NEPHROLOGY 2003; **8**, 80–91

Original Article

Comparison of dipyridamole and fosinopril on renal progression in nephrectomized rats

Chollada BURANAKARL,[1] Anusak KITJTAWONRAT,[1] Somchai PONDEENANA,[1] Boonmee SUNYASUJAREE,[2] Sumolaya KANCHANAPANGKA,[3] Narongsak CHAIYABUTR[1] AND Kenneth C BOVÉE[4]

Departments of [1]Physiology, [2]Pathology and [3]Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand and [4]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA

SUMMARY: There is evidence to suggest that antiplatelet aggregation and inhibition of angiotensin converting enzyme will attenuate the progression of renal disease. In the present study, dipyridamole (DPM; 30 mg/kg per day, p.o.) or fosinopril (FOS; 20 mg/kg per day, p.o.) was given to rats for 5 weeks starting immediately after renal mass reduction (right uninephrectomy and ligation of approximately two-thirds of the blood supply to the left kidney). Renal mass reduction caused increased mean arterial blood pressure, reduced effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), azotemia and proteinuria. Neither proteinuria nor hypertension was affected by DPM, although renal function improved markedly. Rats receiving FOS showed normalization of blood pressure with a significant increase in both ERPF and GFR, along with a lower degree of proteinuria. A histological examination of the remnant kidney detected the presence of vasodilation with a lower degree of podocyte swelling in both treatment groups, with a remarkable effect in the FOS group. These data indicate that both FOS and DPM attenuate the progression of glomerular disease associated with renal mass reduction in rats. However, FOS was more beneficial than DPM because it reduced proteinuria and lowered blood pressure.

KEY WORDS: **dipyridamole, fosinopril, renal disease progression, renal mass reduction rats.**

INTRODUCTION

One of the pathological lesions seen in chronic renal disease is glomerulosclerosis. Glomerulosclerosis appears to be characterized by progressive hyalinization and sclerosis of glomeruli, and thickening and hardening of the glomerular basement membrane, including mesangial hypercellularity. The renal blood flow and glomerular filtration rate will be reduced tremendously while the systemic blood pressure is usually elevated. The mechanism involved may be related to the hyperfiltration of surviving nephrons, and can be induced experimentally by renal mass reduction. Many drugs have been studied to prevent progressive renal disease in renal mass models including antiplatelet aggregating agents, vasodilating agents, calcium channel blockers, and drugs that inhibit the renin-angiotensin system (RAS). However, results are controversial, and the reduction in systemic blood pressure may not be just a primary factor responsible to alleviate the renal disease progression.

Activation of platelets is a key feature that can lead to thromboembolic complications and progression of renal disease in Wistar rats. Platelet activation leads to the formation of the potent aggregation promotor, thromboxane A2 (TXA2), within platelets. Moreover, prostacyclin (PGI2) released by vascular endothelial cells can increase intraplatelet cyclic adenosine monophosphate (cAMP) and block platelet-aggregation. Thus, the inhibition of TXA2 or activation of PGI2 release can inhibit platelet aggregation. Recent studies showed that the thromboxane synthesis inhibitor, OKY 1581, can increase renal blood flow (RBF) and glomerular filtration rate (GFR) in rats with subtotal renal ablation.[1] The urinary protein and thromboxane excretion were also decreased along with improved renal structure. Blood pressure and cardiac index decreased after treatment. However, the results are still controversial because low dose aspirin, which also inhibits both TXA2 and PGI2

Correspondence: Dr Chollada Buranakarl, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Road, Patumwan, Bangkok 10330, Thailand. Email: bchollad@chula.ac.th

Accepted for publication 4 November 2002.

formation, given in combination with a specific thromboxane A2 receptor antagonist failed to improve proteinuria, glomerulosclerosis and hypertension.[2] Thus, the role of balance between renal TXA2 and PGI2 seems to be very important.

Dipyridamole (DPM) is a phosphodiesterase enzyme inhibitor that might be expected to produce anti-aggregation by inhibiting the breakdown of intraplatelet cAMP. In higher doses, DPM can inhibit the red blood cell (RBC) uptake of adenosine and platelet phosphodiesterase, with direct stimulation of the release of PGI2, prostaglandin D2 and inhibition of TXA2 formation. The DPM can improve renal function and reduce proteinuria in puromycin aminoglycoside nephrosis (PAN) rats.[3,4] By using the renal ablation model, DPM (10 mg/kg) plus acetylsalicylic acid (50 mg/kg) was studied along with groups receiving OKY 1581, low dose acetylsalicylic acid alone, heparin or coumarin. The results demonstrate that all groups had lower blood pressure, blood urea nitrogen (BUN) and fewer abnormal glomeruli.[5] However, no information was found when using DPM alone in this model.

The role of RAS on the protection of glomerulosclerosis was studied extensively. The angiotensin converting enzyme inhibitor (ACEI), enalapril, was found to be effective in protecting against the development of renal disease in the renal mass model in rats.[6] By using enalapril and angiotensin II receptor antagonists (AT1RA), losartan alone and in combination with enalapril can slow the progression of renal disease.[7–9] Fosinopril (FOS), another ACEI inhibitor, which has been widely used to reduce blood pressure in humans, has not been studied in a renal failure model.

The objectives of this preliminary study are to evaluate the efficacy of FOS and DPM by using a renal mass reduction model in rats. The study will include a measurement of renal function, blood pressure and histopathology.

METHODS

Four groups of male Wistar rats (National Laboratory Animal Centre, Mahidol University, Salaya, Nakornpratom Province, Thailand) with initial weights of 200–280 g were used in the present study. Rats were allowed free access to a standard rat laboratory diet containing 24% protein by weight and tap water. A 24-h urine specimen was collected from rats while they were in metabolic cages; 1 day before surgery and 1 day 5 weeks after renal ablation. Urine specimens were stored at −20°C for the determination of protein, creatinine concentration and osmolarity.

Surgical procedure for renal mass reduction

Each rat was anaesthetized with pentobarbitone sodium at a dose of 60 mg/kg bodyweight via an intraperitoneal injection. The rats were then placed on a table and the core body temperature was maintained at 37.0°C (±1°C) throughout the experiment. Before renal ablation, blood was collected in a heparinized tube for the determination of creatinine and BUN, to assure normal renal function. Rats were subjected to a five-sixths nephrectomy by the removal of the right kidney and a two-thirds to three-quarters ligation of the arterial supply to the left kidney. Group 1 ($n=8$) served as normal controls. Groups 2–4 were subjected to renal ablation and were fed standard rat chow for 5 weeks after renal ablation. Group 2 ($n=13$) was subjected to renal ablation alone. Group 3 ($n=8$) was treated with dipyridamole (DPM; Persantin®, Olic Ltd, Ayudhaya, Thailand) at a dose of 30 mg/kg bodyweight p.o. once a day. Group 4 ($n=7$) was treated with ACEI, fosinopril (FOS; Monopril ®, Bristol-Myers Squibb Australia Pty Ltd, Noble Park, Victoria, Australia) at a dose of 20 mg/kg bodyweight p.o. The drugs were suspended in 0.2–0.3 mL of water and given via gavage to groups 3 and 4. The drugs were started 1 day after ablation and continued daily for 5 weeks.

