Preliminary Report

Renal Microvascular Changes in Streptozotocin-Induced, Long-Termed Diabetic Rat

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Objective: *To investigate the renal microvascular changes in streptozotocin (STZ)-induced, long-termed diabetic rat.*
Material and Method: *Twelve male Sprague-Dawley rats were used. Each diabetic rat (n = 8) was induced by an intraperitoneal injection of STZ (60 mg/kg) in citrate buffer (pH 4.5). Control rats (n = 4) were injected intraperitoneally with the same amount of the buffer. The animals were sacrificed at 20 weeks after the injections. The kidneys were processed for conventional light microscopy (LM) and vascular corrosion cast technique with scanning electron microscopy (SEM).*
Results: *Under LM, it was found that the glomerular sizes intensively decreased in the long-termed diabetic rat. The thickening of Bowman's basement membrane was demonstrated. Additionally, there were macrophages and capsular drop lesions in renal corpuscles of long-termed diabetes. The sizes of proximal and distal tubules were markedly destroyed, when compared to the control. Moreover, the epithelial necrosis of vacuolated renal tubules was observed. By using vascular corrosion cast with SEM, the glomerular microvascular sizes in the long-termed diabetes were significantly decreased that corresponded to the result under LM. Furthermore, the size of peritubular capillaries decreased. Concerning to vasa recta in the long-termed diabetes, these vessels ran tortuously and decreased in size.*
Conclusion: *Renal microvascular changes, observed in STZ-induced diabetic rats, mimic human diabetic nephropathy (DN). Additionally, the pathological changes of the renal tubules were investigated. Therefore, the present study provides an important basic knowledge for understanding the processes in developing DN, as well as for further study of the therapeutic treatment.*

Keywords: *Renal microvasculature, Diabetic nephropathy, Streptozotocin, Vascular corrosion cast, SEM*

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Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by a chronic elevated blood glucose level. The diabetic patients are at high risk of developing diabetic nephropathy (DN), which is a major cause of morbidity and mortality. Common clinical signs of DN are altered glomerular filtration rate (GFR) and subsequent increases in serum creatinine, albuminuria, proteinuria and end-stage renal failure[1-3]. Accordingly, the kidney is an important organ for the investigation of diabetic pathophysiology and its complications. Several animal models have been conducted to mimic the typical human diabetic lesions. Of these animal models, the streptozotocin (STZ) rat model has been the most widely used[4-6]. The STZ is high specific cytotoxic action on the beta-cells of the islets of Langerhans with rapid necrosis[4]. In addition, the pathological and biochemical features of this model are compatible to those of type 1 diabetes in human. Although a lot of works have been done on the morphological changes of the glomerular capillaries in the diabetes[5-8], the information concerning to

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the alteration of renal tubules is very limited. One of the appropriate techniques to investigate microcirculation in three dimensions is the vascular corrosion cast technique combined with the scanning electron microscopy (SEM). In addition, this technique has not been used to demonstrate the microvascular changes of the vasa recta and peritubular capillaries in any diabetic model. Therefore, it is interesting to employ vascular corrosion cast technique in conjunction with the SEM to elucidate the renal microvascular alterations in the STZ-induced diabetic rat to understand the processes of developing DN.

Material and Method

Twelve male adult Sprague-Dawley rats at the age of 6-8 weeks (National Laboratory Animal Center, Mahidol University, Thailand), initially weighing between 220-240g were used. All animals received care as recommended in the "Guide for the Care and Use of Laboratory Animals"[9]. A week after arrival, each animal was fasted for at least 6 hr, and the glucose concentration in urine was determined by using the urinalysis control strips (Diabur-Test 5000, Roche Ltd., Germany). In addition, the result of the glucose concentration was 0 mg/dL, so that the animal can be used in the experiment.

