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Morphometric analysis of arteriolar diameters in experimental nephropathies: application of microvascular casts

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Summary. Renal arteriolar diameters were measured, using microvascular resin casts, in two hyperfiltration models of rats: the remnants kidney of subtotal nephrectomy (NX) and streptozotocin-induced diabetic kidney (DM). In the NX, the blood pressure was elevated, urinary protein excretion was markedly increased and glomeruli were severely damaged. In the DM, although the blood pressure remained normal, urinary protein excretion was significantly increased and glomeruli were damaged but to a lesser extent than in the NX group. In the NX group, the afferent arteriole was dilated and the efferent arteriole was constricted. In the DM group, the afferent arteriole was dilated, while the efferent arteriole remained unchanged. The results showed that afferent arteriolar dilatation was seen in both the NX and DM groups, possibly leading to the glomerular damage. In the NX group, the systemic high blood pressure and efferent arteriolar constriction augmented glomerular damage significantly.

Key words: Vascular cast – Arteriole – Kidney – Subtotal nephrectomy – Diabetic nephropathy

Introduction

It has been suggested that glomerular haemodynamics play an important role in the initiation and progression of glomerular damage (Hostetter et al. 1981; Brenner 1985; Brenner et al. 1986). Among experimental renal diseases, the remnant kidney in the subtotal nephrectomy model (Hostetter et al. 1981; Herrera-Acosta et al. 1988; Jackson and Johnston 1988; Yoshioka et al. 1988) and diabetic nephropathy (Zatz et al. 1985; Jensen et al. 1987; Cooper et al. 1988) have been assumed to be typical models in which elevation in intraglomerular pressure is a central mechanism for deterioration of glomeruli. Intraglomerular pressure is determined by a transmission of the systemic blood pressure and also by pre- and post-glomerular vascular resistance (Martinez-Maldonado et al. 1987). Morphometric assessment of the arteriolar diameter is, therefore, critical to the understanding of the mechanisms involved in the development of glomerulopathies in these models.

In the present study renal arteriolar diameters in the remnant kidney of rat subtotal nephrectomy and in the rat diabetic kidney were measured. In order to obtain precise analysis of vascular diameters, microvascular casts were used.

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Materials and methods

Fifteen male Wistar rats weighing 250 g were used. The animals were divided into three groups: a control group (C group), a group which underwent subtotal nephrectomy (NX group) and a diabetic group (DM group). Each group consisted of five rats.

In the NX group subtotal nephrectomy was achieved by right uninephrectomy and, after 2 weeks of recovery period, resection of the upper and lower thirds of the left kidney.

In the DM group diabetes was induced by injection of streptozotocin (40 mg/kg body weight) dissolved in citric acid buffer (pH 4.0) through a tail vein (Hostetter et al. 1981; Jensen et al. 1981).

Animals were fed standard rat pellets and were freely accessible to tap water. Fifteen weeks after the surgery or the streptozotocin injection, the systolic blood pressure was measured by the tail cuff method. All animals were then housed individually in metabolic cages for the collection of 24-h-urine samples for the measurement of daily protein excretion. In the C and DM groups, blood samples were taken from a tail vein and the blood glucose level was determined.

Microvascular casts were prepared as previously described (Kimura et al. 1989). Under intraperitoneal pentobarbital anaesthesia, a polyethylene catheter PE-60 was inserted retrogradely into the abdominal aorta with the tip of the catheter placed just below the left renal artery. The systemic blood pressure was monitored via the catheter using a three-way stopcock. Immediately after the proximal aorta had been ligated just above the superior mesenteric artery and the left renal vein opened by a small incision for outflow, 0.9% saline was infused at room temperature for a few seconds, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 3 min to fix the kidney(s). To assure fixation of function-