Procedures of renal function study

After 5 weeks of experiment, renal function was measured in anaesthetized rats by the clearance of inulin and para-aminohippurate (PAH). Immediately after the induction of anaesthesia, a tracheotomy was performed. A midline abdominal incision was made to expose the left kidney. The left ureter was catheterized with polyethylene tubing (PE-10) to allow for the collection of urine samples. The left femoral artery was catheterized with polyethylene tubing (PE-50). This arterial catheter was used for the determinations of baseline-packed cell volume, subsequent collections of blood samples, and for the continuous monitoring of arterial blood pressure. Blood pressure was measured by using a pressure transducer connected to a polygraph (Grass instrument Co., Quincy, MA, USA). A polyethylene catheter was also inserted into the left femoral vein for the infusion of inulin and PAH. A saline solution containing inulin (1%), PAH (0.2%) and mannitol (6%) was infused at a rate of 1 mL/h per 100 g bodyweight throughout the experiment. The period of 45 min was allowed for equilibration. After equilibration, three consecutive urine collections (20–30 min) were made for the determination of urine flow rate and volume. The urine was used to measure inulin, PAH and electrolyte concentrations (Na, K, Cl, Ca and PO_4). The midpoint blood samples were taken from the femoral artery for determinations of PAH, inulin and electrolyte concentrations. Bovine serum albumin (6%) was administered after blood collection at the same volume to replace blood losses. At the end of experiment, a blood sample was collected for the determinations of creatinine, BUN concentration and osmolarity.

When the rats were killed, the remnants of the left kidney were excised, and renal tissue was processed for histological evaluation.

Morphological studies

The renal tissues were fixed *in situ* in randomized rats of each group. These rats were perfused with normal saline, followed by 18 and 3% glutaraldehyde afterwards. Renal tissues were collected and prepared for renal structural alterations at both light and electron microscopic levels.

Laboratory measurements

Urinary and plasma PAH, and inulin concentrations were determined by using the ethylenediamine[10] and anthrone[11] methods,

respectively. The packed cell volume was determined by using the microcentrifugation method. Sodium and potassium in plasma and urine were determined by flame photometry (Flame photometer 410C; Ciba Corning Diagnostics Scientific Instruments, Essex, UK). Plasma and urinary chloride concentration were measured by using a chloridometer (Chloride analyser 925; Ciba Corning, Inc.). Plasma and urine calcium were analysed by the method of Moorehead and Biggs,[12] while inorganic phosphorus concentrations were measured by using the method of Gomori.[13] Plasma urea concentrations were analysed by using a colourimetric method using diacetyl monoxime reagent for colour development.[14] Plasma and urine concentrations of creatinine were analysed by using a colourimetric method using Jaffe's reaction.[15] Plasma and urine osmolarity were measured by the use of an osmometer (Osmometer 3D3; Advance Instruments Inc., Norwood, MA, USA). The urinary protein concentration was measured by using a colourimeter after precipitation with 3% sulfosalicylic acid.

Calculation of renal clearance

Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were calculated by using the clearance of PAH and inulin, respectively. Effective renal blood flow (ERBF) was estimated by ERPF/1-Hct. The filtration fraction (FF) was calculated by the ratio between GFR and ERPF. Renal vascular resistance was calculated by the ratio between mean arterial blood pressure (MAP) and ERPF. The renal electrolyte to the inulin clearance ratio was obtained to represent fractional electrolyte excretion. The mean arterial presusre was obtained by a calculation using the sum of the diastotic blood pressure and one-third of the pulse pressure.

Statistical analysis

All results are expressed as mean ± SEM. Data were analysed by using one way analysis of variance (ANOVA) with Fisher's least-significant difference test. Some data were analysed by using non-parametric Kruskal–Wallis and Dunn's analysis for multiple comparison with controls or the nephrectomized (NPX) group. Statistical significance is found when P values are less than 0.05.

RESULTS

Bodyweight

The bodyweight of rats in each group is shown in Table 1. Rats in groups 2 and 4 had bodyweights significantly lower than that of rats in groups 1 and 3 ($P < 0.05$). There was no difference in bodyweight between either groups 2 and 4 or groups 1 and 3.

Arterial blood pressure

The mean arterial blood pressure in group 1 was 148 ± 5 mmHg (Fig. 1). Blood pressure was increased by

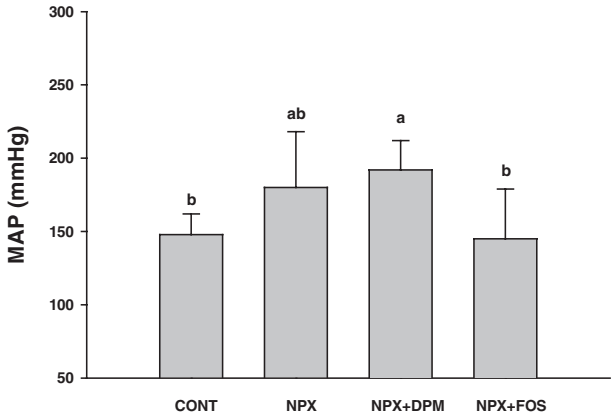

Fig. 1 Mean arterial blood pressure in four groups of rats. Values are reported as mean ± SE. [a,b]Means with different superscripts differ significantly ($P < 0.05$). CONT, control group; DPM, dipyridamole; FOS, fosinopril; MAP, mean arterial pressure; NPX, nephrectomized group.

Table 1 Bodyweight, plasma concentration of electrolytes, creatinine, urea, plasma osmolarity and packed cell volume in four groups of rats

	Control	Ligate	Dipyridamole	Fosinopril
Bodyweight (g)	373.6 ± 16.1[a]	327.5 ± 9.9[b]	372.9 ± 13.6[a]	314.3 ± 8.3[b]
P Na (mEq/L)	141.4 ± 1.6	139.2 ± 0.8	138.4 ± 1.3	139.3 ± 1.3
P K (mEq/L)	3.63 ± 0.21	3.73 ± 0.11	3.89 ± 0.15	3.73 ± 0.13
P Cl (mEq/L)	109.8 ± 2.2[ab]	105.5 ± 1.6 [b]	106.5 ± 2.5[b]	114.0 ± 1.9[a]
P Ca (mg%)	8.44 ± 0.55	8.72 ± 0.33	7.20 ± 0.36	8.80 ± 0.66
P Pi (mg%)	5.14 ± 0.36	6.15 ± 0.38	6.01 ± 0.89	4.74 ± 0.85
Pcr (mg%)	0.620 ± 0.075[b]	1.093 ± 0.079[a]	1.358 ± 0.181[a]	0.891 ± 0.066[ab]
P urea (mg%)	16.44 + 1.38[b]	31.87 + 2.66[a]	35.79 + 3.08[a]	29.27 + 3.02[a]
P osm (mOsm/L)	312.7 ± 4.6	323.9 ± 5.3	316.0 ± 6.8	313.9 ± 5.1
PCV (%)	48.14 ± 1.63	45.74 ± 2.33	47.96 ± 0.94	46.53 ± 0.94

Data reported as mean ± SEM. [a,b]Means in the same row with different superscripts differ significantly ($P < 0.05$). P Ca, plasma concentration of calcium; P Cl, plasma concentration of chloride; Pcr, plasma concentration of creatinine; PCV, packed cell volume; P K, plasma concentration of potassium; P Na, plasma concentration of sodium; P osm, plasma osmolarity; P Pi, plasma concentration of inorganic phosphorus; P urea, plasma concentration of urea.

22% in group 2 with renal mass reduction alone (180 ± 11 mmHg). Group 3 had a 30% (192 ± 7 mmHg; $P < 0.05$) increase in blood pressure. In group 4, blood presssure was not significantly different from group 1 (145 ± 13 mmHg). There was no significant difference among the groups in terms of heart rate (group 1, 333 ± 12; group 2, 367 ± 11; group 3, 375 ± 5; and group 4, 358 ± 16 beats/min).

Plasma urea and creatinine concentrations

Plasma urea concentrations in all three groups with a five-sixths renal reduction for 5 weeks with or without drug treatment were higher than those of the control group ($P < 0.05$; Table 1). However, there were no significant differences in plasma urea concentration among renal mass reduction groups with and without drug treatment. Plasma creatinine concentration was significantly higher in groups 2 and 3 (renal mass reduction alone and with dipyridamole) by 78 and 119%, respectively ($P < 0.05$). In renal mass reduction rats that received fosinopril, plasma creatinine concentration declined compared with groups 2 and 3, and showed no significant difference compared with the control group (Table 1).

