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ORIGINAL ARTICLE

The effect of renal administration of a selective cyclooxygenase-2 inhibitor or stable prostaglandin I_2 analog on the progression of sclerotic glomerulonephritis in rats

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Abstract
Background and methods There is increasing evidence that a change in glomerular hemodynamics may promote the development of glomerulosclerosis. In this study, we focused on the pharmacological effects of 2 contrasting agents, etodolac, a preferential cyclooxygenase-2 inhibitor, and beraprost sodium (BPS), a prostaglandin I_2 analog, delivered renally, on the disease course of progressive anti-Thy-1 (ATS) glomerulonephritis.
Results Intravital microscopic analysis showed that the diameters of glomerular capillaries and glomerular blood flow in unilaterally nephrectomized (Nx) rats treated locally with BPS were significantly increased, as compared to those of Nx rats treated locally with normal saline (NS) or etodolac. We then examined the effects of BPS and etodolac on the course of progressive glomerulosclerosis. Mesangial cell proliferation, adhesion of glomerular capillary tufts and crescent formation in the BPS-treated group appeared to be more severe compared to the ATS + NS and the ATS + etodolac groups. Scoring of mesangial proliferation and glomerulosclerosis revealed that local BPS treatment significantly worsened glomerular pathology. At day 28, there were significant differences in blood flow between the ATS + etodolac group and both the ATS + NS and ATS + BPS groups, indicating that local treatment with etodolac enhanced the recovery of glomerular circulation.
Conclusion This study provides hemodynamic-based evidence showing that disturbance of intraglomerular microcirculation is a critical marker for progressive glomerulonephritis.

Keywords Progressive glomerulosclerosis · Disturbance of glomerular microcirculation · Cyclooxygenase-2 · Prostacyclin · Local drug delivery

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Introduction

Glomerular blood flow is regulated by complex pathways. There is now increasing evidence that changes in glomerular hemodynamics may promote the development of glomerulosclerosis [1, 2]. To stop the progression of glomerulosclerosis leading to renal insufficiency, we should deepen our understanding of the mechanisms regulating glomerular hemodynamics in pathophysiological settings. For this purpose, we have extended our research with an experimental model of progressive glomerulosclerosis [3] using a confocal laser scanning microscope to readily estimate not only changes in glomerular hemodynamics, but also morphological recovery to glomerular circulation [4] and a local drug delivery system [5].

Prostaglandins modulate renal functions, including both hemodynamics and water and salt homeostasis. These eicosanoids are formed by the cyclooxygenase (COX)-dependent metabolism of arachidonic acid. There are 2 isoforms of the COX enzyme, COX-1 and COX-2. COX-1 is expressed constitutively in many tissues, and plays an important function in hemostasis and the protection of the gastric mucosa. COX-2 levels increase in response to

mitogenic and inflammatory stimuli. Harris et al. [6] first demonstrated that COX-2 is constitutively expressed in the macula densa of the juxtaglomerular apparatus and in adjacent epithelial cells of the cortical thick ascending limb of Henle. COX-2 expression in these regions increases in animals that are either chronically salt-depleted [6–8] or receiving subtotal ablation [9].

Here, we focused on the pharmacological effects of COX-2-dependent metabolism of arachidonic acid on the disease course of progressive glomerulosclerosis from the viewpoint of glomerular circulation. As a first step, we examined 2 contrasting agents, a preferential COX-2 inhibitor and a prostacyclin analog, delivered renally. The local delivery system from the subrenal capsular space used in the present study has more advantages than local blockade of the renal renin−angiotensin system; COX-2 dependent metabolism can be effectively induced with no marked effects on systemic blood pressure or serum chemistry, rather than the systemic administration of drugs.

Materials and methods

Animals

All experiments were performed using 6- to 8-week-old female Munich-Wistar rats (Simonsen Laboratories, Gilroy, CA, USA). All animal procedures were conducted with the approval of the Animal Committee of Niigata University.

Subrenal capsular implantation

The preferential COX-2 inhibitor, etodolac ([±]-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-acetic acid) was a kind gift from Nippon Shinyaku Co., Kyoto, Japan. The stable prostaglandin I_2 (PGI_2) analog, beraprost sodium (BPS; sodium-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(E)-(3S)-3-hydroxy-4-methyl-1-octen-6-ynyl]-1H-cyclopenta[b]benzofuran-5-butyrate), a kind gift from Toray Industries, Inc., Tokyo, was used as a prostacyclin analog. As described previously [5], pellets of type-1 collagen (Nitta Gelatin Co., Osaka, Japan) were combined with 50 μl of normal saline (NS) (disease control), BPS (2 mg/ml), or etodolac (100 mg/ml) and implanted in the subrenal capsular pocket.

Experimental design

Rats were divided into 2 major groups, uninephrectomy + anti-Thy-1 (Nx + ATS) group and uninephrectomy (Nx) only group. Rats in the Nx + ATS group were injected intravenously with 1.0 mg of anti-Thy-1.1 antibody (1-22-3 monoclonal antibody) and 30 min after injection unilateral nephrectomy of the right kidney was performed to induce progressive glomerulonephritis with irreversible glomerulosclerotic lesions, as described previously [3]. Rats in the Nx group were injected with 0.5 ml NS and unilateral nephrectomy of the right kidney was performed. Seven days after injection, subrenal capsular implantation of collagen sponge BPS, etodolac, and NS were performed. On day 28 after nephrectomy, all rats were examined for in vivo glomerular microcirculation, with an intravital microscope system as described below, and subsequently killed for immunohistological examination.

Light microscopy

Renal tissue was fixed with 10% neutral buffered formalin, embedded in paraffin, and sections were stained with hematoxylin and eosin, periodic acid-Schiff, and periodic acid methenamine silver. Using periodic acid-Schiff-stained sections, mesangial proliferation and glomerulosclerosis were graded semiquantitatively on a scale of 0 to 4+, as described previously [10].