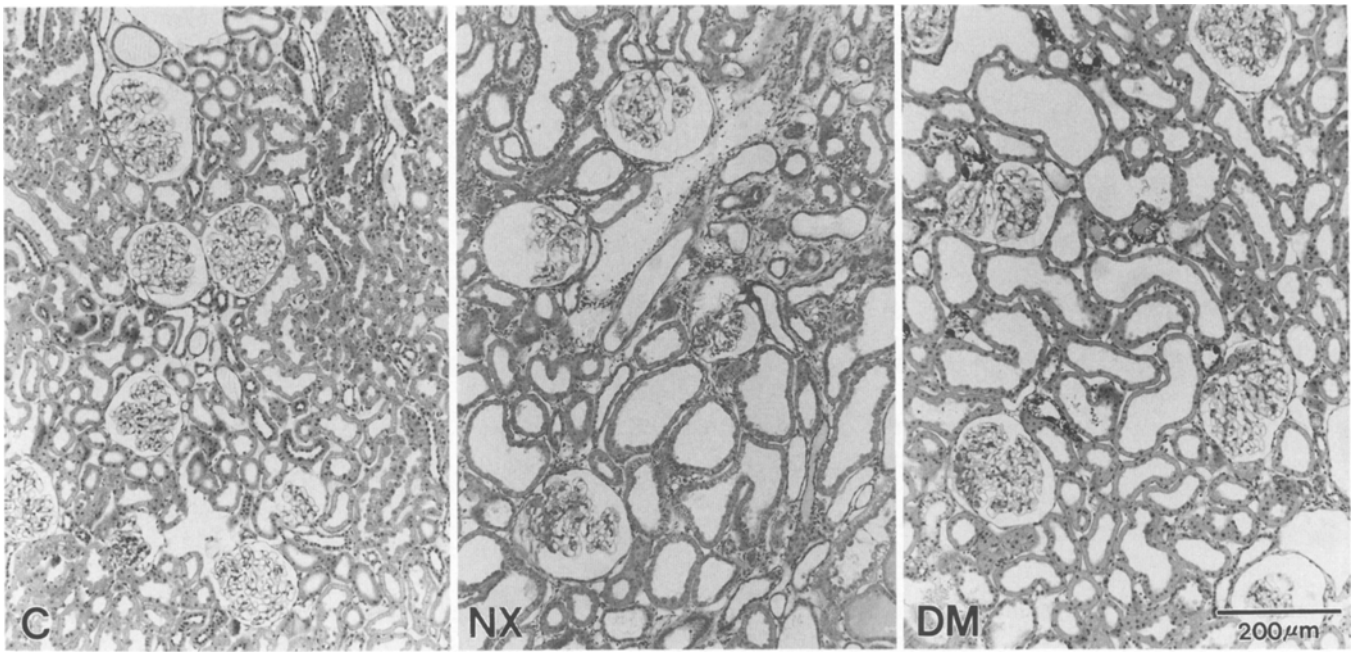

Fig. 1. Light microscopic PAS-stained specimens from three groups. *C*, Control group; *NX*, subtotal nephrectomy group; *DM*, diabetic group. Compared to the C group, in the NX group, glomerular sclerosis, tubular dilatation and interstitial fibrosis and cell infiltration are prominent. In the DM group, glomerular sclerosis and tubular dilatation are also shown but less prominent and interstitial fibrosis and cell infiltration are minimal

al state of the vasculature, the infusion pressure to the kidney(s) was controlled by a hand syringe to be the same as the mean arterial pressure measured just before the ligation of the proximal aorta (Gattone et al. 1983). After the fixation, the acryl resin (Mercox, Dai-Nihon Inki, Tokyo, Japan) was infused for the preparation of a cast of the vascular system in the kidney(s). A portion of the renal tissue was used for conventional light microscopy. The rest of the tissue was soaked in sodium hypochlorite solution and the microvascular casts were obtained.

The glomerular casts were subsequently dissected under a stereomicroscope. Only glomerular casts with both afferent and efferent arteriolar casts were used. A scanning electron microscope (SEM, Hitachi S-450, Hitachi, Japan) was used for the examination and photography of the casts. Three to six glomeruli from each superficial cortex and each deep cortex of one kidney were examined. Arteriolar diameters were measured on the photographic print at five different points in one arteriolar segment, 50 μm from the glomerulus. The mean vessel diameter was determined by averaging the five measurements. The data, pooled in each group of about 50 arterioles, were then analysed.

The blocks for light microscopy were fixed in 10% neutral formalin, dehydrated and embedded in paraffin. Sections were cut (3 μm) and examined after haematoxylin and eosin or periodic acid-Schiff (PAS) staining. For the semi-quantitative evaluation of glomerular damage, the glomerular sclerosis score was defined as previously described (Kimura et al. 1990). On the PAS-stained light microscopic specimens, 100 glomeruli for each kidney were graded according to the degree of sclerosis (mesangial matrix expansion): "0" if there was no mesangial expansion; "1" if mild mesangial expansion was seen (less than 30% of a glomerular area); "2" if moderate mesangial expansion occurred (30–60% of a glomerular area); "3" if marked mesangial expansion was evident (more than 60% of a glomerular area); and "4" if the sclerosis was global. The evaluation was performed by one observer (K.K.) in a blind fashion using coded slides. A composite sclerosis score was then calculated for each kidney according to the following formula: Glomerular sclerosis score = 1 × (number of grade 1 glomeruli) + 2 × (number of grade 2 glomeruli) + 3 × (number of grade 3 glomeruli) + 4 × (number of grade 4 glomeruli).