Plasma electrolytes concentration

Plasma Na^+, K^+, Ca^{2+} and inorganic phosphorus (Pi) were not different among the groups (Table 1). However, plasma Cl- concentrations in groups 2 and 3 were significantly lower than that of group 4. Plasma osmolarity and packed cell volume were not different among rat groups (Table 1).

Renal haemodynamics

No significant differences in urine flow rate was found among the rat groups (Fig. 2). Renal mass reduction alone results in a significant reduction in the glomerular filtration rate in all rat groups. A marked reduction was found in rats subjected to renal mass reduction alone (Fig. 2).Treatment with dipyridamole and fosinopril improved GFR, with a significantly higher than renal mass reduction alone. However, when comparing the left kidneys of control rats with those with renal mass reduction in all three groups, the GFR was not different among the four groups (control, 1.837 ± 0.305; group 2, 1.245 ± 0.229; group 3, 2.289 ± 0.457; group 4, 2.363 ± 0.393 μL/kg per min). The effective renal plasma flow and effective renal blood flow were both significantly lower in groups 2 and 3 by 60 and 42%, respectively, compared with normal control rat groups (Fig. 2). Both groups 3 and 4, which received drug treatment, had significantly higher ERPF and effective renal blood flow (ERBF) compared with renal mass reduction alone. Both values were improved dramatically in group 4 with fosinopril treatment, with no significant difference from that of the control. It was noted that both GFR and renal blood flow to the left remnent kidney of rats in groups 3 and 4, which received drug treatment, were slightly higher than it was in the left intact kidneys of the control group. No significant differences were found in terms of the filtration fraction among all groups.

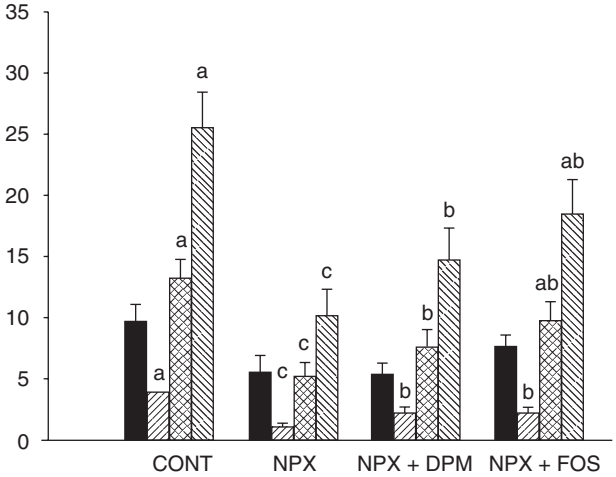

Fig. 2 Renal haemodynamic. (■) Urine flow rate (μL/min), (▨) glomerular filtration rate (μL/g/min), (▩) effective renal plasma flow (μL/g/min) and (▧) effective renal blood flow (μL/g/min) in four groups of rats. Values are reported as mean ± SEM. [a,b,c]Means with different superscripts differ significantly ($P < 0.05$). CONT, control group; DPM, dipyridamole; FOS, fosinopril; NPX, nephrectomized group.

Renal vascular resistance

The renal vascular resistance in renal mass reduction rats with one kidney (group 2) was significantly higher than control rats with two kidneys (23-fold higher, 17.42 + 2.56 control group *vs* 403.31 + 261.12 mmHg/mL per min group 2). By comparing the left renal vascular resistance (RVR) of group 2 and the left RVR of the control group, the values were still ninefold higher (43.11 ± 7.10 mmHg/μL per min for the left kidney of the control group). Rats treated with dipyridamole, although having a lower RVR than group 2, had values still significantly higher than that of the control group (60.28 + 16.54 mmHg/μL per min). The RVR of rats in group 4 was 30.86 + 5.7 mmHg/μL per min with no difference when it was compared with the control group, based upon a calculation using either total or left kidney.

Urinary excretion of electrolytes

The urinary excretion of Na^+ and K^+ were not different among the groups (Table 2). However, the calculated fractional excretion of Na and K was significantly higher by 2.9- and 4.5-fold, respectively, in rats with renal mass reduction alone (group 2; Table 2). No changes were found between the control and group 3, but slightly higher values were found in group 4, although these were not significantly different from the control. The urinary excretion of Cl^- was significantly lower, while fractional excretion of Cl^- was higher in group 2 than in the control. The lower values were found in rats treated with dipyridamole. The urinary excretion of calcium was significantly lower in all renal mass reduction groups. The urinary phosphorus excretion was not different among the groups, although fractional excretion of Pi was significantly higher in groups 2 and 4 compared with the control group (Table 2).

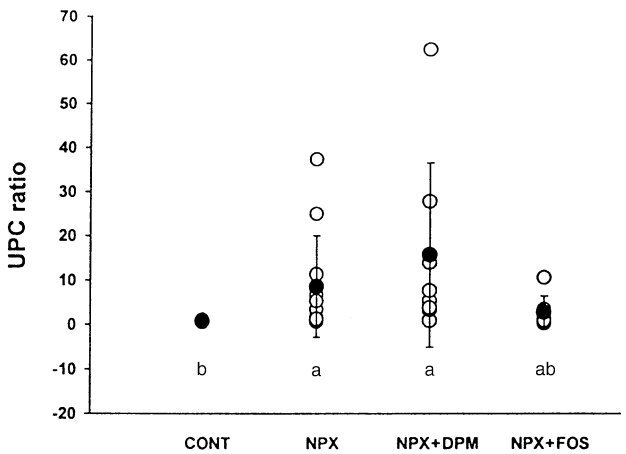

Fig. 3 Urinary protein creatinine (UPC) ratio in four groups of rats. Values are reported as mean ± SEM. [a,b]Means with different superscripts differ significantly ($P < 0.05$). (●) Mean value in each group, (○) data obtained from individual rats. CONT, control group; DPM, dipyridamole; FOS, fosinopril; NPX, nephrectomized group.

Urinary protein creatinine ratio

The urinary protein creatinine ratio in the control group was 0.82 ± 0.08. The ratio was significantly higher; by 10-fold in rats with renal mass reduction (group 2), and by almost 20-fold in rats with renal mass reduction who received dipyridamole for 5 weeks (group 3). However, this ratio was lower in rats who received fosinopril treatment, and this was not significantly different from that of the control group (Fig. 3; Table 2). There was no significant difference in terms of the ratio of urinary and plasma osmolarity among rat groups (Table 2).

Structural and ultrastructural alterations

Glomerular alteration

Group 2 (nephrectomized rats) had an obliterated glomerular tuft lumen when compared with the control rat group (Figs 4a,b; 5a,b). The glomerular epithelia (podocytes) were swollen, thus, no space was observed between each glomerular capillary. Granules with a Periodic Acid Schiff (PAS) positive reaction were found in the podocyte cytoplasm (Fig. 4c). Glomerular capillaries in the DPM-treated group (group 3) were more apparent (Figs 4d, 5c) than those in group 2, and podocytes, endothelial cells and mesangial cells were able to defy. How-

Table 2 Urinary and fractional excretion of electrolytes, urinary and plasma osmolarity ratio (Uosm; Posm), and urinary protein creatinine (UPC) ratio in four groups of rats

	Control (total)	Ligate	Dipyridamole	Fosinopril
UNaV (μEq/min)	13.345 ± 1.873	8.269 ± 1.864	6.375 ± 1.378	11.593 ± 1.280
UKV (μEq/min)	2.046 ± 0.183	1.464 ± 0.219	1.473 ± 0.249	1.745 ± 0.144
UClV (μEq/min)	10.691 ± 1.857[a]	6.032 ± 1.564[b]	4.272 ± 1.181[b]	9.084 ± 1.041[ab]
UCaV (μg/min)	4.078 ± 0.759[a]	2.090 ± 0.491[b]	0.998 ± 0.350[b]	1.916 ± 0.347[b]
UPiV (μg/min)	34.361 ± 3.915	21.362 ± 3.810	24.700 ± 4.865	31.336 ± 3.275
FENa (%)	6.751 ± 0.779[b]	19.353 ± 4.608[a]	6.228 ± 1.486[b]	12.808 ± 2.525[ab]
FEK (%)	42.07 ± 3.92[b]	188.03 ± 81.32[a]	51.34 ± 8.88[b]	70.54 ± 8.55[ab]
FECl (%)	6.751 ± 0.863[bc]	14.655 ± 1.987[a]	5.129 ± 1.330[c]	12.295 ± 2.496[ab]
FEPi (%)	52.22 ± 8.11[c]	105.22 ± 13.96[ab]	54.21 ± 9.31[c]	111.24 ± 13.20[a]
Uosm/Posm	0.797 ± 0.124	1.509 ± 0.228	0.942 ± 0.132	1.136 ± 0.187
UPC ratio	0.822 ± 0.080[b]	8.528 ± 3.291[a]	15.642 ± 7.350[a]	2.743 ± 1.361[ab]