The animals were randomly divided into two groups. In the STZ-induced diabetic group, each of eight rats was injected intraperitoneally with a single dose of 60 mg/kg body weight of STZ (Acros Organics, Janssen Pharmaceutical, Belgium) in the citrate buffer at pH 4.5[4,6]. Four animals in the control group were injected intraperitoneally with the same amounts of the buffer. Then, after fasting for 10 to 12 hr, each animal was measured the urine glucose level every morning. All animals were sacrificed at 20 weeks after intraperitoneal injection as a long-termed period. In addition, these rats were divided into two groups. In the first group, two control and two STZ-induced diabetic rats were processed for the histological study. In the second group, two control and six STZ-induced diabetic rats were used for renal microvascular study by using vascular corrosion cast technique with SEM. Each animal was anesthetized by halothane inhalation, before the thoracic cage was cut to expose the heart. After 0.05 ml of heparin (Leo 5,000 iu/mL) was injected into the left ventricle, 500 ml of 0.9% NaCl solution was perfused through the ascending aorta. Then, the right atrium was cut as the efferent port. In the histological group, 300 ml of Bouin's solution was immediately injected after 0.9% NaCl solution injection. Next, the kidneys were removed and processed in the conventional histological technique, serially sectioned at 6-7 μm thick and stained with hematoxylin and eosin. In the microvascular investigation, processes of Batson's no. 17 plastic mixture injection and preparation of renal microvascular corrosion cast had been performed as previously described in Bamroongwong et al[10].

Results

With the LM observations of the kidneys in the STZ-induced diabetic and control groups, it was roughly divided into two distinct regions: outer cortex and inner medulla. The renal cortex contained renal corpuscles and renal tubules, whereas the renal medulla consisted of entirely parallel bundles of renal tubules and collecting ducts. At the high magnification of the renal corpuscles, it was found that the glomeruli decreased in size and were destroyed in the long-termed DM. Additionally, the thickening of the Bowman's basement membrane was demonstrated, when compared to those in the age-matched control. In addition, there were macrophages and capsular drop lesions in the renal corpuscles of the diabetic rats (Fig. 1). At the high magnification of the renal medulla, it was observed that the proximal (PT) and distal tubules (DT) were markedly destroyed, when compared to those in the age-matched control. Furthermore, the vacuolization and epithelial necrosis were demonstrated in the renal tubules of this late stage (Fig. 2).

By using the vascular corrosion cast technique with the SEM, the renal microvascular casts were compared between the diabetic and control groups. The sizes of the glomeruli were decreased and rapidly destroyed in the long-termed DM (Fig. 3). In addition, the morphology of the peritubular capillary plexus was distinctly destroyed, compared to those in the age-matched control groups (Fig. 4). Concerning to both descending and ascending limbs of the vasa recta, these vessels ran tortuously. Additionally, the sizes of these vessels were decreased in the long-termed DM, when compared to those in the age-matched control (Fig. 5).

Discussion

In the long term of DM, it was found in the present study that the sizes of glomeruli, diameter of glomerular capillary, afferent and efferent arterioles decreased. Moreover, macrophages, capsular drop lesion, and increased thickness of Bowman's basement membrane occurred. During this stage, advanced glycosylation end product (AGE) not only is intensively

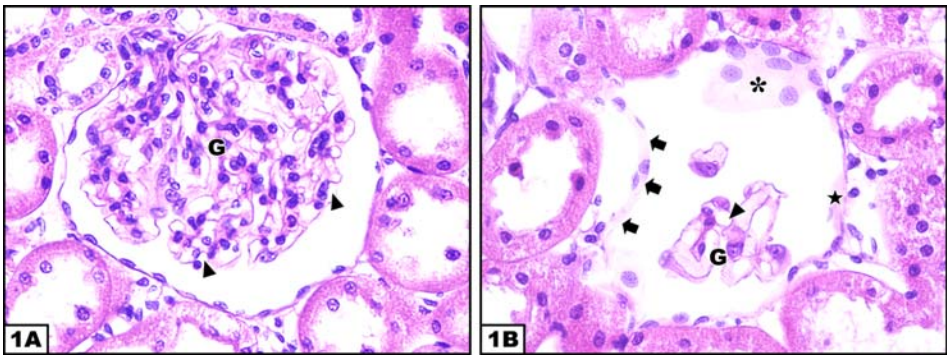

Fig. 1 Light micrographs of renal corpuscles in age-matched control (1A) and long-termed DM (1B)
G, glomerulus; arrowheads, glomerular capillaries; arrows, Bowman's basement membrane thickening; a star, capsular drop lesion; an asterisk, macrophage. X400