Immunofluorescent microscopy

As described previously [11], frozen sections were incubated with rabbit anti-rat COX-2 antibody (Abcam, Cambridge, UK), rabbit-anti rat type-1 collagen antibody (Abcam), monoclonal mouse anti-rat α-smooth muscle actin (α-SMA) antibody (Sigma-Aldrich), monoclonal mouse anti-rat platelet-endothelial cell adhesion molecule-1 (PECAM-1) antibody (Serotec Ltd., Kidlington, UK). Fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulin antibody and fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin antibody were used as secondary antibodies (Dako, Denmark). These sections were then doubly stained with the marker, biotinylated mouse Ab-OX-7 (Abcam) for identifying glomerular mesangial cells. The sections were incubated with tetramethyl rhodamine B isothiocyanate-conjugated streptavidin (KPL Inc., Gaithersburg, MD, USA). Stained sections were examined with confocal laser scanning immunofluorescent microscopy (Nikon, Tokyo, Japan).

Urinary analysis

The amount of urinary protein excreted was determined by the Biuret method, using bovine serum albumin as a standard [3].

Serum biochemical analysis

Serum total protein, serum albumin, total cholesterol, blood urea nitrogen, and creatinine concentrations were measured.

Intravital observation of the renal microcirculation

Observation of the microcirculatory blood flow was made with an intravital microscope system (Nikon) equipped with a real-time confocal scanner unit model CSU-10 (Yokogawa Electric Corporation, Tokyo, Japan), and image processing devices, according to our previously described methods [3, 12]. The inner diameter of the glomerular capillary was measured by intravenous injection (10 mg/ml, 2 ml/kg) of a solution of fluorescein isothiocyanate-labeled dextran (M_W 150000; Sigma Chemical Co., St Louis, MO, USA) as described previously [13].

Statistical analysis

All values were expressed as mean ± SD. The Bonferroni test was used for comparison of urinary protein excretion, serum biochemical analysis, and red blood cell (RBC) velocity. The Mann–Whitney *U* test was used for comparison of mesangial proliferation index and glomerulosclerosis index. All data analysis was performed using SPSS software. *P* values <0.05 were considered significant in all statistical tests.

Results

Effects of renal local drug delivery systems in experimental groups

As shown in Table 1, ATS antibody injection with unilateral nephrectomy induced marked proteinuria at day 3. Urinary protein excretion decreased at day 7 and then gradually increased at day 28. ATS glomerulonephritis with unilateral nephrectomy was treated by the implantation of a collagen sponge containing BPS, etodolac, and NS at day 7 after disease induction. At day 14 in the ATS + BPS group, urinary protein excretion was significantly higher than that in the ATS + NS group. The urinary protein level in the ATS + etodolac group was similar to that of the ATS + NS group. At day 28, the urinary protein level in the ATS + BPS group was the highest among the 3 treatment groups, but this was not a significant difference. There was no apparent increase in urinary protein excretion in the Nx groups treated by implantation of a collagen sponge containing BPS, etodolac, and NS.

Blood urea nitrogen and creatinine in the ATS + etodolac group tended to be lower than in the ATS + NS and ATS + BPS groups at day 28 (Table 2). However, there were no significant differences in serum total protein, total cholesterol, blood urea nitrogen, albumin, or creatinine levels among all groups at days 14 and 28 after disease induction.

Adverse effects of NS and BPS, delivered locally, on renal histopathology in progressive glomerulonephritis

As reported previously [3, 12], light microscopic findings revealed diffuse mesangial cell proliferation and mesangial matrix expansion at day 14 in the ATS + NS group (Fig. 1b). Following local BPS treatment for 7 days in the ATS + BPS group, pathological lesions, such as mesangial cell proliferation, exudation into the urinary space, and the adhesion of glomerular capillary tufts, appeared to be more severe, and crescent formation was found more often than in the ATS + NS group (Fig. 1c). At day 28, mesangial proliferative change and crescent formation worsened, in addition to remarkable cell infiltration into the interstitium in the ATS + BPS group (Fig. 2c); glomerular pathology was apparently maintained at the same degree, from day 14 to day 28, in the ATS + NS group (Figs. 1b, 2b). The glomerulosclerosis indexes in the ATS + BPS group were significantly higher than those of the ATS + NS group at both days 14 and 28 (Table 3). In contrast, in the etodolac treatment group, mesangial proliferative changes appeared to be milder at days 14 and 28 (Figs. 1d, 2d). There were significant differences in mesangial matrix expansion with cell proliferation and glomerulosclerosis between the ATS + NS and the ATS + etodolac groups at day 28 (Table 3).

Table 1 Urinary protein excretion (mg/day)

	Day 0	Day 3	Day 7	Day 10	Day 14	Day 28
Nx + NS ($n = 13$)	1.2 ± 2.7	1.9 ± 3.4	1.3 ± 2.9	0.7 ± 1.7	0.8 ± 1.6	0.5 ± 1.1
Nx + BPS ($n = 9$)				0.3 ± 0.5	1.5 ± 2.4	0.3 ± 0.4
Nx + etodolac ($n = 3$)				3.7 ± 2.2	2.4 ± 0.7	3.3 ± 0.7
ATS + NS ($n = 21$)	1.4 ± 2.9	105.8 ± 29.0	59.0 ± 20.0	65.1 ± 29.1	62.7 ± 41.4	77.0 ± 43.4
ATS + BPS ($n = 16$)				73.8 ± 25.5	78.4 ± 32.2*	95.4 ± 50.7
ATS + etodolac ($n = 7$)				60.0 ± 24.8	62.0 ± 32.0	80.1 ± 21.7

