Glomerular Lesions in Unilateral Nephrectomized and Diabetic (UN-D) Mice

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ABSTRACT. Experimental diabetes was induced in both control and unilaterally nephrectomized male mice by injecting streptozotocin (SZ) (50 mg/kg x 5 days) one week after nephrectomy. The time course changes in the glomerular lesions were examined for up to 12 weeks after completion of the SZ-injection (12WAI). In unilateral nephrectomized and diabetic mice, mild segmental expansion of the mesangial area developed at 4WAI, and it progressed to prominent segmental glomerulosclerosis at 12WAI. In the electron microscopic examination at 12WAI, marked expansion of the mesangial area, segmental thickening of the glomerular basement membrane, fusion of the foot processes of podocytes and a prominent increase in the number of microvilli of capillary endothelial cells were observed. On the other hand, mild to moderate expansion of the glomerular mesangial area was only sporadically found in unnephrectomized diabetic mice at 12WAI. Interestingly, Bowman's capsules of diabetic mice were generally lined with flattened epithelia but those of non-diabetic mice with cuboidal or low columnar epithelia.---KEY WORDS: diabetes, glomerular lesion, mouse, streptozotocin, unilateral nephrectomy.

Streptozotocin (SZ) was originally developed as an antibiotic and antitumor agent. Since it was revealed that SZ had a toxic action on islet beta cells [6], SZ has been attracting great attention as a useful tool for either the induction of insulin-dependent diabetes mellitus (DM) or the investigation of its complications in rodent species, especially in rats [1, 3, 4, 6, 8]. However, a long induction period is required to produce marked glomerular lesions, one of the most important complications of DM, in rats or mice. In this connection, Steffes et al. [9] reported that SZ produced prominent glomerular lesions in a shorter time period (6 months) in unilaterally nephrectomized rats [9]. Recently we succeeded in inducing severer glomerular lesions in a much shorter period (12 weeks) in male mice by a similar experimental procedure to that reported in rats by Steffes et al. [9]. In addition, a very interesting morphological feature was found in Bowman's capsules in these mice. The purpose of this study is to describe the details of glomerular changes in these mice.

MATERIALS AND METHODS

One hundred 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 throughout the experimental period.

The mice were divided equally into the following 4 groups: control (C), SZ-induced diabetes (D), unilateral (left kidney) nephrectomy (UN), and unilateral nephrectomy-SZ-induced diabetes (UN-D) groups. As shown in Fig. 1, mice in the D and UN-D groups received SZ at 50 mg/kg of body weight per day for 5 consecutive days from 1 week after nephrectomy. SZ (Lot No. 78R-5017, Sigma, St Louis, MO, U.S.A.) was dissolved in 0.1 M citrate buffer solution (pH 4.5) just before daily intraperitoneal injections.

Body weight and food and water consumptions for 24 h were recorded weekly throughout the experimental period. Seven to ten mice from each group were killed by heart puncture under ether anesthesia at 4, 8 and 12 weeks after completion of the series of SZ-injections (4, 8 and 12WAI), respectively. Blood glucose, blood urea nitrogen (BUN) and creatinin (CRNN) levels were measured on each blood sample obtained at autopsy by means of an autoanalyzer, Monach (Instrumentation Laboratory, U.S.A.).

At autopsy, after measuring the weight, the kidneys were fixed in 10% neutral buffered formalin, and 4 μm paraffin sections were stained with hematoxilin and eosin (HE), periodic acid-Schiff
(PAS) or periodic acid methenamine silver (PAM) for light microscopic examination.

For electron microscopic examination, small pieces of renal cortex obtained from the C and UN-D mice (2 mice each) killed at 12WAI were fixed in the mixture of 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 double stained with uranyl acetate and lead citrate and observed under a JEOL-1200EX electron microscope (JEOL Co., Ltd., Tokyo).

RESULTS

Food and water consumption: There were no significant changes in food and water consumption in the C and UN groups throughout the experimental period. On the other hand, in the D and UN-D groups, food and water consumption increased from 2WAI and reached very high levels at 12WAI (food: about twice higher than the level of the C group, water: about 8 times higher than the level of the C group). In these groups, prominent polyuria was also observed with the increase in water consumption from 2WAI to the end of the experiment.

Body and kidney weight: A significant depression of body weight gain was recorded in the D and UN-D groups throughout the experimental period (Fig. 2). The right kidney weight was significantly greater in the UN and UN-D groups than in the C and D groups (Fig. 3).