Data reported as mean ± SEM. [a,b,c]Means in the same row with different superscripts differ significantly ($P < 0.05$). Comparisons between treatment groups were compared with the control group. FECl, fractional excretion of chloride; FEK, fractional excretion of potassium; FENa, fractional excretion of sodium; FEPi, fractional excretion of inorganic phosphorus; UCaV, urinary excretion of calcium; UClV, urinary excretion of chloride; UKV, urinary excretion of potassium; UNaV, urinary excretion of sodium; UPiV, urinary excretion of inorganic phosphorus.

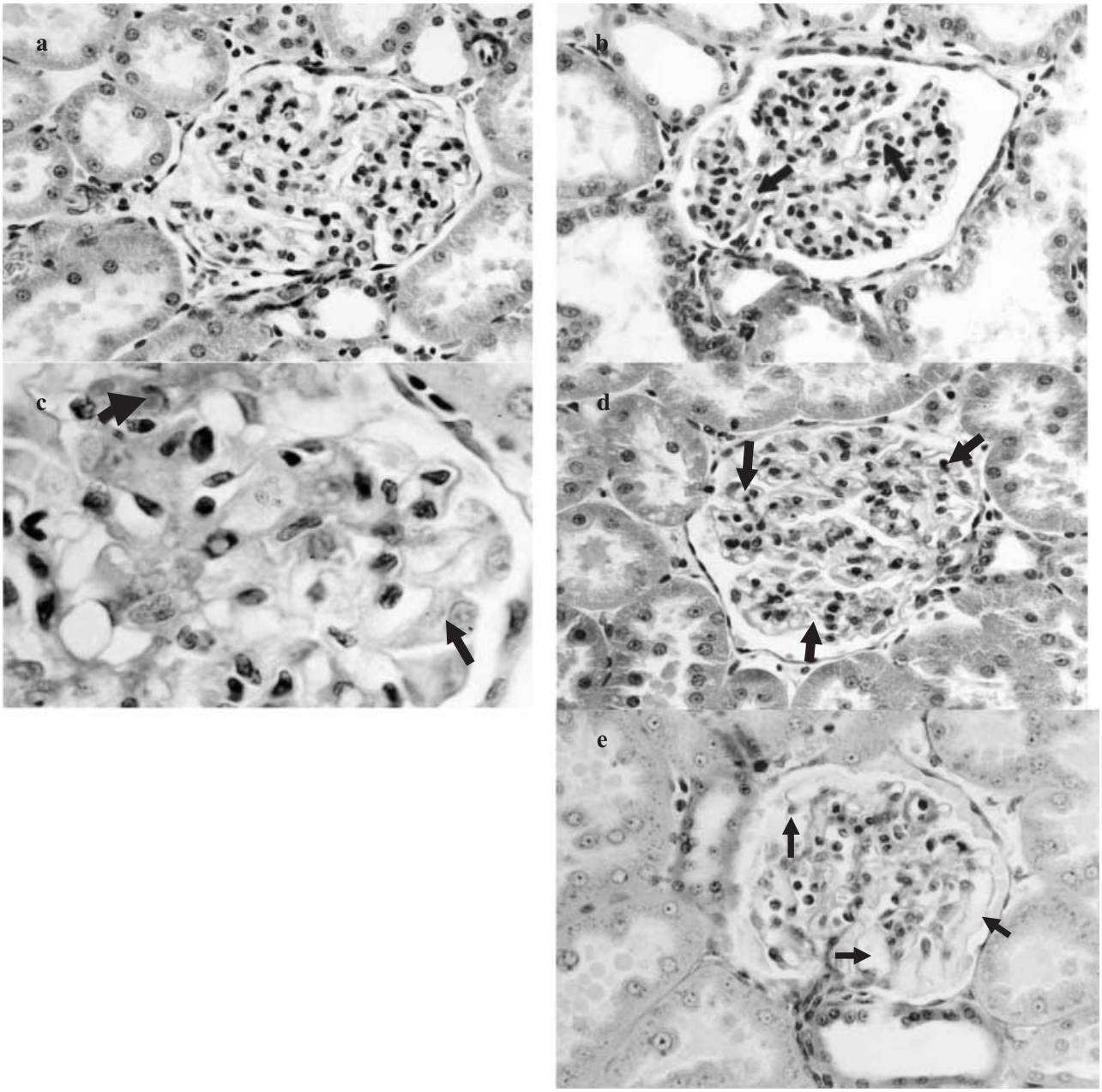

Fig. 4 Glomerular alterations of rats at the light microscopic level. (a) Group 1. Control glomerulus. HE 400×. (b) Group 2 (nephrectomized and kidney mass reduced). Swollen and fusion of glomerular epithelia (arrows) HE 400×. (c) Group 2. Positive PAS granules are observed in the podocyte cytoplasm (arrows) PAS 1000×. (d) Group 3 (dipyridamole treated). Glomerular capillaries are apparent (arrows). HE 400×. (e) Group 4 (fosinopril treated). Glomerular capillaries are apparent (arrows) and an outline of podocyte is observed. PAS, periodic acid Schiff.

ever, PAS positive granules continued to be found in the cytoplasm of glomerular cells. Group 4 (FOS treated) rats had glomerular appearances similar to that of the DPM-treated rats (Figs 4e,5d), and an outline of podocytes was observed. A histochemical reaction was unable to demonstrate the thickening of the glomerular basement membrane (GBM).

Ultrastructural alterations

An electron micrograph from group 2 rats revealed swollen podocytes and a fusion of the cell foot processes when compared with the control rats (Figs 6a,b). Splits (separation) within the GBM were frequently found. Rats treated with DPM and FOS (groups 3 and 4) also dis-