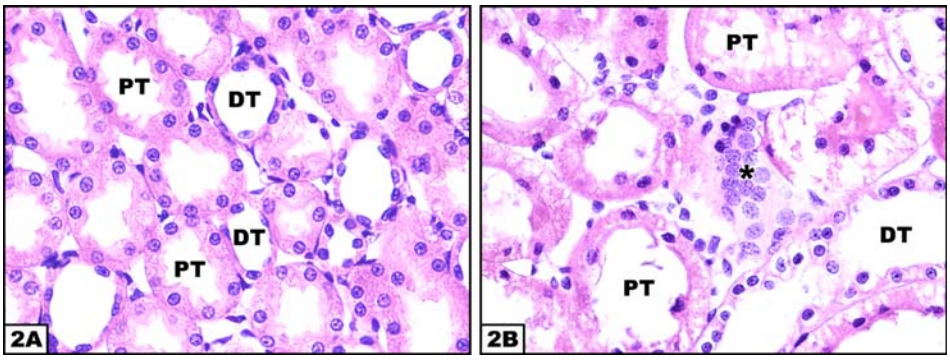

Fig. 2 Light micrographs of renal medulla in age-matched control (2A) and long-termed DM (2B)
PT, proximal tubule; DT, distal tubule; an asterisk, macrophage. X400

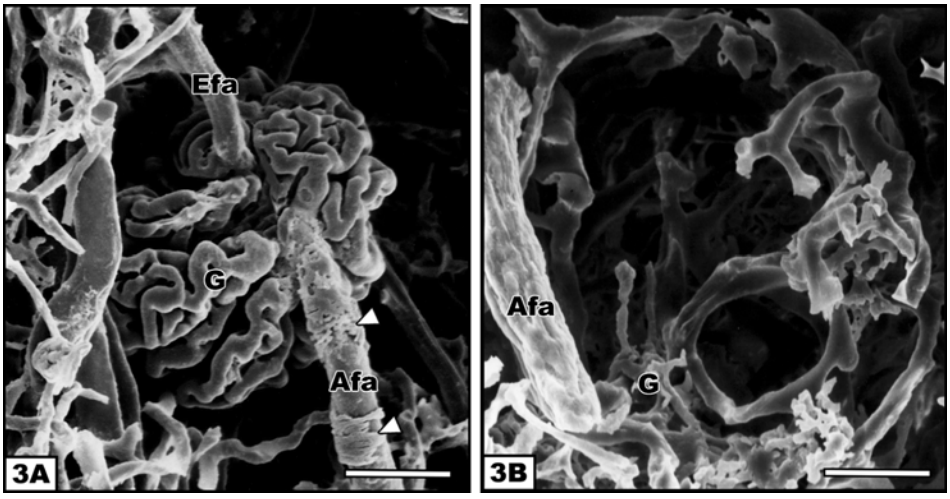

Fig. 3 SEM micrographs of glomerular microvascular casts in age-matched control (3A) and long-termed DM (3B)
G, glomerulus; Afa, afferent arteriole; Efa, efferent arteriole; arrowheads, undigested smooth muscle cells, Bar = 50 μm

