Expression of Sialic Acid Residues in Renal Tubule of Streptozotocin-induced Diabetes Rats

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Abstract: The distribution of sialic acid residues in renal tubule of streptozotocin-induced diabetes rats was investigated using lectin histochemical method to compare with specimens from control animals. Sialic acid specific lectins, *Limax flavus* (LFA), *Sambucus nigra* (SNA), and *Maackia amurensis* (MAA) were applied. The sialic acid residues of the renal tubule in diabetes rats were markedly different from those in the control. The epithelial cells of the entire proximal tubule in normal rats were positive for LFA and SNA, and negative for MAA. Meantime, the proximal tubule in diabetes rats was negative for these lectins. The medullary thick ascending limb in normal rats showed negative for LFA, SNA and MAA. In contrast, that limb in diabetes rats showed positive for these lectins. Moreover, the degenerated epithelial cells in the cortical thick ascending limb were intensely positive for LFA and MAA, and negative for SNA. The present study revealed that the different distribution of epithelial sialic acid residues in the renal tubule might be related to the functional diversity exhibited by these tubular regions between diabetic and normal rats. (J Toxicol Pathol 1998; 11: 49~53)

Key words: diabetes mellitus, rats, renal tubule, streptozotocin, lectin histochemistry

Introduction

Diabetes mellitus, a common metabolic disorder in man, is associated with a relative or absolute deficiency in insulin and is characterized by hyperglycemia, glycosuria, and disturbances in protein as well as lipid metabolism¹. It is well established that sialic acid residues present on glycoproteins and glycolipids are involved in a variety of biological interactions (for review see Schober E *et al*²). Several studies have demonstrated that the distribution of sialic acid residues in diabetes kidney glomerulus^{3,4}. However, the sialic acids residues in the renal tubule have not been well established.

Streptozotocin (STZ), a drug commonly used for experimental induction of diabetes mellitus in several animal species has a diabetogenic effect by its specific action on B cells of the pancreas⁵. Lectin is a carbohydrate-binding protein of nonimmune origin with no enzyme activity that bind to specific sugar residues⁶. The well characterized sialic acid-binding lectin which has been used in histochemistry is *Limax flavus* (LFA)⁷. Furthermore, the detailed sialic acid binding pattern to internal galactose can be recognized using *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA); SNA recognizes Neu 5Ac alpha 2, 6 Gal/Gal NAc sequence, and MAA recognizes Neu 5Ac alpha 2, 3 Gal β 1 sequence^{8,9}. This approach allowed us to detect a gross difference in the sialic acid residues composition among different segments of the renal tubule. In the present study, we have used lectin histochemical technique for the high resolution localization of sialic acid residues, using LFA, SNA, and MAA, in

various segments of renal tubule in normal and STZ induced diabetes rats.

Materials and Methods

A total of 30 male 6-week-old Wistar rats (SLC Inc., Shizuoka) were used. The animal were housed at the Azabu University Research Institute of BioScience under the supervision of the Azabu University School of Veterinary Medicine. Rats were housed four per plastic cage on hard wood chip bedding in an environment-controlled room, maintained at 12 h. light/dark cycle, 22 $\pm$ 2C and 55 $\pm$ 5% relative humidity, fed conventional diet (F-1, Funabashi Form), and tap water *ad libitum* throughout the experimental period. On Day 1, 24 rats were injected intravenously with Streptozotocin (Sigma, St Louis, MO, USA), 65 mg/kg body weight, in 0.3 ml of 50 mmol/l citrate buffer (pH 4.5). Six rats served as controls, and were injected intravenously with citrate buffer alone. After 12 weeks all rats were killed by decapitation. Blood glucose levels were measured by an autoanalyzer system¹⁰. The specimen were fixed with a 10% solution of buffered formalin for 12 hours. After dehydration in graded alcohol solution, the tissues were embedded in paraffin wax. For histological evaluation, 4 μ m sections were cut and stained with Mayer haematoxylin and eosin, Masson trichrome, or periodic acid-Schiff (PAS) with and without prior digestion with 1 mg/ml alpha-amylase (Sigma) for 1 h at 37C.