Urinary protein was measured by the pyrogallol red method (Watanabe et al. 1986). Blood glucose was measured using the multilayer-film analytical element method as reported by Ohkubo et al. (1981) (Fuji Dri-Chem System, Fuji Film Co., Japan).

The data were expressed as mean ± standard error of the mean (SEM). Comparisons among groups were assessed by one-way analysis of variance, and $p < 0.05$ was considered significant.

Results

The blood pressure in the NX group was significantly higher than that in the C group (151 ± 4 and 121 ± 4 mm Hg respectively, $p < 0.001$), whereas the blood pressure in the DM group was similar to that in the C group (120 ± 4 mm Hg) (Table 1). The daily urinary protein excretion in the NX group was remarkably aug-

Table 1. Relationship of glomerular sclerosis, blood pressure and arteriolar diameter ratio

	BP	a/e	GSS
C	121 ± 4	1.2 ± 0.1	36 ± 4
NX	151 ± 4***	1.5 ± 0.1**	146 ± 14***
DM	120 ± 4	1.4 ± 0.1*	67 ± 11*, ****
SHR[a]	197 ± 10	0.9 ± 0.1	47 ± 4

BP, Blood pressure (mm Hg); a/e, arteriolar diameter ratio (afferent to efferent); GSS, glomerular sclerosis score (see text); C, control rats; NX, rats with subtotal nephrectomy; DM, rats with streptozotocin-induced diabetes; SHR, spontaneously hypertensive rats
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs C; **** $p < 0.01$ vs NX

[a] The SHR data are cited from Kimura et al. (1989) for comparison

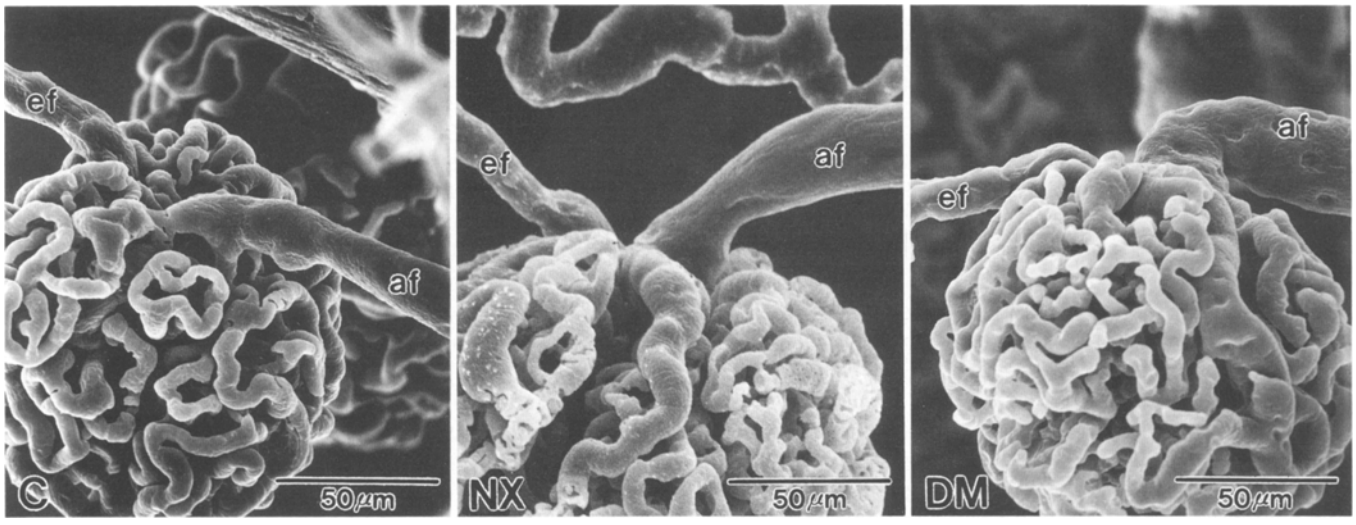

Fig. 2. Representative vascular casts from three groups. *C*, *NX* and *DM* indicate the same groups as in Fig. 1. Note that in NX and DM the afferent arterioles are significantly enlarged. *af*, Afferent arterioles; *ef*, efferent arterioles