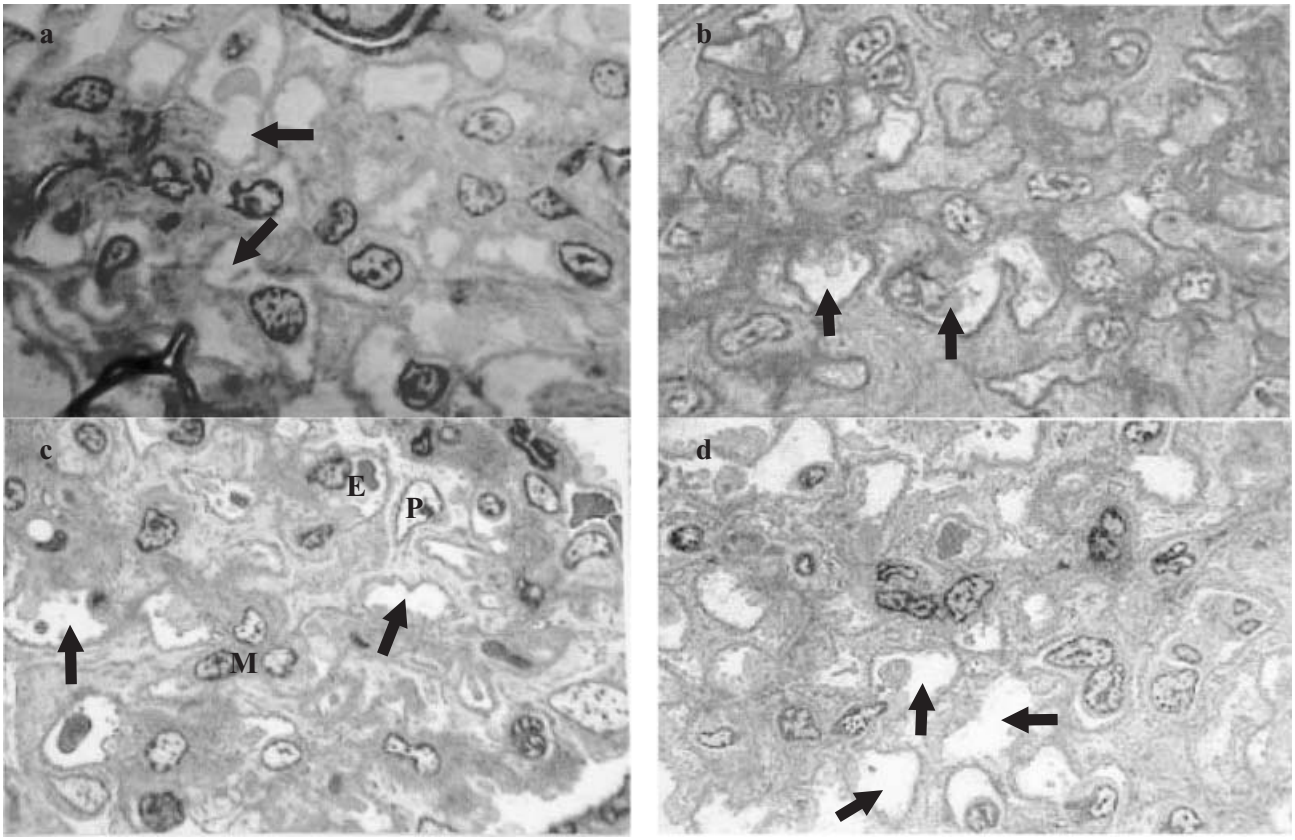

Fig. 5 Thick section (1 μm) of glomerular alterations. (a) Group 1. Control glomerulus with clear glomerular capillaries (arrows). Toludine Blue (TB) 1000×. (b) Group 2. Obliterated glomerular capillaries are cleared up (arrows). Podocytes are swollen and cellular outline is obscured. TB 1000×. (c) Group 3. Glomerular capillaries are cleared up (arrows) and podocyte (P) cytoplasmic outline is apparent. E, endothelial; M, mesangial cell. TB 1000× . (d) Group 4. Glomerular capillaries are observed (arrows) and the podocyte cytoplasmic outline is observed. TB 1000×.

played splits GBM (Fig. 6c), although podocytes appeared less swollen.

DISCUSSION

The present study indicates that rats subjected to 70% of renal mass reduction developed hypertension, proteinuria and reduced renal function. Plasma urea and creatinine concentration are almost doubled, while a significant reduction in GFR and RBF was found. The blood pressure was significantly higher in group 2 compared with that of the control group. The glomerular and tubular lesions were observed by both light and electron microscopy. The podocytes were swollen, and an obliterated glomerular capillary lumen was observed. Interestingly, the glomerular basement membrane did not reveal any thickening. However, frequent splits GBM were apparent in the NPX group. A previous study showed that urinary protein excretion in Sprague–Dawley rats subjected to renal mass ablation increased significantly 30 days after surgery. Serum creatinine concentration also progressively increased. However, the focal glomerulosclerosis affected only 8% of glomeruli at day 30, and 24% of glomeruli at day 120.[16] The previous report on spontaneously hypertensive rats also showed that only the animal with a five-sixths nephrectomy and hypertension became uraemic, and only 71% of total glomeruli was sclerotic at 26 weeks.[17] The medial thickening of the arterial walls was increased in these rats, and the diameter of the normal-appearing glomeruli was also increased and consistent with the degree of nephrectomy. Because the spontaneously hypertensive rats were used, the degree of azotemia and sclerotic lesion may be more pronounced than when normotensive rats were used. Also, the duration after the nephrectomized period was longer compared with the present study.

The study by Cortes *et al.*[18] demonstrated that the major determinants of glomerular volume expansion include capillary wall tension, basal glomerular volume and intrinsic distensibility, which is markedly influenced by the character of extracellular matrix. The prolifera-

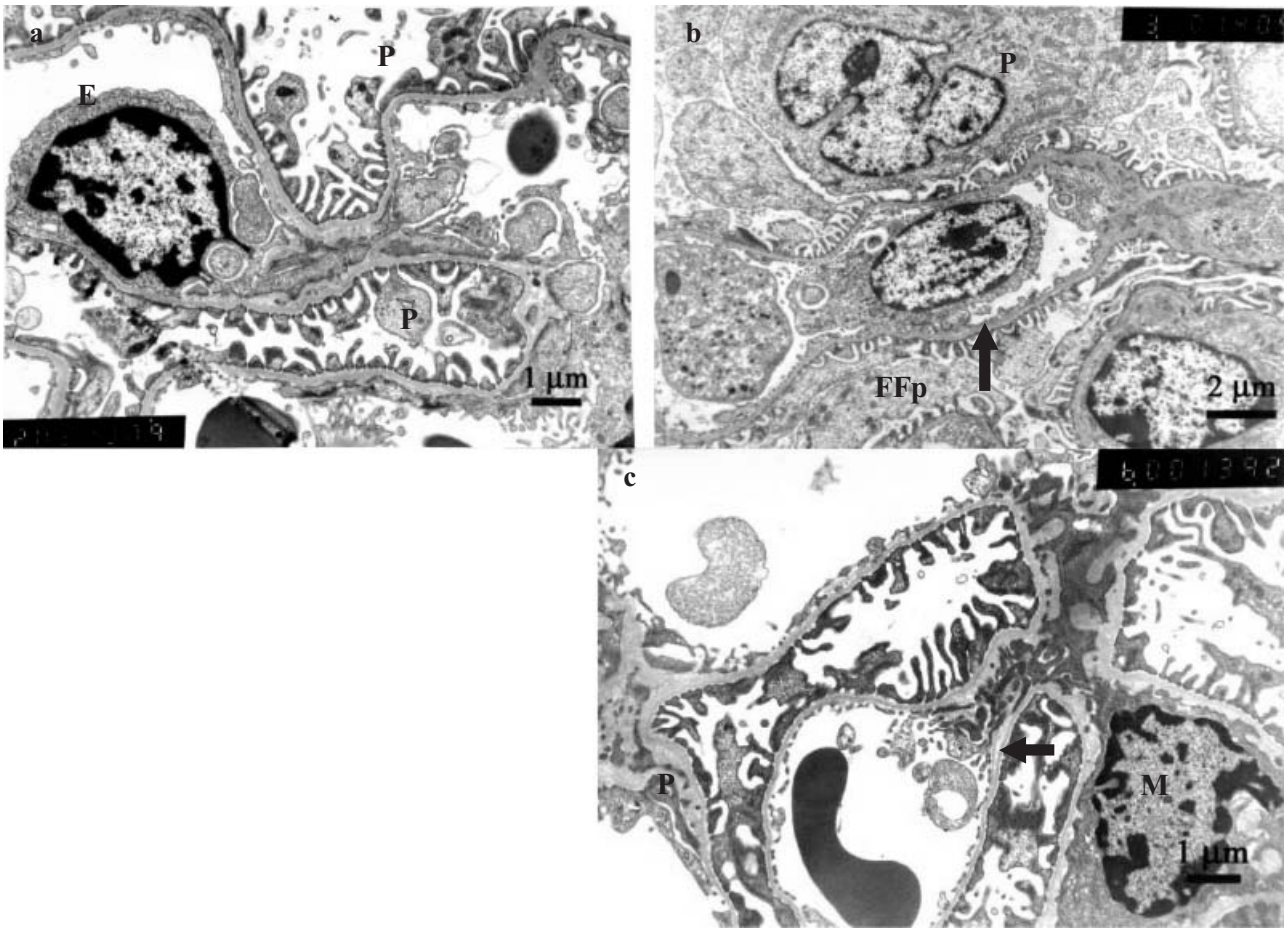

Fig. 6 Electromicrographs of glomerular alterations. (a) Group 1. Control glomerulus. E, endothelium; P, podocyte. 9000×. (b) Group 2. Swollen podocyte (P) obliterate the glomerular space. Fusion of podocyte foot processes (FFp) and splits of GBM (arrows) are occassionally found. 5500×. (c) Group 4. Podocyte (P) appears less swollen compared to group 2. Some split GBM is observed (arrow). M, mesangial cell. 9000×. GBM, glomerular basement membrane.