* $P < 0.05$ vs ATS + NS group; each value is expressed as mean ± SD

Table 2 Serum biochemical analysis

	TP (g/dl)	Alb (g/dl)	TC (mg/dl)	BUN (mg/dl)	Cr (mg/dl)
Nx + NS					
Day 14 ($n = 5$)	4.4 ± 0.7	3.2 ± 0.5	61.2 ± 15.2	31.8 ± 6.9	0.42 ± 0.06
Day 28 ($n = 9$)	5.4 ± 1.3	4.0 ± 1.0	66.2 ± 13.9	30.0 ± 2.2	0.42 ± 0.07
Nx + BPS					
Day 14 ($n = 5$)	4.5 ± 0.5	3.3 ± 0.4	65.4 ± 9.9	29.3 ± 5.8	0.40 ± 0.05
Day 28 ($n = 8$)	5.4 ± 1.1	4.0 ± 0.6	77.0 ± 20.9	26.7 ± 7.9	0.34 ± 0.06
Nx + etodolac					
Day 14 ($n = 3$)	4.3 ± 0.3	3.2 ± 0.3	62.0 ± 5.0	25.9 ± 1.1	0.34 ± 0.03
Day 28 ($n = 3$)	4.0 ± 0.7	2.9 ± 0.5	55.3 ± 11.5	25.9 ± 3.3	0.36 ± 0.11
ATS + NS					
Day 14 ($n = 9$)	4.4 ± 1.0	2.9 ± 0.7	104.4 ± 14.2	74.1 ± 41.3	0.89 ± 0.44
Day 28 ($n = 11$)	4.5 ± 0.9	2.9 ± 0.6	132.5 ± 22.9	117.2 ± 104.7	1.35 ± 0.89
ATS + BPS					
Day 14 ($n = 7$)	4.2 ± 0.7	2.7 ± 0.3	109.1 ± 25.6	87.7 ± 41.0	0.95 ± 0.38
Day 28 ($n = 9$)	4.6 ± 1.1	2.7 ± 0.7	153.9 ± 42.7	123.7 ± 81.8	1.32 ± 0.83
ATS + etodolac					
Day 14 ($n = 4$)	3.6 ± 0.4	2.4 ± 0.4	106.0 ± 16.9	61.6 ± 23.1	0.71 ± 0.23
Day 28 ($n = 6$)	4.3 ± 1.0	2.9 ± 0.2	122.7 ± 32.3	57.1 ± 16.2	0.68 ± 0.20

Each value is expressed as mean ± SD

TP total protein, *Alb* albumin, *TC* total cholesterol, *BUN* blood urea nitrogen, *Cr* creatinine

No pathological findings were observed in the Nx + NS, Nx + BPS, or Nx + etodolac groups at days 14 and 28 (Figs. 1a, 2a; Table 3).

Immunofluorescence findings

We examined the expressions of COX-2, α-SMA, type-1 collagen, and PECAM-1 in 20–30 glomeruli and peri-glomerular areas per rat by immunofluorescence. The findings of the immunofluorescence study were semi-quantitatively analyzed and summarized in Table 4. Renal cortex COX-2 expression was localized to the macula densa at the juxtaglomerular region, but not within the glomerulus (Fig. 3a, b) in the Nx + NS group, as described originally in the normal rats [6]. COX-2 was similarly expressed in the macula densa region in all experimental groups. COX-2 expression in glomeruli was observed in all ATS nephritic groups (Fig. 3c–h; Table 4). Glomerular COX-2 expression was apparently enhanced in the ATS + BPS group compared with other ATS groups (Fig. 3c–h) although there was no significant difference (Table 4). Immunofluorescent intensity of COX-2 staining in the ATS + BPS group appeared to be increased and there was an increasing number of yellow fluorescence-positive cells, indicating colocalization of COX-2 and mesangial cell marker, OX-7, at day 28 (Fig. 3f).

In the physiological setting, there is no glomerular expression of α-SMA (data not shown). Furthermore, in the Nx + NS group, there was negative staining for α-SMA within glomeruli (Fig. 4a, b). Its expression in the ATS + BPS group and in the ATS + etodolac group appeared to have increased and decreased, respectively, compared to the ATS + NS group (Fig. 4c–h), although there was no significant difference (Table 4). The expression of α-SMA was found around the glomeruli at day 28, and the degree of immunofluorescent intensity was apparently higher in the ATS + BPS group and lower in the ATS + etodolac group, compared to the ATS + NS group (Fig. 4d, f, h).

There was negative staining for type-1 collagen within glomeruli and in the Bowman's capsule in the normal (data not shown) and Nx + NS rats (Fig. 5a, b). In contrast, type-1 collagen expression was found in and around the glomeruli in the ATS groups (Fig. 5c–h; Table 4). Type-1 collagen expression was concentrated in the mesangial area, which seemed to correspond to the sclerotic lesions found by light microscopy (Fig. 5c–h). As shown in Fig. 5d, f, and Table 4, its expression in the ATS + NS and ATS + BPS groups was enhanced with time. Type-1 collagen expression, in particular, was markedly extended around glomeruli, mainly in crescentic lesions found by light microscopy, at day 28. Interestingly, this type of abnormal type-1 collagen expression was significantly attenuated by local treatment with etodolac (Fig. 5g, h; Table 4).

