks after the last SZ-injection}  
\textit{C: Control mice, SZ: SZ-injected mice}  
\textit{* No. of mice showing lesion/No. of mice examined}
hepatic lobule (Fig. 2). Hyperplastic bile ducts were lined with epithelia of various types. Namely, some were lined with squamous epithelia, and others with cuboidal or ciliated columnar ones (Fig. 3). They accompanied no apparent connective tissue proliferation around them.

At 12WAI, in one of three cases (Table 1), bile duct hyperplasia sometimes built up pseudolobules. Pseudolobules were mainly composed of hepatocytes with almost normal appearance, which were surrounded with a narrow belt composed of cytomegalic hepatocytes (Fig. 4). Pseudolobules showed no distinct arrays of hepatic cords. In the remaining 2 cases, cytomegalic hepatocytes diffused almost all over the hepatic lobules with no bile duct hyperplasia.

The most characteristic ultrastructural picture of cytomegalic hepatocytes was a prominent increase in number of mitochondria (Fig. 5), and a light microscopic intranuclear inclusion was an invagination of cytoplasm into a nucleus (Fig. 6).

Although cytomegalic hepatocytes generally share fundamental histopathology with the above-mentioned acidophilic granular hepatocytes, they...
Fig. 4. Liver of an SZ-injected mouse at 12WAI. Pseudolobule composed of hepatocytes with almost normal appearance. HE ×125.

Fig. 5. Hepatocyte of an SZ-injected mouse at 12WAI. A prominent increase in number of mitochondria. ×5,000.

seem to step widely forward a degenerative process. On the other hand, although the pathogenesis of bile duct hyperplasia is obscure, it is noteworthy that bile duct hyperplasia was always accompanied with the appearance of cytomegalic hepatocytes in the present material.

Bile duct hyperplasia was also reported in SZ-induced diabetic Chinese hamsters⁸ and Jcl: SD rats¹⁰ at a high frequency 6 months or more after the SZ-treatment. In those cases, however, bile duct hyperplasia was not multifocal and the hyperplastic ducts were lined with columnar epithelia. In SZ-induced diabetic Chinese hamsters and Jcl: SD rats, cholangioma was also found in a high incidence one year or more after the SZ-treatment. This suggests a possibility that bile duct hyperplasia has some relation to the later development of cholangioma in SZ-induced diabetic animals. Now long-term observations on the liver of SZ-induced diabetic mice are in progress.
Fig. 6. Hepatocyte of an SZ-injected mouse at 8WAI. Note invagination of cytoplasm into nucleus. × 3,000.

References