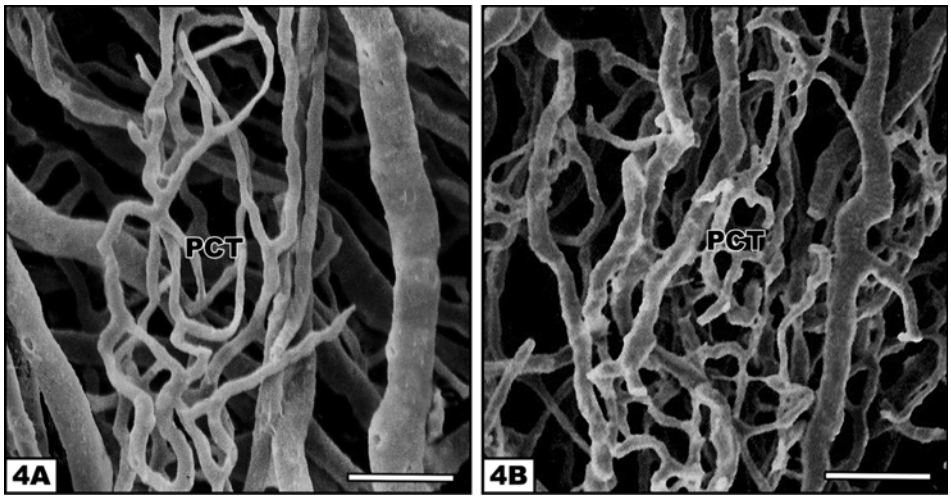

Fig. 4 SEM micrographs of peritubular capillaries in age-matched control (4A) and long-termed DM (4B)
PTC, peritubular capillaries, Bar = 50 μm

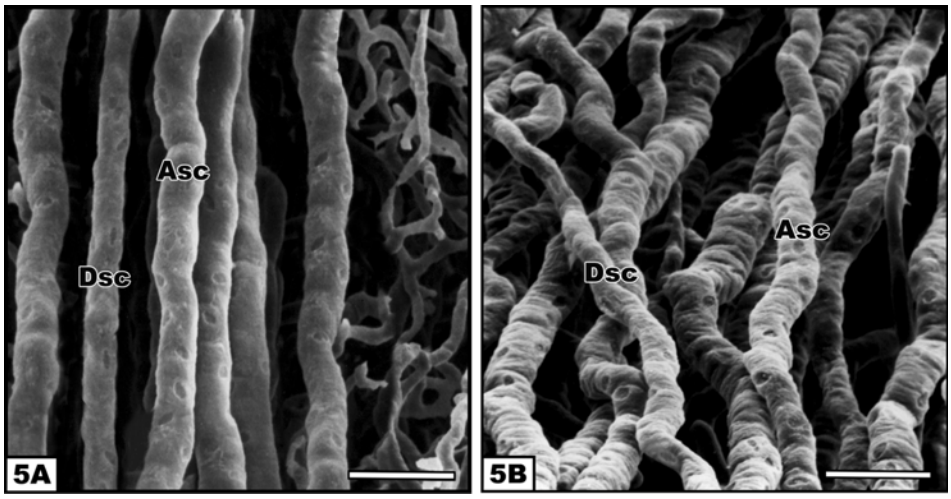

Fig. 5 SEM micrographs of vasa recta in age-matched control (5A) and long-termed DM (5B)
Dsc, descending limb of the vasa recta; Asc, ascending limb of the vasa recta, Bar = 50 μm

produced in the basement membrane of capillary, but also attracts monocyte and macrophage to eliminate the AGE[11,12]. After glomerular capillary was destroyed, the capsular drop lesion appeared and the glomerular size decreased. Moreover, AGE in the glomerular basement membrane (GBM) attaches to plasma proteins such as albumin, immunoglobulin, and lipoprotein causing GBM thickening[6,7]. In renin-angiotensin-aldosterone system, levels of angiotensin and its receptor are low[13,14]. Accordingly, GFR decreases in the long-termed DM. Furthermore, heparan sulfate and glycosaminoglycans, carrying negative charges, decrease in the GBM of DM[5,15]. The alteration of the charges in the GBM induces podocyte detachment, loss of podocyte foot processes, and decrease in podocyte number[6,7]. These lead to abnormal permeability in the GBM that causes microalbumin and glucose in the filtrate[16]. Even though GFR decreases in this stage of DM, hyperfunction of the renal tubules occurs to reabsorb the leaked substances in the filtrate by increased length of PT and DT[8]. Because of continuation of hyperfunction in these renal tubules, the

epithelial necrosis was observed, and these tubules were finally destroyed by macrophages. Therefore, renal tubules were destroyed in the long term of DM.

Concerning to renal tubules and vessels, it was observed that the renal tubules were obviously destroyed. The diameters of peritubular capillary and vasa recta decreased; furthermore, the vasa recta ran tortuously. The macrophages were exhibited among the renal tubular vacuolization. Consequently, the epithelial necrosis was also seen in this stage. It has been known that GFR in the late stage decreases, so functions of renal tubules increase. However, loss of property in glomerular filtration barrier occurs, the microalbuminuria is found in the filtrate. In addition, it is shown that the lengths of PT and DT increase[8,17,18]. Therefore, it might be possible that even peritubular capillary was destroyed, tortuous course of vasa recta generally lead to increase surface area for reabsorption of leaked substances.