As described previously [3], it seemed that there was a positive association between the impairment of vascular

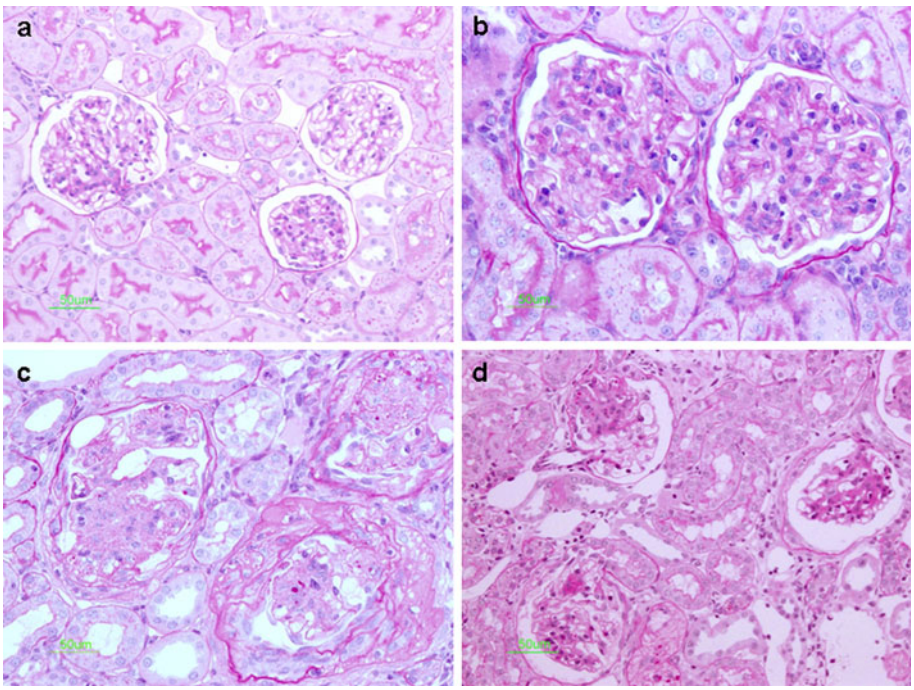

Fig. 1 Light microscopic findings of the kidney in the Nx + NS, ATS + NS, ATS + BPS, and ATS + etodolac groups at day 14. **a** There was no pathological finding in the Nx + NS group. **b** Diffuse mesangial cell proliferation and mesangial matrix expansion were found in the ATS + NS group. **c** Mesangial cell proliferation, exudation into urinary space, adhesion of glomerular capillary tufts appeared to be more severe, and crescent formation was more often found in the ATS + BPS group. **d** There were mild mesangial proliferative changes in the ATS + etodolac group (*bar* 50 μm)

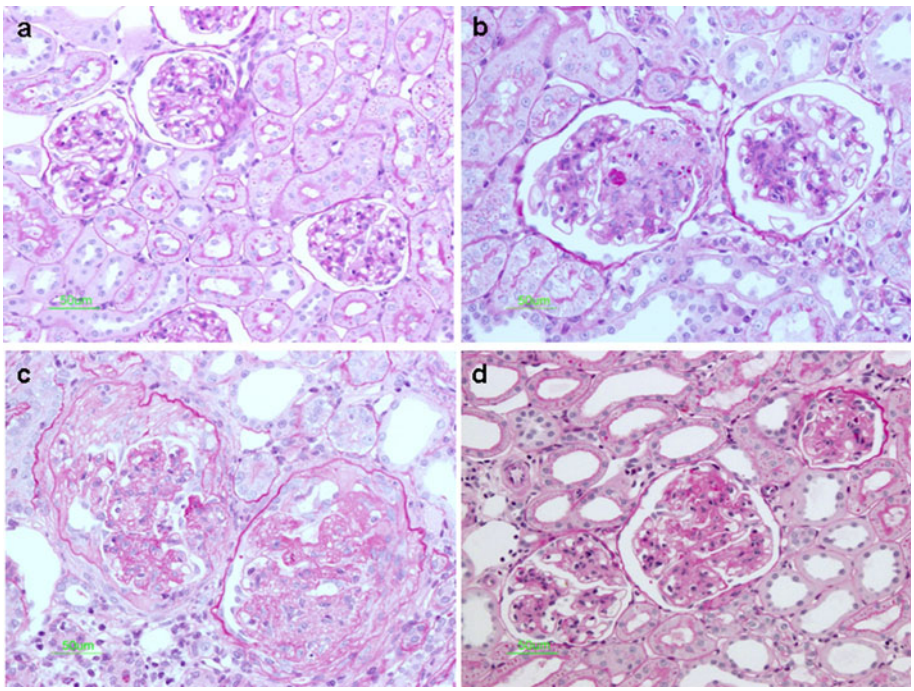

Fig. 2 Light microscopic findings of the kidney at day 28. **a** There was no pathological finding in the Nx + NS group. **b** Glomerular pathology seemed to be at the same degree as at day 14 in the ATS + NS group. **c** Mesangial proliferative change and crescent formation became worse, in addition to remarkable cell infiltration into the interstitium in the ATS + BPS group. **d** Mesangial proliferative changes in the ATS + etodolac group appeared to be milder than in other nephritic groups (*bar* 50 μm)

regeneration and the development of glomerulosclerosis. At day 28, the number of PECAM-1-positive capillaries in the ATS + etodolac group was significantly greater than that in the ATS + BPS and ATS + NS groups (Fig. 6d, f, h; Table 4).