tion of renal mesangial cells, and to a lesser degree endothelial cells, from day 5 to week 4 can be detected by immunostaining for the proliferating cell nuclear antigen (PCNA). The glomerular sclerotic changes and leucocyte infiltrate consisted of monocytes/macrophages are increased markedly at week 10 in rats who had undergone renal ablation.[19] Because our rats were subjected to 5 weeks of renal ablation, the histological results obtained from light microscopy of sclerotic glomeruli were not pronounced compared with previous studies. However, the impaired renal function with less GFR and ERPF corresponded to the higher renal vascular resistance and obliterated glomerular capillary lumen.

Rats receiving DPM showed improved renal function, with an increase in both GFR and renal blood flow. However, blood pressure was still high and urinary protein loss was even higher when compared with nephrectomized rats that did not receive treatment. It was found that there is a relationship between the degree of hypertension and proteinuria. A study on the effect of blood pressure on proteinuria in spontaneous hypertensive rats subjected to renal mass reduction showed that a larger nephrectomy is related to higher proteinuria and a lower total serum protein and albumin.[17] Also, with the same extent of nephrectomy, these changes were severe in the group of rats with untreated hypertension. Thus, the higher urinary protein creatinine ratio in the DPM group may be caused by the hypertensive state, even though the renal vascular resistance was reduced compared with the NPX group.

When comparing the results with other antiplatelet aggregating agents, it was shown that by using a low dose of aspirin, which also inhibits both TXA2 and PGI2 formation, failed to improve proteinuria and glomerulosclerosis, while hypertension still persisted in rats that had a surgical reduction of renal mass.[2] However, by giving a thromboxane synthesis inhibitor (OKY 1581) alone without blocking PGI2, an increased RBF and GFR occurred in rats that had a subtotal renal ablation.[1] The urinary protein and thromboxane excretion were also

decreased along with an improved renal structure. Both blood pressure and cardiac index decreased after treatment. Another thromboxane A2 synthase inhibitor (FCE22178) was given to rats for 35 days starting 10 days after surgical ablation, which showed an improvement in renal function in comparison to rats receiving vehicle alone.[20] The systolic blood pressure was significantly lower than it was in animals given the vehicle. The urinary thromboxane B2 excretion was significantly decreased, but an increase in urinary 6-keto-prostaglandin F1 alpha was observed. It was concluded that the role of balance between renal TXA2 and PGI2 seems to be very important, particularly increased PGI2 on antiplatelet aggregation.

Although the blocking of TXA2 action alone seems to have beneficial effects on renal disease progression, the controversial results were reported by using daltroban, a thromboxane receptor antagonist, which showed that the drugs cannot protect against the development of renal disease in renal mass model.[6] Neither the proteinuria nor the hypertension was affected by daltroban administration. A histological examination of the remaining kidney demonstrated no beneficial effect of daltroban.

When comparing the result with other studies using DPM, it was found that in puromycin aminoglycoside nephrosis (PAN) rats, DPM can scavenge hydroxyl radicals and thus alleviate the PAN nephrosis.[3] The suppressive effect of DPM on proteinuria of aminoglycoside nephrosis rats was also found.[4] In the model of renal ablation, DPM (10 mg/kg) plus acetylsalicylic acid (50 mg/kg) was studied along with groups receiving OKY 1581, low-dose acetylsalicylic acid alone, heparin or coumarin. The results demonstrate that all groups had lower blood pressure, BUN and fewer abnormal glomeruli.[5] These studies were not consistent with the present study, especially on renal protein excretion, blood pressure and BUN. It is possible that the dose of DPM used in the present study caused the inhibition of the phosphodiesterase enzyme without stimulation of the release of PGI2, prostaglandin D2, and the inhibition of TXA2 formation. Furthermore, the study using DPM solely to slow renal disease in this model has not yet been reported. We proposed that the mechanism involved in improvement of renal function is anti-intraglomerular thrombosis rather than antihypertensive action, which is implicated in the pathogenesis of glomerulopathy. From a light microscopical examination after rats received DPM, renal tissues showed an increase diameter of glomerular capillary lumen compared with nephrectomized rats alone. The podocytes were less swollen. Thus, blood flow through the kidney could be higher and more plasma could be filtered.

In the present study, rats with renal mass reduction had less urinary excretion of all electrolytes. However, the remnent kidney can compensate by increasing the fractional excretion of all electrolytes. Nephrectomized rats administered DPM can reduce the fractional excretion of all electrolytes significantly, resulting in a reduction of the urinary to plasma osmolarity ratio (Uosm/Posm). The suppression of fractional excretion of all electrolytes caused by DPM is not surprising because DPM is a phosphodiesterase inhibitor, which inhibits the accumulation of c-AMP. Under the chronic treatment of DPM, a dose of 75 mg four times daily from 3 to 12 months can decrease the renal phosphate leak and increase serum phosphorus in patients with an idiopathic low renal phosphate threshold.[21] The 24 h calcium excretion decreased under DPM treatment. However, DPM administered 4.4 mg/kg per day in X-linked hypophosphataemia for a 12-week period had no effect on serum phosphorus and urinary excretion.[22] The controversy may depend on the dose and duration of DPM used in each experiment. The effect on renal electrolytes transport is dependent on c-AMP because the potentiation of NAD action by DPM caused decreasing GFR, CPAH and electrolyte excretion in anaethetized rats, and an associated increasing plasma adenosine level was also demonstrated.[23] Extracellular c-AMP (10–1000 μmol/L) inhibited Na-dependent phosphate uptake in a time- and concentration-dependent manner.[24] The effect of cAMP was reduced by DPM, which inhibits adenosine uptake. *In vivo* DPM abolished the phosphaturia induced by exogenous cAMP infusion in acutely parathyroidectomized (APTX) rats, intact rats, and phosphaturia induced by PTH infusion in APTX rat.[24] Thus, PTH may be inhibited by this manner. However, in the present study, because the calcium excretion is also decreased, the mechanism of DPM on calcium and phosphorus transport may not be mediated by the inhibition of PTH action.

Our study demonstrated that FOS can alleviate the glomerular lesions compared with rats subjected to nephrectomay alone. The blood pressure of NPX rats that received FOS reduced to a normal range compared with the control rats. A study in conscious normotensive sodium-repleted dogs showed that fosinopril, at a dose of 1 mg/kg per day, decreased the systolic pressure and mean arterial pressure significantly.[25] The blood pressure lowering effect of FOS was reported previously in conscious dogs, sodium-depleted Cynomolgus monkeys, spontaneously hypertensive rats, two-kidney, one-clip hypertensive rats, deoxycorticosteroid acetate (DOCA)-salt hypertensive rats and bilateral perinephritis monkeys.[26] Thus, it was not surprising to find that fosinopril could normalize blood pressure in NPX rats in this experiment.

