LECTIN HISTOCHEMICAL STUDY OF THE KIDNEY IN UNINEPHRECTOMIZED AND DIABETIC (UN-D) MICE

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Abstract: Lectin histochemical characteristics of the kidney of experimentally induced diabetic mice were investigated. Mice were divided into the following 4 groups: control (C), streptozotocin (SZ)-induced diabetes (D), unilateral nephrectomy (UN), and unilateral nephrectomy-SZ-induced diabetes (UN-D) groups, and killed at 12 weeks after completion of the SZ-injection. No lectin histochemical differences were observed in the glomeruli of all groups. Stainabilities of Bowman's capsules lined with cuboidal to columnar epithelia seen in C and UN groups were the same as those of proximal tubules, and they were different from those of Bowman's capsules lined with flattened epithelia generally seen in D and UN-D groups. In D and UN-D groups, distal tubules and collecting ducts increased their affinities to Canavalia ensiformis A, Dolichos biflorus agglutinin, Griffonia simplicifolia agglutinin-I, Glycine max agglutinin, and Triticum vulgaris agglutinin, compared with C and UN groups. Conclusively, this study showed that unilateral nephrectomy enhanced SZ-induced kidney lesions without a prominent histochemical modification. (J Toxicol Pathol 6: 67~71, 1993)

Key words: Diabetes mellitus, Lectin histochemistry, Mice, Streptozotocin, Unilateral nephrectomy

Introduction

Streptozotocin (SZ) has been used 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. However, a long induction-period is required to produce marked glomerular lesion, one of the most important complications of DM, in rats or mice. Recently, we succeeded in the induction of marked glomerular lesions within a short time period (12 weeks) by SZ-inoculation to unilaterally nephrectomized mice.

The purpose of this study is to clarify the lectin-binding characteristics of the kidney of this newly developed model for diabetic nephropathy, in which many lectin histochemical changes have been reported.

Materials and Methods

Twenty 8-week-old ICR: CD-1 male mice (Charles River Japan Inc., Kanagawa) weighing about 38 g were used. Animals were housed using an isolator caging system (Niki Shoji Co., Tokyo) in an animal room under controlled condition (temperature: 23±2°C; humidity: 55±5%) and fed commercial pellets (MF: Oriental Yeast Co. Ltd., Tokyo) and tap water ad libitum throughout the experimental period.

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. Mice of D and UN-D groups received SZ at 50 mg/kg of body weight per day for 5 consecutive days from 1 week after ne-
phrectomy. SZ (Lot No. 78F-5017, Sigma, St Louis, MO, USA) was dissolved in 0.1 M citrate buffer solution (pH 4.5) just before daily intraperitoneal injection.

All mice of each group were killed by heart puncture under ether anesthesia at 12 weeks after completion of the SZ-injection. Blood glucose levels were measured on blood samples obtained at autopsy using an autoanalyzer, Monach (Instrumentation Laboratory, USA). Kidneys were fixed in 10% neutral buffered formalin and 4-μm-paraffin sections were stained with hematoxylin and eosin (HE) or periodic acid-Schiff (PAS).

For lectin histochemistry, sections were also stained with Bauhinia purpurea agglutinin (BPA), Dolichos biflorus agglutinin (DBA), Griffonia simplicifolia agglutinin (GSA)-I, GSA-II, Maclura pomifera agglutinin (MPA), Arachis hypogaea (PNA), Glycine max agglutinin (SBA), Ulex europaeus agglutinin (UEA)-I, and Triticum vulgaris agglutinin (WGA) (horseradish peroxidase (HRP)-conjugated form, E-Y laboratories, San Mateo, CA, USA), and with unconjugated Canavalia ensiformis A (Con A; Sigma, USA) as previously reported.11,12

Results

Changes in Blood Glucose Levels

Blood glucose levels in D and UN-D groups were more than 600 mg/dl, while those in C and

Fig. 1. Kidneys of mice in UN-D group. Expansion of mesangial area. PAS ×600.

Fig. 2. Kidneys of mice in UN (a) and UN-D (b) group. Cuboidal or columnar epithelial cells of Bowman's capsule (arrow) have the same stainability as proximal tubules (P). BPA ×300.
UN groups were about 200 mg/dl.

