

Brief communication (Original)

Improved vascular repair is relevant to enhanced renal function with vasodilators in early stage of chronic kidney disease

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Background: Treatment with vasodilators can improve renal function in early stage of chronic kidney disease (CKD) patients.

Objective: Study the mechanism of vascular repair in 20 CKD patients associated with actual creatinine clearance greater than 60 mL/min/1.73m² (mean 84±24 mL/min/1.73m²) who had been under treatment with vasodilators.

Results: Initial study on angiogenic factors revealed a low value of VEGF, no significant change in VEGF-R1, whereas antiangiogenic factors showed elevated angiopoietin-2 and no significant change in VEGF-R2. Initial actual creatinine clearance was significantly depleted and fractional excretion of magnesium (FE Mg) was elevated significantly. Follow-up study showed improved VEGF and a significant decline in angiopoietin-2. Such improved vascular repair coincided with enhanced creatinine clearance.

Conclusion: Improved renal function can be achieved by vasodilators under environment favourable for adequate vascular repair.

Keywords: Angiopoietin, CKD, vascular repair, VEGF, VEGF-R1

There is consensus that treatment under present common practice initiated at the late stage of chronic kidney disease (CKD) due to the insensitiveness of the available diagnostic marker such as serum creatinine determination fails to improve renal function [1]. Recent study of the mechanism of vascular repair in late stage of CKD patients revealed multiple defects that would not be capable of inducing neoangiogenesis, and thus fail to restore renal perfusion and function in response to vasodilators [2-4]. In contrast, the authors observed that treatment with vasodilators at the early stage of CKD patients such as type-2 diabetic nephropathy associated with normoalbuminuria, or early stage of nephrotic patients associated with focal segmental glomerulosclerosis, would increase renal perfusion and function [5-7].

In this paper, we studied the mechanism of vascular repair in the patients who improved renal

function with vasodilators, and attempted to explain the relevance of improved vascular repair in enhanced renal function with vasodilators in the early stage of CKD.

Material and methods

The Research and Ethics Committee of the King Chulalongkorn Memorial Hospital approved this study.

The present diagnostic marker such as serum creatinine is quite insensitive, thus only detects late stage (CKD stages 3-5). In those cases, and the therapeutic result usually fails to restore renal function. Therefore, we selected a group of CKD patients at the early stage (CKD stages 1, 2) with the assistance of fractional excretion of magnesium (FE Mg). As demonstrated previously, it directly correlates with the magnitude of tubulointerstitial fibrosis [1, 8]. Thus, this can screen the early stage of CKD.

We performed a perspective cross-sectional study in 20 early stage CKD patients with mean actual creatinine clearance of 84±24 mL/min/1.73m² and

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with the mean value of FE Mg of $3.4 \pm 0.9\%$. The abnormally elevated FE Mg and decreased creatinine clearance observed in these patients reflect the hemodynamic abnormality and reduction in renal perfusion. This is a crucial determinant inducing renal disease progression [9]. The therapeutic strategy aimed to improve renal perfusion and to restore renal function at the early stage of CKD with vasodilators, such as Enaril 10-40 mg/day, Telmisartan 40-80 mg/day, or Losartan 50-100 mg/day, calcium channel blocker 10-20 mg/day, and antioxidants such as vitamin C 1000-2000 mg/day. All patients were advised to drink water ad lib (3000 mL/day). All patients complied well with the study. The study on vascular homeostasis included the initial and follow-up studies. The renal function checks were repeated at regular intervals thereafter.

Enzyme-linked immunosorbent assay (ELISA) for vascular endothelial growth factor (VEGF)

This assay employs the quantitative sandwich enzyme immunoassay technique. Standards and samples were pipetted into the wells. Any VEGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF was added to the wells. Following a wash to remove any unbound antibody enzyme reagent, a substance solution was added to the wells and color develops in proportion to the amount of VEGF bound in the initial step. The color development was stopped, and the intensity of the color was measured.

Immunoassay of human angiopoietin-2, VEGF-R1, and VEGF-R2

These assays employ the quantitative sandwich enzyme immunoassay technique in a similar manner described in the above.

Renal function study

Renal function study was performed under 10-hour urinary collection. No diuretic was administered during or within 24 hours before the test. Briefly, after a regular supper, no additional food except drinking water ad lib was allowed. The patients were instructed to void at 7 pm, and the urine was collected from 7 pm to 5 am. Clotted blood from venipuncture was drawn at the end of the test for analysis of creatinine and magnesium levels. The Renal Metabolic Laboratory unit, King Chulalongkorn Memorial Hospital, analyzed urine samples and blood sample.