While renal functional parameters improved, the plasma creatinine concentration was also reduced in NPX rats that received FOS. A study in post-transplant hypertensives showed that GFR was reduced 4 and 12 months after fosinopril was given along with reduced proteinuria. However, the GFR response to acute protein loading was enhanced during fosinopril treatment.[27] Given fosinopril 10 mg/day for 10 days in nine patients

with chronic renal insufficiency (creatinine clearance <1.8 L/h) showed that renal blood flow, glomerular filtration rate and renal plasma flow were not reduced.[28] Fosinopril reduced proteinuria by 21–23% from baseline in patients with proteinuric renal disease.[29,30] Moreover, FOS administered intravenously to normotensive, concious, sodium-repleted dogs increased PAH clearance and GFR significantly.[26] The oral administration of FOS 1 mg/kg per day for 7 days in conscious normotensive dogs increased both GFR ($P < 0.05$) and effective renal plasma flow.[25]

The role of the renin-angiotensin system on the protection of glomeruloslerosis was studied extensively. The angiotensin converting enzyme inhibitor (ACEI), enalapril, can slow the progression of renal disease. Enalapril (25–100 mg/L in drinking water) resulted in a significant attenuation of the proteinuria, hypertension and glomerular lesions associated with partial renal ablation.[6,8,9,31] It was also seen by using angiotensin II receptor antagonists (AT1RA), losartan alone,[32] and in combination with enalapril.[7] These raise the question of whether renoprotective effects is caused by blood pressure reduction. If this feature occurs because of the normalization of blood pressure, giving antihypertensive therapy should slow down the renal disease progression. However, a study on the effect of a calcium channel blocker, nifedipine, showed that the drug can normalize blood pressure but induce a greater pressure transmission to the glomeruli and cause the development of glomerulosclerosis, compared with enlapril group.[8] Micropuncture studies, using a combination of reserpine, hydralazine and hydrochlorothiazide, which can reverse systemic blood pressure, cannot reduce proteinuria and glomerular capillary pressure with a high prevalence of glomerular sclerotic lesions compared with the group receiving enalapril or losartan.[32,33] The hyperfiltration measured by an exaggerated glomerular filtration rate was found in these three drugs regimen.[34] One study using a micropuncture technique also showed that enalapril prevents systemic hypertension but maintained the mean glomerular transcapillary hydraulic pressure gradient without significantly compromising the single nephron glomerular filtration rate (SNGFR) and the glomerular capillary plasma flow rate, compared with the untreated group.[9] These results indicate that the effect on glomerular function and structure depend on glomerular pressure reduction mediated by the inhibition of AII activity, and are not attributable simply by the normalization of systemic blood pressure. However, our study found increased GFR and RPF in NPX rats with FOS treatment. We proposed that the improved GFR was not caused by the increased glomerular hydraulic pressure, but it may be caused by the reduction in precapillary resistance, which was shown by a reduction in total renal resistance. Angiotensin II is thought to mediate the glomerular hypertension associated with partial nephrectomy, which has been shown both *in vivo*[35–37] and *in vitro*[38] studies to contrict the renal efferent arteriole. As shown in a previous study, the administration of endothelin-1 blockade (bosentan) and angiotensin receptor antagonist (losartan) prevented the increase in systemic blood pressure, glomerular capillary pressure and efferent renal resistance, and the fall in the ultrafiltration coefficient in rats with systemic NO synthase (NOS) inhibition.[39] Therefore, a reduction in efferent arteriolar resistance that enhanced renal blood flow to the kidney and increased the glomerular capillary ultrafiltration coefficient may be the crucial mechanism to enhance GFR without changing the capillary hydraulic pressure, and it may become a key factor in protecting glomerular hypertension and glomerulosclerosis.

The present study is the first one to demonstrate the renoprotective effect of fosinopril on glomerular lesions. The light microscopic study showed glomerular capillary vasodilation, and the swelling of podocytes was minimal. The role of angiotensin II-induced podocyte swelling is still unclear. The mesangial cells did not change remarkably in the present study, although a previous study showed that mesangial cell expansion, accompanied with hyperfiltration, occurred in rats with spontaneous hypertension and underwent a reduction of one- and five-sixths of renal mass that can be inhibited by enalapril.[34]

Although the urinary excretion of Na in rats receiving fosinopril increased slightly compared with rats receiving NPX alone, the fractional Na excretion was not elevated. A single oral dose of fosinopril, 40–640 mg, completely inhibited plasma ACE activity for at least 24 h in normotensive volunteers and hypertensive patients.[40] Fosinopril 5–40 mg/day was administered over 1–12 weeks to normotensives or hypertensives who showed either a decrease or unaltered plasma aldosterone.[41] A similar study was conducted by giving fosinopril 5–10 mg/day to 17 hypertensive patients who showed a significant reduction in ACE activity after 4 weeks. Plasma aldosterone levels were unaffected by fosinopril.[42] In patients with renal impairment, ACE activity remained inhibited 24–48 h postdose, but plasma aldosterone levels were decreased or unchanged with increased plasma renin activity when a single oral dose of fosinopril 10 mg for 4 days was received,[43] or after the last dose of fosinopril 10–20 mg/day was taken in a 12-week trial.[44,45] Thus, although fosinopril can inhibit ACE activity, it may only have a slight effect on aldosterone and renal electrolyte transport. One report of a significant increase in urinary excretion with a slight increase in fractional excretion of sodium was found in normotensive dogs receiving fosinopril at the dose of 1 mg/kg per day for 5 days.[25] Nevertheless, many mechanisms are involved in the regulation of electrolytes transport, which should be further investigated.

In conclusion, the present study indicates that both antiplatelet aggregation using DPM and blockage of renin-angiotensin system using FOS can ameliorate the progression of renal diseases associated with a five-sixths nephrectomy. However, DPM did not normalize blood

pressure or reduce proteinuria. Nephrectomized rats receiving FOS improved in terms of renal function, urinary protein creatinine ratio, plasma creatinine concentration, and renal histopathological lesions. We concluded that FOS is more beneficial than DPM in protecting against the progression of renal deterioration. Whether the mechanism is caused by intrarenal haemodynamic changes involving angiotensin II needs further investigation.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Veterinary Science, Chulalongkorn University. The authors wish to thanks Mrs Chongkol Chiansuwan for her technical assistance. The authors also wish to thank Miss Hathaithip Parkinsee for her help with the preparation of this manuscript.