Intravital observation of the glomerular microcirculation

We analyzed 5–10 glomeruli from each of 3–6 rats in each group, 1–5 microvessels per glomerulus, in order to

examine the effect of locally delivered drugs on the inner diameters of glomerular capillaries and glomerular blood flow. At first, we examined the effects of BPS and etodolac on the diameters of glomerular capillaries at days 14 and 28 in unilaterally Nx rats. There was no substantial difference in glomerular hemodynamics between the data at day 14 and day 28. Figure 7 shows the differences in inner diameters of glomerular capillaries among the Nx + NS, Nx + BPS, and Nx + etodolac groups. The diameters in Nx + BPS rats (8.3 ± 1.0 μm) were significantly increased compared to Nx + Ns rats (6.8 ± 1.1 μm) and Nx + etodolac (7.4 ± 0.7 μm), respectively ($P < 0.05$), indicating dilatation of glomerular capillaries by BPS. In ATS-induced glomerulonephritis at day 28, the diameters of ATS + NS and ATS + BPS rats were 6.8 ± 1.0 μm and 6.8 ± 1.2 μm, respectively. The diameters of ATS + etodolac rats were 7.6 ± 0.9 μm, significantly larger than those of ATS + NS and ATS + BPS rats. We then examined the effects of BPS and etodolac on blood flow at days 14 and 28 in the unilaterally Nx rats. As shown in Fig. 8, the mean RBC velocity in the Nx + BPS group was 884.3 ± 150.9 μm/s, significantly higher than in the other Nx groups (Nx + NS 674.8 ± 167.3 μm/s; Nx + etodolac 735.9 ± 100.3 μm/s). In the nephritic settings, as shown in Fig. 8, there were significant differences in RBC velocity between the ATS + etodolac group (651.0 ± 123.0 μm/s) and both the ATS + NS (572.0 ± 146.0 μm/s) and ATS + BPS groups (530.9 ± 164.8 μm/s), respectively. This result shows that local treatment with etodolac significantly enhanced the recovery of glomerular circulation when compared to other groups ($p < 0.05$).

Table 3 Mesangial proliferation index and glomerulosclerosis index

	Mesangial proliferation index	Glomerulosclerosis index
Nx + NS		
Day 14 ($n = 5$)	0.02	0.00
Day 28 ($n = 5$)	0.02	0.00
Nx + BPS		
Day 14 ($n = 5$)	0.00	0.00
Day 28 ($n = 5$)	0.02	0.00
Nx + etodolac		
Day 14 ($n = 3$)	0.00	0.00
Day 28 ($n = 3$)	0.02	0.00
ATS + NS		
Day 14 ($n = 8$)	1.88	0.63
Day 28 ($n = 8$)	1.89	0.71
ATS + BPS		
Day 14 ($n = 7$)	2.01	0.94**
Day 28 ($n = 6$)	2.18	1.07**
ATS + etodolac		
Day 14 ($n = 5$)	1.77	0.36*
Day 28 ($n = 5$)	1.68*	0.34*

* $P < 0.05$ vs ATS + NS group

** $P < 0.01$ vs ATS + NS group

Discussion

The present study shows that local delivery of preferential COX-2 inhibitor or PGI_2 analog with adverse effects on prostaglandin metabolism resulted in an altered course for potentially progressive glomerulonephritis. The timing of subrenal capsular administration at day 7 after disease induction was selected on the basis of our previous work from histopathological and hemodynamic aspects [3, 12]; it is considered to be a temporal turning point—delineating the point of progression of glomerulonephritis to irreversible glomerulosclerosis.

Table 4 Summary of immunofluorescence of frozen kidney sections

	COX-2	α-SMA		Type-1 collagen		PECAM-1
	Intraglom	Intraglom	Periglom	Intraglom	Periglom	Intraglom
Nx						
Day 14	0.09*	0.00*	0.00**	0.00**	0.00**	3.00**
Day 28	0.34**	0.00**	0.00**	0.00**	0.00**	3.00**
ATS + NS						
Day 14	0.80	0.71	1.29	1.38	1.25	1.80
Day 28	1.25	1.14	1.43	2.29	1.86	1.71
ATS + BPS						
Day 14	1.50	0.75	1.50	2.20	1.60	1.60
Day 28	2.20	1.80	2.20	2.38	2.75*	1.13**
ATS + etodolac						
Day 14	0.43	0.56	0.78	0.89	0.78	2.33
Day 28	0.50	0.55	0.82	1.00**	0.60*	2.50*

The mean degree of immunofluorescent intensity was expressed semiquantitatively as follows: −, almost negative; +, slightly positive; 2+, moderately positive; 3+, markedly positive

α-SMA alpha smooth muscle cell actin, *intraglom* intraglomrular region, *periglom* periglomerular region