Lectin histochemistry was performed with biotinylated lectins, avidin-biotin-peroxidase complex, and 3,3'-diaminobenzidine tetrahydrochloride-H₂O₂¹¹. The sections were counter stained with Methyl green. *Limax flavus* (LFA) (concentration 10 μ g/ml), *Sambucus nigra* (SNA) (concentration 10 μ g/ml), and *Maackia amurensis* (MAA) (concentration 10 μ g/ml) were applied (biotinylated lectins were obtained from E.Y. Laboratories, Inc., USA)¹¹. The

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following controls were used: (1) replacement of the lectins by the corresponding buffer; (2) sections were preincubated with neuraminidase (0.1 units/ml of type VI from *Clostridium perfringens*, Sigma, USA) using the methods of Jones *C et al.*¹²

Results

At the end of the study, STZ-treated rats had a significantly increased plasma glucose concentration, glycemia of 470 mg/dl on an average, while those in normal rats were about 200 mg/dl, and a reduced body weight compared to the normal rats.

In STZ induced diabetes rats, mild to moderate, focal to segmental expansion of glomerular mesangial areas was observed (Fig. 1). Degenerated epithelial cells were observed in the cortical thick ascending limb, and these cells were PAS positive (Fig. 2), and negative for digestion with α -amylase materials (data not shown). These changes were not observed in control normal rats.

In normal rats, the glomeruli showed binding with LPA and SNA (Fig. 3), and were negative for MAA (Fig. 4). In STZ-induced diabetes rats, almost all glomeruli were negative for these lectins (Figs. 5, 6).

Lectin binding patterns in the renal tubules are summarized in **Table 1**. The different sites of lectin binding between STZ-induced diabetes and control rats were as follows. In normal rats, microvilli and apical zone of the entire proximal tubule showed binding with LPA and SNA (Fig. 3). The medullary thick ascending limb was negative for LPA, SNA, and MAA (Fig. 7). In STZ-induced diabetes rats, the entire proximal tubule showed negative binding with LPA, SNA, and MAA (Figs. 5, 6). The apical zone of medullary thick ascending limb showed binding with LPA, SNA, and MAA (Figs. 8, 9). The degenerated epithelial cells in the cortical thick ascending limb showed intense binding with LPA and MAA (Fig. 6), and was negative for SNA (Fig. 5). Pre-incubation of sections with neuraminidase, eliminated staining of renal tubules with LPA, SNA, and MAA (data not shown).

Fig. 1. Kidney at 12 weeks after induction of diabetes showing expansion of mesangial areas. Masson trichrome $\times 300$

Fig. 3. Kidney of control rat. LPA binding with glomerulus, microvilli and apical zone of proximal tubules and cortical thick ascending tubules. Methyl green counterstain. $\times 480$

Fig. 2. Kidney at 12 weeks after induction of diabetes. The cortical thick ascending limb showing severe tubular vacuolar degeneration, containing intense PAS positive materials. PAS $\times 300$

Fig. 4. Kidney of control rat. MAA binding with cortical thick ascending tubules. The proximal tubules and glomerulus are negative for MAA. Methyl green counterstain. $\times 480$

Fig. 5. Kidney at 12 weeks after induction of diabetes. SNA binding with apical zone of cortical thick ascending tubule. The degenerated epithelial cells of cortical thick ascending tubule (*), proximal tubules, and glomerulus are negative for SNA. Methyl green counterstain. $\times 480$

Fig. 6. Kidney at 12 weeks after induction of diabetes. MAA binding with degenerated epithelial cells of cortical thick ascending tubule (*). The proximal tubules and glomerulus are negative for MAA. Methyl green counterstain. $\times 480$

Table 1. Lectin Binding Sites in Renal Tubules of Normal and Streptozotocin-induced Diabetic Rats

	LFA		SNA		MAA	
	N	DM	N	DM	N	DM
Proximal tubule						
-convoluted	#	—	#	—	—	—
-recta	+	—	+	—	—	—
Thin limb	#	#	+	#	#	#
Thick Ascending limb						
-medullary	—	#	—	#	—	#
-cortical	$\pm \pm$	$\pm \pm$	$\pm \pm$	$\pm \pm$	$\pm \pm$	$\pm \pm$
degenerated cell		#		—		#
Distal convoluted tubule	#	#	#	#	#	#
Collecting duct						
-initial	$\pm \pm$	$\pm \pm$	$\pm \pm$	$\pm \pm$	—	—
-medullary	—	—	—	—	—	—

#, moderate to intense reaction; +, weak reaction; $\pm$, a few reactive cells; —, no reaction; N: normal rats; DM: streptozotocin-induced diabetic rats.