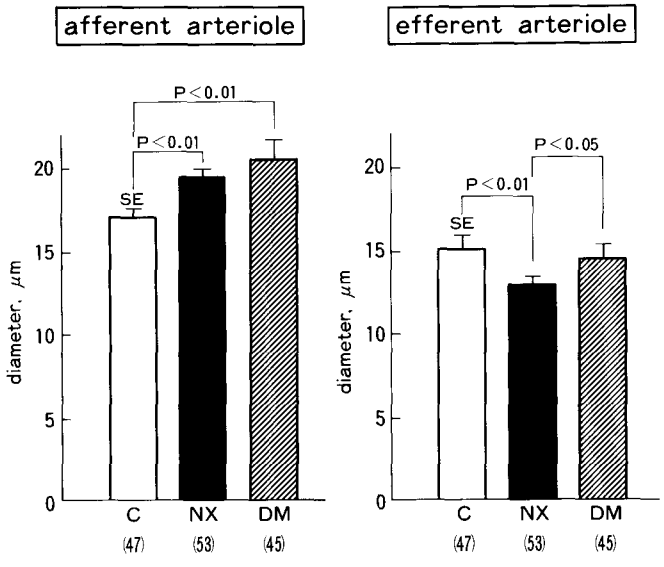

Fig. 3. The arteriolar diameters. The afferent arteriolar diameters are shown in the *left panel* and the efferent arteriolar diameters in the *right panel*. *C*, *NX*, and *DM* indicate the same groups as in Fig. 1. Note that the afferent arterioles are larger in the NX and DM groups than in the C group, and that the efferent arteriolar diameters are smaller in the NX group than in the C group. Data are expressed as mean ±SEM

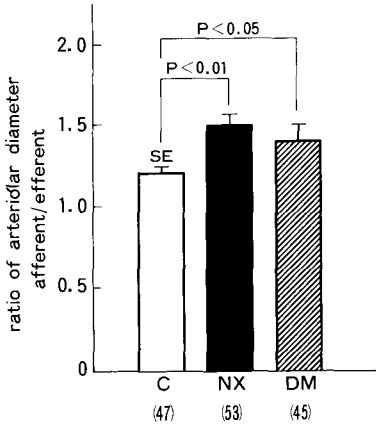

Fig. 4. Ratio of the afferent arteriolar diameter to the efferent arteriolar diameter. *C*, *NX* and *DM* indicate the same groups as in Fig. 1. Note that the diameter ratios are larger in the NX and DM groups than in the C group. Data are expressed as mean ±SEM

mented (238±48 mg/day, $p<0.001$ and C group, 27± 5 mg/day) and that in the DM group was also increased but to a lesser extent (92±15 mg/day, $p<0.02$ compared to C group). The blood glucose levels were 500±25 mg/dl in the DM group and 98±4 mg/dl in the C group ($p<0.001$).

Light microscopic photographs from the three groups are shown in Fig. 1. In the NX group, marked glomerular sclerosis and tubular dilatation and atrophy were observed. Distinct interstitial fibrosis and cell infiltration were also revealed. In the DM group, similar renal damage was observed but to a lesser extent than in the NX group, and interstitital fibrosis and cell infiltration were minimal. Glomerular sclerosis scores were 146±14 in the NX group ($p<0.001$; C group, 36±4) and 67±11 in the DM group ($p<0.02$ compared to C group, $p<0.01$ compared to NX group) (Table 1).

Representative casts from three groups are shown in Fig. 2. The results of the quantitative evaluation of arteriolar diameters are shown in Figs. 3 and 4. The average diameters of afferent and efferent arterioles in the C group were 17.2±0.4 and 15.2±0.8 μm, respectively. In the NX group, the diameter of the afferent arteriole was increased (19.6±0.4 μm, $p<0.01$ compared to C group), whereas that of the efferent arteriole was decreased (13.0±0.4 μm; $p<0.01$ compared to C group). In the DM group, the diameter of the afferent arteriole was also increased (20.5±1.1 μm, $p<0.01$ compared to C group). However, the diameter of the efferent arteriole was comparable with that in the C group (14.6±0.8 μm) (Fig. 3). The ratios of diameters of affer-

ent to efferent arterioles were elevated in both the NX group (1.5 ± 0.1, $p < 0.01$ compared to C group, 1.2 ± 0.1) and DM group (1.4 ± 0.1, $p < 0.05$ compared to C group) (Fig. 4, Table 1).