REFERENCES

1. Purkerson ML, Joist JH, Yates J, Valdes A, Morrison A, Klahr S. Inhibition of thromboxane synthesis ameliorates the progressive kidney disease of rats with subtotal renal ablation. *Proc. Natl Acad. Sci. USA* 1985; **82**: 193–7.
2. Zoja C, Benigni A, Livio M *et al*. Selective inhibition of platelet thromboxane generation with low dose aspirin does not protect rats with reduced renal mass from the development of progressive disease. *Am. J. Pathol.* 1989; **134**: 1027–38.
3. Nakamura K, Kojima K, Arai T *et al*. Dipyridamole and dilazep suppress oxygen radicals in puromycin aminonucleoside nephrosis rats. *Eur. J. Clin. Invest.* 1998; **28**: 877–83.
4. Kimura K, Endou H, Sakai F. Suppressive effect of dipyridamole on the proteinuria of aminonucleoside nephrosis in rat. *J. Toxicol. Sci.* 1979; **4**: 1–10.
5. Purkerson ML, Joist JH, Yates J, Klahr S. Role of hypertension and coagulation in the progressive glomerulopathy of rats with subtotal renal ablation. *Miner. Electrolyte Metab.* 1987; **13**: 370–6.
6. Brooks DP, Contino LC, Trizna W, Edwards RM, Ohlstein EH, Solleveld HA. Effect of enalapril or the thromboxane receptor antagonist, daltroban, in rats with subtotal renal ablation. *J. Pharmacol. Exp. Ther.* 1990; **253**: 119–23.
7. Ots M, Mackenzie HS, Troy JL, Rennke HG, Brenner BM. Effects of combination therapy with enalapril and losartan on the rate of progression of renal injury in rats with 5/6 renal mass ablation. *J. Am. Soc. Nephrol.* 1998; **9**: 224–30.
8. Griffin KA, Picken MM, Bidani AK. Deleterious effects of calcium channel blockade on pressure transmission and glomerular injury in rat remnant kidneys. *J. Clin. Invest.* 1995; **96**: 793–800.
9. Anderson S, Meyer TW, Rennke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J. Clin. Invest.* 1985; **76**: 612–19.
10. Brun C. A rapid method for the determination of para-aminohippuric acid in kidney function test. *J. Lab. Clin. Med.* 1951; **37**: 955–8.
11. Young MK Jr, Raisz LG. An anthrone procedure for determination of inulin in biological fluids. *Proc. Soc. Exp. Biol. Med.* 1952; **80**: 771–4.
12. Moorehead UR, Biggs HG. 2-Amino-2 methyl-1-propanol as the alkalinizing agent in an improve of continuous flow cresolphthaline complex one procedure for calcium in serum. *Clin. Chem.* 1974; **20**: 1458–60.
13. Gomori GA. Modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Lab. Clin. Med.* 1942; **27**: 955–60.
14. Ritcher HJ, Lapointe YS. Urea in blood serum or urine (diacetyl mono-oxime procedure). *Clin. Chem.* 1962; **8**: 335.
15. Hawk PB, Oser BL, Summerson WH. *Practical Physiological Chemistry*, 13th edn. New York: Blakinton, 1954.
16. Orisio S, Benigni A, Bruzzi I *et al*. Renal endothelin gene expression is increased in remnent kidney and correlates with disease progression. *Kidney Int.* 1993; **43**: 354–8.
17. Tsuruda H, Okuda S, Onoyama K, Oh Y, Fujishima M. Effect of blood pressure on the progress of renal deterioration in rats with renal mass reduction. *J. Lab. Clin. Med.* 1986; **107**: 43–50.
18. Cortes P, Zhao X, Riser BL, Narins RG. Regulation of glomerular volume in normal and partially nephrectomized rats. *Am. J. Physiol.* 1996; **270**: F356–70.
19. Floege J, Bruns MW, Alpers CE *et al*. Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnent kidney model. *Kidney Int.* 1992; **41**: 297–309.
20. Zoja C, Perico N, Corna D *et al*. Thromboxane synthesis inhibition increases renal prostacyclin and prevents renal disease progression in rats with remnant kidney. *J. Am. Soc. Nephrol.* 1990; **1**: 799–807.
21. Prie D, Blanchet FB, Essig M, Jourdain JP, Friedlander G. Dipyridamole decreases renal phosphate leak and augments serum phosphorus in patients with low renal phosphate threshold. *J. Am. Soc. Nephrol.* 1998; **9**: 1264–9.
22. Seikaly MG, Quigley R, Baum M. Effect of dipyridamole on serum and urinary phosphate in X-linked hypophosphatemia. *Pediatr. Nephrol.* 2000; **15**: 57–9.
23. Redlak M, Szczepanska-Konkel M, Stepinski J, Angielski S. Effects of dipyridamole and furosemide on renal function during adenine dinucleotide infusion in rats. *Acta Physiol. Pol.* 1986; **37**: 1–7.
24. Friedlander G, Couette S, Coureau C, Amiel C. Mechanisms whereby extracellular adenosine 3′ 5′-monophosphate inhibits phosphate transport in cultured opossum kidney cells and in rat kidney. Physiological implication. *J. Clin. Invest.* 1992; **90**: 848–58.
25. Buranakarl C, Kijtawornrat A, Nampimoon P. Effects of Fosinopril on renal function, baroreflex response and noradrenaline pressor response in conscious normotensive dogs. *Vet. Res. Comm.* 2001; **25**: 355–66.
26. DeForrest JM, Waldron TL, Harvey C *et al*. Blood pressure lowering and renal hemodynamic effects of fosinopril in conscious animal models. *J. Cardiovasc. Pharmacol.* 1990; **16**: 139–46.
27. Bochicchio T, Sandoval G, Ron O, Perez-Grovas H, Bordes J, Herrera-Acosta J. Fosinopril prevents hyperfiltration and decreases proteinuria in post-transplant hypertensives. *Kidney Int.* 1990; **38**: 873–9.
28. Sica DA, Cutler RE, Parmer RJ, Ford NF. Comparison of the steady-state pharmacokinetics of fosinopril, lisinopril and enalapril in patient with chronic renal insufficiency. *Clin. Pharmacokinet.* 1991; **20**: 420–7.
29. Keilani T, Schlueter WA, Levin ML, Batlle DC. Improvement of lipid abnormalities associated with proteinuria using fosinopril, an angiotensin-converting enzyme inhibitor. *Ann. Intern. Med.* 1993; **118**: 246–54.
30. Maschio G, Cagnoli L, Claroni F *et al*. ACE inhibition reduces proteinuria in normotensive patients with IgA nephropathy: a

multicentre, randomized, placebo-controlled study. *Nephrol. Dial. Transplant.* 1994; **9**: 265–9.
31. Brunner FP, Thiel G, Hermle M, Bock HA, Mihatsch MJ. Long-term enalaril and verapamil in rats with reduced renal mass. *Kidney Int.* 1989; **36**: 969–77.
32. Lafayette RA, Mayer G, Park SK, Meyer TW. Angiotensin II receptor blockade limits glomerular injury in rats with reduced renal mass. *J. Clin. Invest.* 1992; **90**: 766–71.
33. Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J. Clin. Invest.* 1986; **77**: 1993–2000.
34. Raij L, Chiou XC, Owens R, Wrigley B. Therapeutic implications of hypertension-induced glomerular injury. Comparison of enalapril and a combination of hydralazine, reserpine, and hydrochlorothiazide in an experimental model. *Am. J. Med.* 1985; **79**: 37–41.
35. Hall JE, Guyton AC, Jackson TE, Coleman TG, Lohmeier TE, Trippodo NC. Control of glomerular filtration rate by renin- angiotensin system. *Am. J. Physiol.* 1977; **233**: F366–72.
36. Hsu CH, Kurtz TW, Slavicek JM. Effect of exogenous angiotensin II on renal hemodynamics in the awake rat. *Circ. Res.* 1980; **46**: 646–50.
37. Myers BO, Deer WM, Brenner BM. Effects of norepinephrine and angiotensin II on the determinants of glomerular ultrafiltration and proximal tubule fluid reabsorption in the rat. *Circ. Res.* 1975; **37**: 101–10.
38. Edwards RM. Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. *Am. J. Physiol.* 1983; **244**: F526–34.
39. Qiu C, Bayliss C. Endothelin and angiotensin mediated most glomerular responses to nitric oxide inhibition. *Kidney Int.* 1999; **55**: 2390–6.
40. Duchin KL, Waclawski AP, Tu JI, Manning J, Frantz M, Willard DA. Pharmacokinetics, safety, and pharmocologic effects of fosinopril sodium, an angiotensin-converting enzyme inhibitor in healthy subjects. *J. Clin. Pharmacol.* 1991; **31**: 58–64.
41. Murdoch D, McTavish D. Fosinopril: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in essential hypertension. *Drugs* 1992; **43**: 123–40.
42. Forslund T, Franzen P, Backman R. Comparison of fosinopril and hydrochlorothiazide in patients with mild to moderate hypertension. *J. Intern. Med.* 1991; **230**: 511–17.
43. Gehr T, Sica D, Grasela D, Duchin K. Pharmacodynamics of fosinopril in chronic ambulatory peritoneal dialysis (CAPD) and hemodialysis (HD) patients. *Clin. Pharmacol. Ther.* 1991; **49**: 194 (Abstract).
44. Keilani T, Schlueter W, Molteni A, Battle DC. Converting enzyme inhibition with fosinopril does not suppress plasma aldosterone and may not cause hyperkalemia despite moderate renal impairment. *J. Hypertens. Suppl.* 1992; **4**: 245 (Abstract).
45. Keilani T, Schlueter W, Batlle D. Selected aspects of ACE inhibitor therapy for patients with renal disease: impact on proteinuria, lipids and potassium. *J. Clin. Pharmacol.* 1995; **35**: 87–97.