**Histopathological Findings**

Focal and segmental expansion of glomerular mesangial area was marked in UN-D group (Fig. 1) and mild to moderate in D group. Luminal dilatation in some distal tubules and collecting ducts in the cortex was observed more frequently in UN-D group than in D-group (Fig. 3). These changes were not observed in C and UN groups.

Most of the Bowman's capsules were partially or completely lined with cuboidal or low columnar

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**Fig. 3.** Kidneys of mice in C (a, c, e, and g) and UN-D (b, d, f, and h) groups. Stainability of distal tubules or collecting ducts to each lectin in UN-D group is enhanced. Con A (a and b), GS-I (c and d), SBA (e and f), and WGA (g and h). a and b: ×100, c: ×400, d-h: ×200.
epithelial cells in C and UN groups while they were generally lined with flattened epithelia in D and UN-D groups (Figs. 1 and 2).

**Lectin Histochemical Findings**

Lectin histochemical observations were focused on the cortex where histopathological changes were mainly seen.

Renal corpuscle (Fig. 2): Being common to all groups, capillary endothelial cells were slightly to moderately stained with BPA, Con A, GSA-I, and WGA, no mesangial area was stained with any lectins, and visceral epithelial cells were moderately stained with BPA, Con A, MPA, and WGA. Flattened epithelial cells of Bowman’s capsules in D and UN-D groups were slightly to moderately stained with BPA, Con A, and MPA while cuboidal or columnar epithelial cells of Bowman’s capsules in C and UN groups showed the same stainability as that of epithelial cells of proximal tubules. Shortly, the brush border was stained with BPA, Con A, PNA, and SBA, and the cytoplasm was stained with BPA, Con A, and PNA.

Proximal tubules (Figs. 2, 3a, 3b): No lectin histochemical differences were observed among all groups. The brush borders of most epithelial cells were moderately to strongly stained with BPA and Con A, and those of some cells were slightly stained with PNA and SBA. The cytoplasm of some epithelial cells was slightly stained with BPA, Con A, and PNA.

Distal tubules and collecting ducts (Fig. 3): In C group, the cytoplasm of distal tubule epithelial cells was slightly to moderately stained with 7 lectins other than BPA, DBA, and GSA-I. That of collecting duct epithelial cell was slightly to moderately stained with 7 lectins other than DBA, GSA-I, and UEA-I. The apical border of both tubules was slightly to moderately stained with 7 lectins other than DBA, GSA-I, and UEA-I. Both tubules in UN group showed similar stainabilities to those in C group. However, in UN-D group, the cytoplasm of all cells of both tubules was strongly stained with Con A, GSA-I, SBA, and WGA and that of some cells with DBA. The apical border of both tubules also increased its stainability to these lectins. In D group, similar but weaker lectin-binding activities were observed in comparison with those in UN-D group.

**Discussion**

In this study, we clarified the lectin-binding characteristics of the kidney of unilaterally nephrectomized diabetic mice. Histopathological findings were the same as those previously reported.

Compared with C-group, affinities of distal tubule and collecting duct epithelial cells to Con A, DBA, GSA-I, SBA, and WGA increased in D and especially in UN-D group but they did not change in UN group. This suggests that such changes in lectin binding activities in these epithelial cells common to diabetic mice might be enhanced by unilateral nephrectomy.

Accumulation of WGA-positive substance in the glomerular basement membrane and mesangium and expression of GS-I A4 binding sites in the sclerotic areas of glomeruli were reported in SZ-induced diabetic rats and mice, respectively. In this study, however, no lectin histochemical changes were observed in any glomeruli of diabetic mice of D and UN-D groups. In the present case, changes of glycocompounds in the glomeruli, if any, might be under detectable limit by the direct HRP-conjugation method.

Bowman’s capsules of sexually mature mice are generally lined with a cuboidal to low columnar epithelium. They are called “male-type of Bowman’s capsules”, and their epithelial cells are suggested to be similar to those of proximal tubules functionally as well as morphologically. Such “male-type” capsules mainly seen in C and UN groups exhibited the same lectin stainabilities as those of proximal tubules. On the other hand, lectin stainabilities of Bowman’s capsules composed of flattened epithelial cells generally seen in D and UN-D groups were different from those of proximal tubules. This suggests a close relationship between cell morphology and histochemical characteristics.

In conclusion, this study showed that unilateral nephrectomy enhanced SZ-induced kidney lesions without a prominent histochemical modification.
References