Fig. 7. Kidney of control rat. MAA binding with apical zone of some epithelial cells of collecting ducts. The medullary thick ascending limbs are negative for MAA. Methyl green counterstain. $\times 480$

Discussion

Decrease in the sialic acid content of the glomeruli in human diabetes has been consistently reported¹³, and this decrease has also been observed in rat alloxan¹⁴ or STZ³ - induced diabetes. The present study also revealed that a decrease of sialic acid residues in the glomeruli was found in diabetes rats, as compared with normal ones.

The present lectin histochemical study revealed that the distribution of renal tubular sialic acid residues in STZ-induced diabetes and normal rats was markedly different with each other. The most prominent features in diabetes rats, as compared with normal ones, were a significant reduction of SNA, recognizing Neu 5Ac alpha 2,6 Gal/Gal NAc sequence^{8,9}, reactivity at the proximal tubules, and significant increment of SNA and MAA, recognition of Neu 5Ac alpha 2,3 Gal β 1 sequence^{8,9}, reactivity at the medullary thick

ascending limb. It has previously been shown that the sialidase activity in diabetes rats kidney selectively increases at the proximal tubule¹⁵, and the increased level is much more sialidase specific for Neu 5Ac alpha 2,6 Gal than that for Neu 5Ac alpha 2,3 Gal¹⁶. The decrease of SNA reactivity in the proximal tubule of diabetes rats in this study might be associated with the increment of sialidase especially for Neu 5Ac alpha 2,6 Gal in that tubule. As the detailed sialidase activity in the thick ascending limb of diabetes rats has not been elucidated, the functional relationship between sialic acid and sialidase level in that limb is still unclear.

Sialic acids are anion and can conduct the ionic transport in renal tubule¹⁷. In normal animals, proximal tubule where active sodium and passive chloride and also water uptake take place, sialic acid residues could be involved in

Fig. 8. Kidney at 12 weeks after induction of diabetes. SNA binding with apical zone of medullary thick ascending limbs. Methyl green counterstain. $\times 480$

Fig. 9. Kidney at 12 weeks after induction of diabetes. MAA binding with apical zone of medullary thick ascending limbs. Methyl green counterstain. $\times 480$

attracting cations such as sodium as well as water to the cell surface prior to their transepithelial movement¹⁸. On the other hand, in normal rats, the absence of sialic acid residues in thick ascending limbs could be functionally important since active reabsorption of chloride, an anion, and passive sodium take place therein^{18,19}. In the present study revealed a reverse distribution of sialic acid residues in the proximal tubule and the thick ascending limb of diabetes and normal rats. These findings suggest that the ionic transport formation through sialic acid in the proximal tubule and the thick ascending limb in diabetes and normal rats is quite different from each other.

In diabetes rats, tubular excess-reabsorption of glucose has been observed in the degenerated epithelial cells of the cortical thick ascending limb²⁰. The present study revealed a marked LFA and MAA reactivity in these degenerated epithelial cells. Entry of glucose into renal tubular epithelial cells occurs via a glucose-sodium symporter which is dependent on the active extrusion of sodium through the basolateral cell membrane by the sodium pump system²¹. Moreover, the thick ascending limb in diabetes rats, compared to other renal tubule segments, shows the highest Na⁺-

K⁺-ATPase activity which is responsible for sodium reabsorption^{21,22}. Expression of sialic acids, especially Neu 5Ac alpha 2, 3 Gal β 1 sequence, in such degenerated renal tubular epithelial cells might be related to glycogen accumulation through the increase of active sodium reabsorptive level.

Even though further studies are required to elucidate the precise physiological role of cellular sialic acid residues in each segment of renal tubule, the present results already provide valuable insight into the histogenesis and pathogenesis of diabetic alteration of renal tubules in rats.

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