It was summarized that renal histological and microvascular changes were observed in the STZ-induced diabetic rats that imitate the human DN. Therefore, this induced rat is a suitable model to understand the processes in development of the DN. So that, the present finding provides an important basic knowledge for further study of the pathophysiology and therapeutic treatment of the DN.

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การเปลี่ยนแปลงของระบบไหลเวียนเลือดอย่างละเอียดในไตของหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานในระยะเรื้อรังด้วยสาร streptozotocin

ศิรินุช ศรีเจริญเวช, ยุทธพงษ์ ทองพบ, ภัสรา ลานเหลือ, สิทธา ปิยะวินิจวงศ์, จันทิมา รุ่งเรืองชัย, อิทธิพล พวงเพชร

วัตถุประสงค์*: เพื่อศึกษาการเปลี่ยนแปลงของระบบไหลเวียนเลือดอย่างละเอียดในไตของหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานในระยะเรื้อรังด้วยสาร streptozotocin (STZ)*

วัสดุและวิธีการ*: หนูพันธุ์ Sprague-Dawley เพศผู้จำนวน 12 ตัว โดยแบ่งเป็นกลุ่มเบาหวาน (n = 8) ซึ่งเหนี่ยวนำโดยการฉีด STZ (60 mg/kg) ใน citrate buffer (pH 4.5) เข้าทางช่องท้อง และกลุ่มควบคุม (n = 4) ซึ่งฉีดด้วย buffer ชนิดเดียวกันในปริมาณเท่ากัน ทำการ sacrificed ที่ 20 สัปดาห์หลังจากเหนี่ยวนำ จากนั้นนำไตไปผ่านกระบวนการเตรียมชิ้นเนื้อด้วยเทคนิคทางเนื้อเยื่อวิทยาเพื่อศึกษาด้วยกล้องจุลทรรศน์ (LM) และโครงหลอดเลือดของไตนั้น ศึกษาด้วยเทคนิค vascular corrosion cast ร่วมกับจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM)*

ผลการศึกษา*: จากการศึกษาด้วยกล้องจุลทรรศน์พบว่าในหนูเบาหวานระยะเรื้อรังมีขนาดของ glomerulus เล็กลงอย่างมาก นอกจากนี้ยังพบมีการหนาตัวของ Bowman's basement membrane, macrophage และ capsular drop lesion ที่ renal corpuscle ในท่อไตส่วน proximal และ distal พบว่ามีขนาดเล็กลงและมีการเสื่อมสลายของท่อไต จากการศึกษาด้วยเทคนิค vascular corrosion cast ร่วมกับ SEM พบว่าขนาดของ glomerulus, glomerular และ peritubular capillaries, afferent และ efferent arterioles รวมทั้ง vasa recta ในหนูเบาหวานระยะเรื้อรังมีขนาดเล็กลงและถูกทำลาย ซึ่งสอดคล้องกับผลการศึกษาด้วยกล้องจุลทรรศน์ นอกจากนี้ยังพบการขดงอตัวของ descending และ ascending limb ของ vasa recta*

สรุป*: ผลจากการศึกษาการเปลี่ยนแปลงของระบบไหลเวียนเลือดอย่างละเอียดในไตของหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานในระยะเรื้อรังด้วย STZ นี้ นอกจากได้แสดงให้เห็นการเปลี่ยนแปลงของ renal tubule ซึ่งยังไม่มีรายงานที่ชัดเจนมาก่อน ยังเป็นประโยชน์อันจะสามารถนำมาอธิบายการดำเนินของโรคในผู้ป่วยเบาหวานที่มีภาวะแทรกซ้อนทางไตได้เป็นอย่างดี ตลอดจนนำไปสู่การดูแลรักษาผู้ป่วยเบาหวานที่มีภาวะแทรกซ้อนทางไต เป็นลำดับต่อไป*