* $P < 0.05$ vs ATS + NS group

** $P < 0.01$ vs ATS + NS group

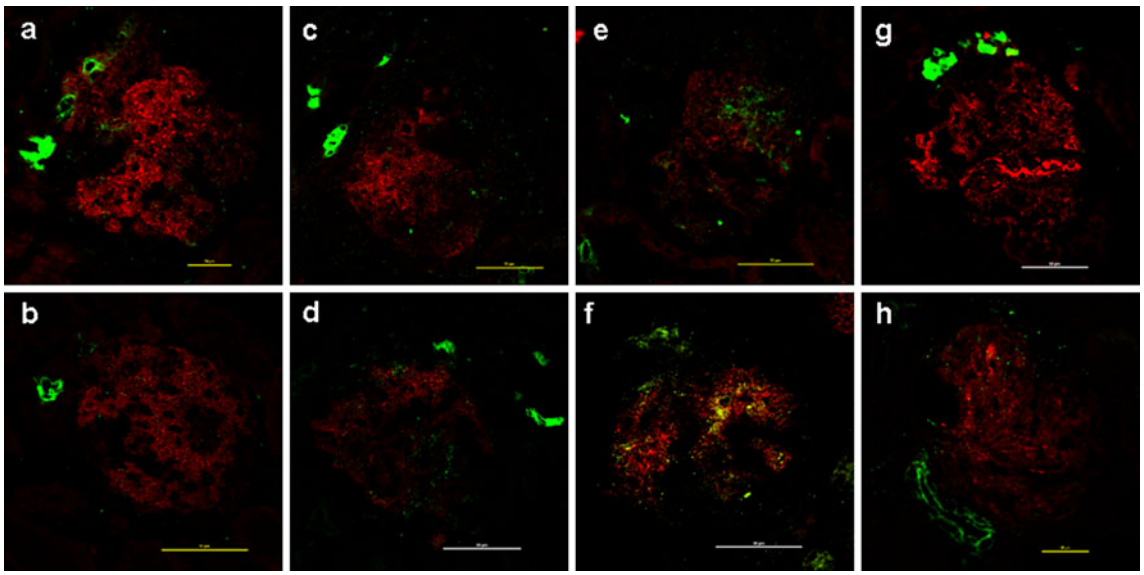

Fig. 3 Immunofluorescent analysis of COX-2 in the Nx + NS, ATS + NS, ATS + BPS, and ATS + etodolac groups at days 14 and 28. Staining for mesangial cells (OX-7, *red*) was performed as a localization marker. **a**, **b** Staining for COX-2 (*green*) was found intrinsically at the macula densa at the juxtaglomerular region, **c**, **d** COX-2 expression was not found within the glomerulus. **c**–**h** Staining for COX-2 expression in glomeruli was positive in all ATS nephritic groups, and was particularly enhanced in the ATS + BPS group (**e**, **f**): **a**, **c**, **e**, **g** day 14, **b**, **d**, **f**, **h** day 28 (*bar* 50 μm)

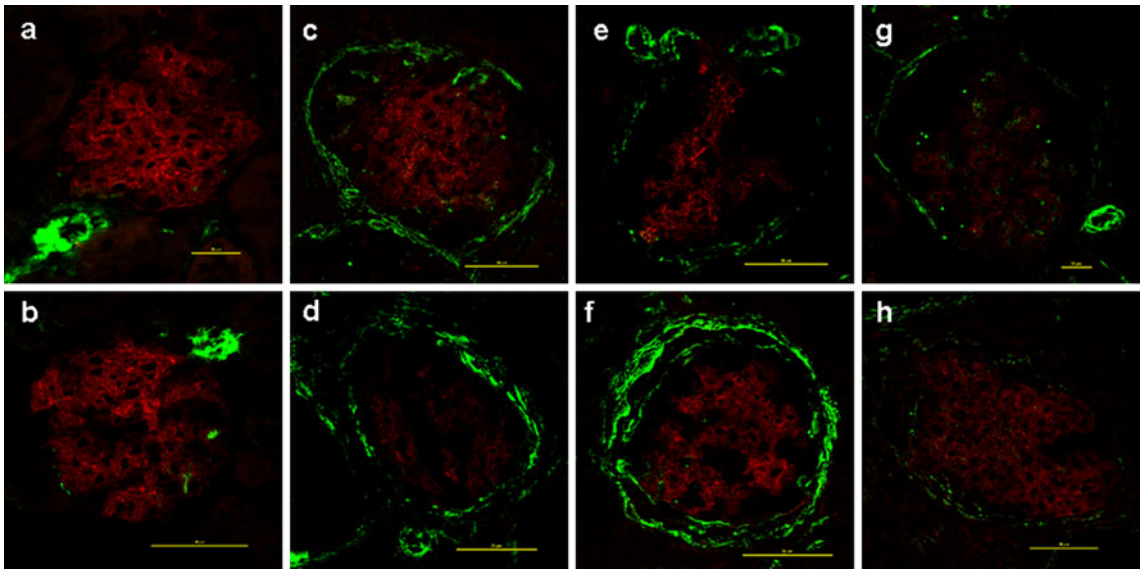

Fig. 4 Immunofluorescent analysis of α-SMA in the Nx + NS, ATS + NS, ATS + BPS, and ATS + etodolac groups at days 14 and 28. Staining for mesangial cells (OX-7, *red*) was performed as a localization marker. **a**, **b** Staining for α-SMA (*green*) was negative within glomeruli in the Nx + NS group, **c**, **d** ATS + NS group, **e**, **f** ATS + BPS, group; α-SMA expression around glomeruli was enhanced in the ATS + BPS group compared to the ATS + NS and ATS + etodolac groups, **g**, **h** the expression of α-SMA was decreased in the ATS + etodolac compared to other groups: **a**, **c**, **e**, **g** day 14, **b**, **d**, **f**, **h** day 28 (*bar* 50 μm)

There is increasing experimental and clinical evidence that COX-2 plays a pivotal role in maintaining vascular tone and water salt balance, and in inflammation [14, 15]. Wang et al. [9] reported that COX-2 selectively increased in the region of the macula densa and in the loop cells of the surrounding cortical thick ascending limb of Henle following subtotal renal ablation, while there was no change in COX-1 expression. Harris's group then extended their work to examine the pharmacological effect of a selective COX-2 inhibitor on the disease course of subtotal renal ablation [16]. Administration of the selective COX-2 inhibitor, SC58236, which was started on day 3 after