Serum biochemical findings: In the D and UN-D groups, blood glucose increased markedly at 4WAI and remained over 550 mg/dl until the end of the series of experiments. In contrast, blood glucose levels in the C and UN groups were about 200 mg/dl at any time. In addition, the BUN level in the UN-D group and CRNN levels in the D and UN-D groups were significantly higher than those in the C and UN groups (Table 1).

Light microscopic findings of the kidney: In the UN-D group, mild segmental expansion of the mesangial area was found in some glomeruli in the juxtamedullary cortex of a small number of mice at 4WAI. At 8WAI, glomerular lesions extended to all
parts of the cortex in many mice and were characterized by mild to moderate segmental expansion of the mesangial area due to an increase in matrix materials positively stained by PAS and/or PAM methods (Fig. 4). At 12WAI, almost all mice had prominent glomerulosclerosis (Fig. 5), and partial adhesion of glomerular tufts to Bowman's capsule was sometimes observed.

In the D group, glomerular lesions similar to those in the UN-D group at 8WAI were sporadically found in a few mice at 12WAI (Fig. 6). Such glomerular changes as observed in the D and UN-D groups were not detected in the C and UN groups throughout the experimental period.

Bowman's capsules were, in general, partially or completely lined with cuboidal or low columnar epithelial cells in the C and UN groups (Fig. 7). However, those of the D and UN-D mice were
generally lined with flattened epithelia, and cuboidal cells were rarely observed at the urinary pole (Figs. 4–6).

Electron microscopic findings: In mice in the UN-D group at 12WAI, consistent with the light microscopic findings, segmental expansion of the mesangial area due to an increase in basement membrane-like materials was conspicuous (Fig. 8). There was, however, no amyloid fibrils in such an expanded mesangial area. In addition to mesangial alterations, segmental thickening of basement mem-

brane (Fig. 9) and fusion of foot processes of podocytes (Fig. 10) were sometimes observed. In some parts of these glomeruli, a large number of microvilli were seen at the luminal surface of capillary endothelial cells (Fig. 11).

Glomeruli of mice in the C group showed no significant ultrastructural changes.
pathy as proposed concerning UN-D rats [9].

At 12WA1, almost all mice in the UN-D group developed prominent segmental glomerulosclerosis, which was apparently severer than that in UN-D rats at 6 months after unilateral nephrectomy and SZ-treatment [9].

Ultrastructural changes in the UN-D mice were also severer than those in the UN-D rats. In addition to the changes in the mesangial area and basement membrane common to the UN-D mice and rats [9, 10], fusion of foot processes of podocytes which is one of the main diabetic glomerular alterations [10] and a prominent increase in the number of microvilli of some glomerular capillary endothelial cells, *i.e.* "archade-formation" [7], were detected in the UN-D mice. Although the latter is generally said to be a reflection of an increased absorptive function of endothelial cells [7], its real meaning is still obscure.

In some glomeruli of the UN-D mice, there was a small nodular sclerotic lesion probably due to a marked increase in the amount of mesangial matrix materials, which was a major histopathological indicator of human diabetic glomerulopathy. Such a nodular lesion, on the other hand, is generally said to develop at a later stage in diabetic glomerulopathy [10]. Therefore there remains the possibility that UN-D mice and rats may be seen to develop the nodular lesion if observed longer. From this point of view, mice seem to be a more useful model than rats because glomerular lesions progressed faster, as shown in the present study.

Bowman's capsules in sexually mature male mice are usually lined with cuboidal or low columnar epithelial cells and are called "male-type Bowman's capsule". This type of capsular epithelium is considered to be a male hormone-dependent feature and has been reported to change into a flattened epithelium after castration [5]. Interestingly, Bowman's capsules of male mice in SZ-induced diabetic groups were generally lined with a flattened epithelium, i.e. "female-type Bowman's capsule" [5]. This suggests that diabetic conditions may also influence the morphology of male-type epithelial cells of Bowman's capsules probably through continuously increased hydrostatic pressure in the glomerular cavity due to marked and persistent urine production. Flattened epithelia of Bowman's capsules in ICR: CD-1 female mice were not influenced by SZ-induced diabetic conditions [2].

**Fig. 10.** A glomerulus of a UN-D mouse at 12WA1. Fusion of foot processes of podocytes. Bar=1 μm.

**Fig. 11.** A glomerulus of a UN-D mouse at 12WA1. A large number of microvilli at the luminal surface of capillary endothelial cells. Bar=1 μm.

**DISCUSSION**

Although the serum glucose level was similar in the D and UN-D groups and mice in the UN group exhibited no significant changes in glomerular morphology, glomerular lesions appeared earlier and were prominently severer in the UN-D group than in the D group. This suggests that microcirculatory dynamics altered by unilateral nephrectomy may accelerate the development of diabetic glomerulo-
REFERENCES