Discussion

The results showed that the afferent arterioles were significantly dilated in rats with subtotal nephrectomy and also in rats with diabetes. In rats with subtotal nephrectomy, the efferent arterioles were constricted, whereas in the diabetic rats the efferent arterioles remained unchanged.

In subtotal nephrectomy, micropuncture studies have shown that the decrease in the number of nephrons induces hyperfiltration or intraglomerular hydraulic pressure elevation in residual nephrons, which then leads to glomerular sclerosis and the progression of renal deterioration (Hostetter et al. 1981; Brenner 1985; Brenner et al. 1986; Herrera-Acosta et al. 1988; Jackson and Johnston 1988; Yoshioka et al. 1988). The present cast study revealed that the afferent arteriole was dilated, which is consistent with the results of micropuncture studies where the calculated afferent arteriolar vascular resistance is reduced. Yoshioka et al. (1988) reported that the calculated efferent arteriolar resistance is elevated in this model, which is also consistent with the present cast study where the efferent arteriole was constricted.

As previously discussed, the diameter ratio (afferent to efferent) and the systemic blood pressure are the main determinants of the glomerular pressure (Martinez-Maldonado et al. 1987; Kimura et al. 1989); when the ratio is elevated, the glomerular pressure is increased, and vice versa. In this model, as both the systemic blood pressure and the diameter ratio were elevated, the glomerular pressure is assumed to rise markedly. This assumption, based upon morphological assessment, is supported by the results of previously reported micropuncture studies (Hostetter et al. 1981; Brenner et al. 1986; Yoshioka et al. 1988). These findings are in sharp contrast with those of spontaneously hypertensive rat (SHR) kidneys. In Table 1, the present findings are compared with data from 20-week-old SHR from our previous vascular cast study (Kimura et al. 1989). In SHR kidneys, when the systemic blood pressure is elevated the afferent arterioles are constricted and the efferent arterioles are dilated, just the opposite to the findings in the remnant kidney. As a result, the arteriolar diameter ratio is significantly lowered in SHR. These differences in arteriolar diameter alterations could explain the differences in the degree of glomerular sclerosis; in the kidneys of SHR, the glomerular sclerosis score was much lower than that of the remnant kidneys from the subtotal nephrectomy model, in spite of the marked elevated blood pressure. The decrease in the diameter ratio in the SHR would contribute to the protection of glomeruli from the sclerotic deterioration by reducing the glomerular pressure. The autoregulation of glomerular filtration has been proposed as the primary mechanism of afferent arteriolar constriction in SHR (Martinez-Maldonado et al. 1987; Dilley et al. 1984). In contrast, in the subtotal nephrectomy model, the arteriolar diameter ratio was elevated, in spite of the presence of systemic hypertension, possibly indicating dysfunction of the autoregulation. Further study is necessary to reveal the mechanism of this arteriolar dysfunction.

Unlike subtotal nephrectomy, in diabetic nephropathy the efferent arteriole remained unchanged, while the afferent arteriole was dilated. In this model, as the arteriolar diameter ratio was also increased, the glomerular pressure was assumed to be elevated in spite of normal systemic blood pressure. The elevated glomerular pressure would lead to glomerular sclerosis. These results are consistent with earlier micropuncture studies where the measured intraglomerular hydraulic pressure was elevated and the calculated afferent arteriolar vascular resistance was decreased (Zatz et al. 1985; Jensen et al. 1987; Cooper et al. 1988). However, to date, the morphological observation of the arteriolar diameter in the diabetic kidney has only been reported by Volkmann and Wehner (1986), who showed renal arteriolar dilatation in genetic diabetic mice using serial light microscopic specimens. Hormonal factors and myogenic factors have been suggested as mechanisms for the afferent arteriolar dilatation in diabetic nephropathy (Bank et al. 1987, 1988; Ortola et al. 1987; Wiseman et al. 1987).

In conclusion, the present vascular cast study revealed renal arteriolar diameter alterations in the subtotal nephrectomy model as well as in diabetic nephropathy. Although these arteriolar diameter alterations are considered to lead to glomerular sclerosis, further study is necessary to reveal the precise mechanisms.

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