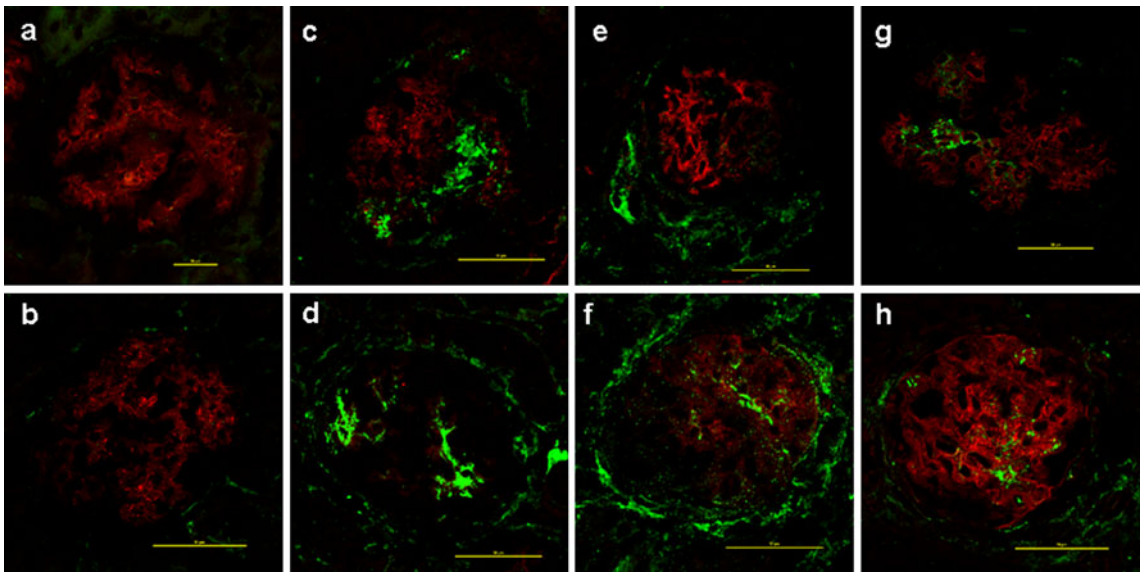

Fig. 5 Immunofluorescent analysis of type-1 collagen in the Nx + NS, ATS + NS, ATS + BPS, and ATS + etodolac groups at days 14 and 28. Staining for mesangial cells (OX-7, *red*) was performed as a localization marker. **a**, **b** There was negative staining for type-1 collagen (*green*) within the glomeruli and in the Bowman's capsule in the Nx + NS group. **c**, **d** Type-1 collagen expression (*green*) was found around and in the glomeruli in the ATS + NS group. **e**, **f** Type-1 collagen expression was enhanced over time in the ATS + BPS group. **g**, **h** Type-1 collagen expression appeared to be attenuated in the ATS + etodolac group: **a**, **c**, **e**, **g** day 14, **b**, **d**, **f**, **h** day 28 (*bar* 50 μm)

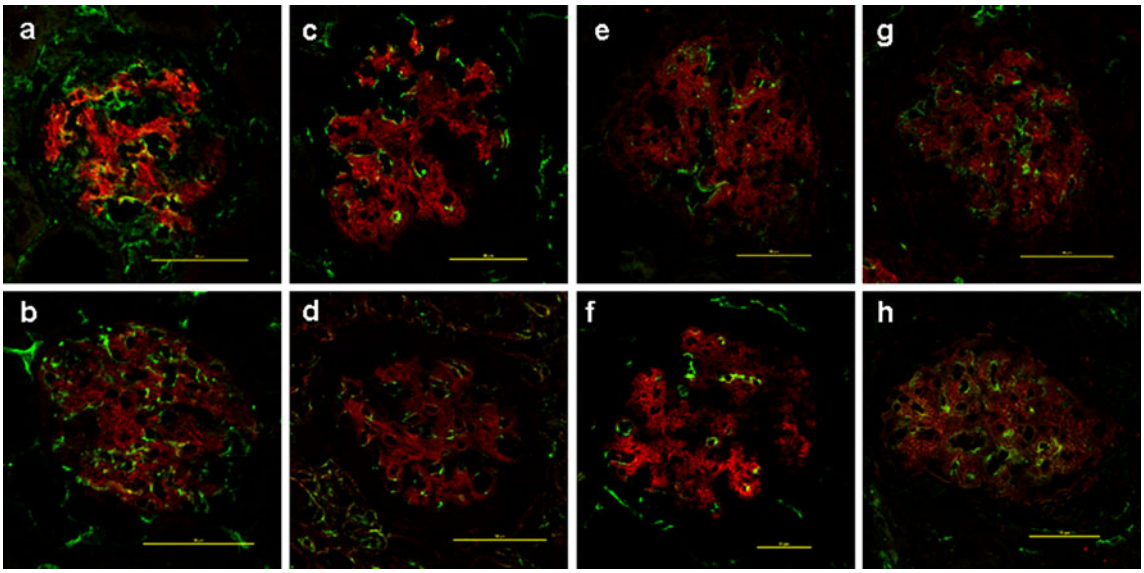

Fig. 6 Immunofluorescent analysis of endothelial cells (PECAM-1) in the Nx + NS, ATS + NS, ATS + BPS, and ATS + etodolac groups at days 14 and 28. Staining for mesangial cells (OX-7, *red*) was performed as a localization marker. **a**, **b** Nx + NS, **c**, **d** ATS + NS, **e**, **f** ATS + BPS, **g**, **h** ATS + etodolac; **a**, **c**, **e**, **g** day 14, **b**, **d**, **f**, **h** day 28. At day 28, the number of PECAM-1-positive capillaries in the ATS + etodolac group appeared to be greater than in the ATS + BPS and ATS + NS groups (*scale bar* 50 μm)

ablation, continued for 6–10 weeks and resulted in significant reduction in proteinuria at 6 and 10 weeks after ablation. COX-2 inhibitor treatment also significantly reduced the level of glomerulosclerosis. We present here evidence that the preferential COX-2 inhibitor, etodolac, has a renoprotective effect on potentially progressive glomerulosclerosis. This conclusion was drawn from histological findings, including mesangial proliferation and glomerulosclerosis indices, immunofluorescent studies for expressions of α-SMA and type-1 collagen, and analysis of glomerular hemodynamics showing recovery of glomerular capillary diameter and glomerular blood flow. It is not yet

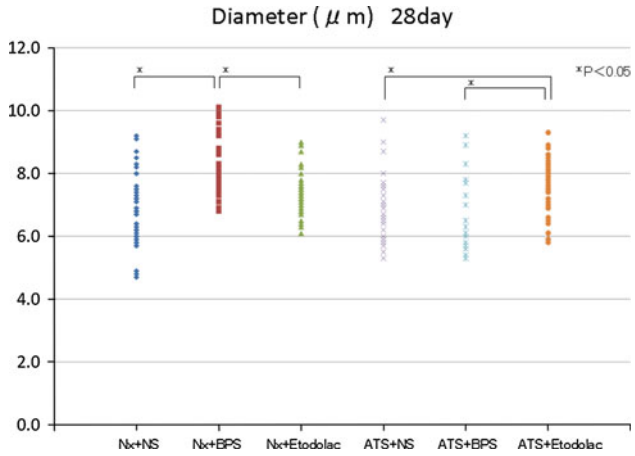

Fig. 7 The effect of BPS and etodolac delivered renally on the diameters of glomerular capillaries at day 28. To measure vessel diameter, rats were injected with FITC-labeled dextran. The diameters in Nx + BPS rats were significantly increased compared to Nx + NS rats and Nx + etodolac rats ($P < 0.05$). The diameters of ATS + etodolac rats were significantly larger than those of ATS + NS and ATS + BPS rats ($P < 0.05$)

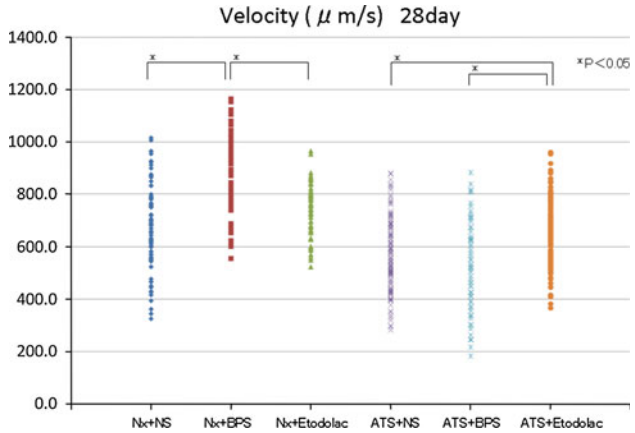

Fig. 8 The effect of BPS and etodolac, delivered renally, on RBC velocity in glomeruli at day 28. To measure RBC velocity, rats were injected with their own FITC-labeled RBCs. The mean of RBC velocity in the Nx + BPS group was significantly higher than in other Nx groups ($P < 0.05$). In the nephritic settings, there were significant differences in RBC velocity between the ATS + etodolac and the ATS + NS group and also between the ATS + etodolac and the ATS + BPS groups ($P < 0.05$)

clear whether sustained disturbance of glomerular blood flow is a cause or result of glomerular pathology, resulting in progressive glomerulosclerosis. However, it is reasonable to consider that the earlier recovery of glomerular circulation and the attenuation of glomerular pathology were caused by local treatment of COX-2 inhibitor, from the viewpoint of glomerular hemodynamics as described previously [5, 12].

On the other hand, it is known that COX-2 inhibitors induced acute renal failure, similar to that observed with nonselective COX inhibitors [17]. In anti-Thy 1.1 glomerulonephritis, selective COX-2 inhibitors, rofecoxib or celecoxib, impaired glomerular capillary repair after mesangiolysis, probably due to the inhibition of endothelial cell migration, and shape change [18]. In our present study, the number of PECAM-1-positive glomerular capillaries in the ATS + etodolac group appeared to be higher than in the ATS + BPS and ATS + NS groups at day 28. The discrepancy between these 2 studies could be explained by the different timing and pathway of drug delivery. Compared to the oral administration of selective inhibitor of COX-2, beginning 18 h after disease induction and continuing up to day 5 in the former study, we administered it only once beneath the renal capsule at a fully established stage of glomerular inflammation on day 7 when it was the critical turning point leading to irreversible glomerulosclerosis without treatment [3, 12].

PGI_2 plays a major physiological role in systemic circulation as a potent mediator of vasodilation and inhibitor of platelet aggregation. There is accumulating evidence of the therapeutic effects of PGI_2 analogs on glomerular injuries such as ATS glomerulonephritis [19], systemic lupus erythematosus-like nephritis in NZB/W F_1 mice [20], streptozotocin-induced diabetes in rats [21], and anti-glomerular basement membrane serum-induced nephritis in rats [22]. On the contrary, our present study showed the 'vicious circle' effect of PGI_2 analog on glomerular injury in the progressive model of ATS glomerulonephritis. It is worthwhile to discuss the distinctly different effects of the PGI_2 analog seen in these studies for understanding the cellular and molecular mechanisms leading to progressive glomerulosclerosis. In our present study, PGI_2 analog was administered renally at day 7 after ATS antibody injection, resulting in significantly increased urinary protein excretion at day 14 after disease induction compared to other nephritic groups. At day 28, the urinary protein level in the ATS + BPS group was the highest among the 3 nephritic groups. The results of the increased diameter of glomerular capillaries and enhanced glomerular blood flow in the Nx + BPS group at day 28 suggested that the load of hyperperfusion may continue to be rendered on the inflamed glomeruli, leading to aggravated lesions showing an abnormal expression of α-SMA and type-I collagen, and enhanced crescent formation in the ATS + BPS group. Taken together, it is reasonable to assume that timing of treatment with PGI_2 analog after disease induction and the method of PGI_2 delivery could determine the extent of adverse effects and the progression of glomerulonephritis. However, we have not yet examined the exact roles of local renin, nitric oxide and other prostanoids such as prostaglandin E_2 and thromboxane A_2, which might be involved in the deviation of renal pathology induced by local delivery of COX-2 inhibitor and PGI_2 analog. Further study

is needed to clarify the cellular and molecular mechanisms of preventing the progression of glomerulonephritis leading to renal death.

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