For analysis of creatinine and magnesium, we used the methods described by Faulkner & King, and Atomic Absorption Spectrophotometer (model 1100G; Perkin Elmer, Norwalk, USA), respectively. A reflection of tubular cell function or tubulointerstitial structure was derived from the determination of FE Mg, which was calculated using the formula:

$$\text{FE Mg} = (\text{urine magnesium}) / (\text{plasma magnesium}) \times (\text{plasma creatinine}) / (\text{urine creatinine}) \times 100 (\%).$$

Statistical analysis

Comparison of the sample mean of two quantitative variables was determined by the non-parametric method using the Mann-Whitney test. P-values below 0.05 were considered significantly different.

Table 1. Pre-treatment and post-treatment studies on vascular repair and renal function.

	Pre-treatment	P-value	Post-treatment	P-value	Normal
Angiogenic factors					
VEGF (pg/mL)	193 \pm 230	<0.05	450 \pm 234	NS	429 \pm 220
VEGF-R1 (ng/mL)	62 \pm 19	NS	76 \pm 13	NS	54 \pm 10
Antiangiogenic factors					
Angiopoietin-2 (pg/mL)	4506 \pm 1114	<0.01	2372 \pm 501	<0.01	1850 \pm 1320
VEGF-R2 (ng/mL)	7841 \pm 1273	<0.001	1171 \pm 243	<0.001	6747 \pm 3554
Creatinine clearance (mL/min/1.73m ²)	84 \pm 24	<0.05	103 \pm 32	NS	118 \pm 32
FE Mg (%)	3.4 \pm 0.9	NS	2.8 \pm 0.8	<0.05	1.6 \pm 0.6

NS=not significantly different.

Results

The initial study on angiogenic factors revealed a low value of VEGF, whereas no significant change in VEGF-R1 compared to the control (Table 1, Fig. 1 and 2). With respect to anti-angiogenic factors, a significant elevation was observed in angiopoietin-2, whereas no significant change was observed in VEGF-R2. With respect to the renal function

study, the initial creatinine clearance was significantly depleted, whereas the value of FE Mg was significantly elevated. During our follow-up study, a significant improvement in VEGF was observed. The antiangiogenic factors such as angiopoietin-2 declined significantly toward the normal level. There was an improvement in vascular repair, and a documented significant improvement in creatinine clearance.

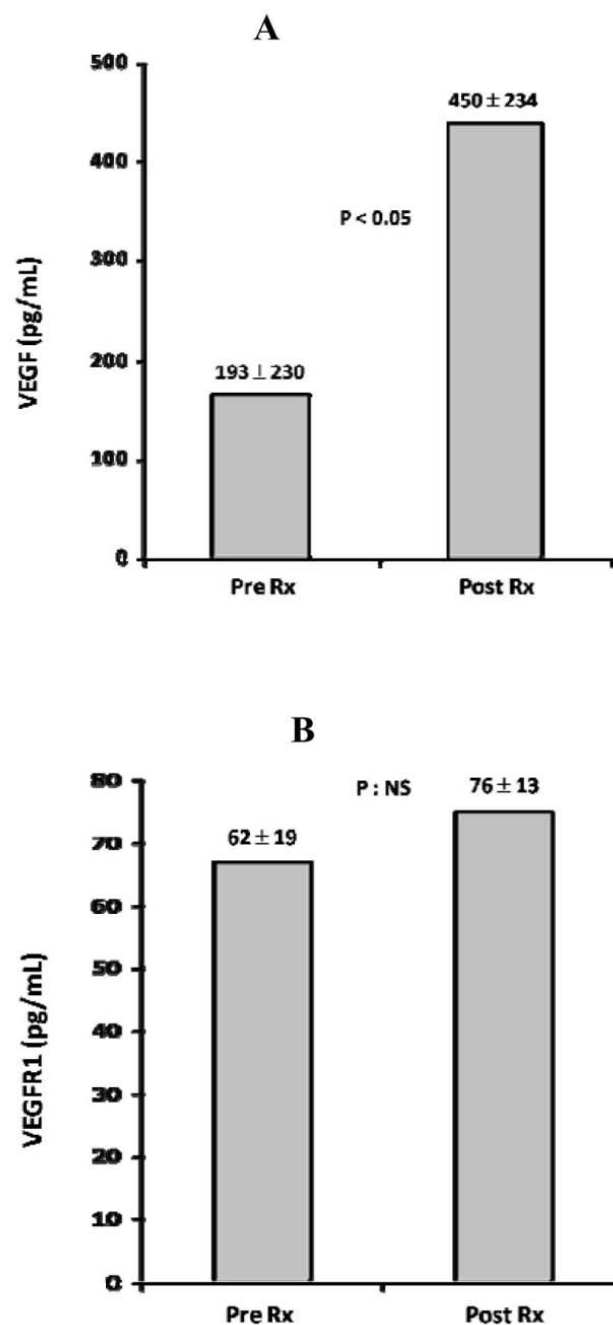


Fig. 1 Pre- and post-values of angiogenic factors, VEGF (A) and VEGF-R1 (B), before and after treatment (Rx). Increase in VEGF level indicates a significant improvement after treatment. NS=not significantly different.

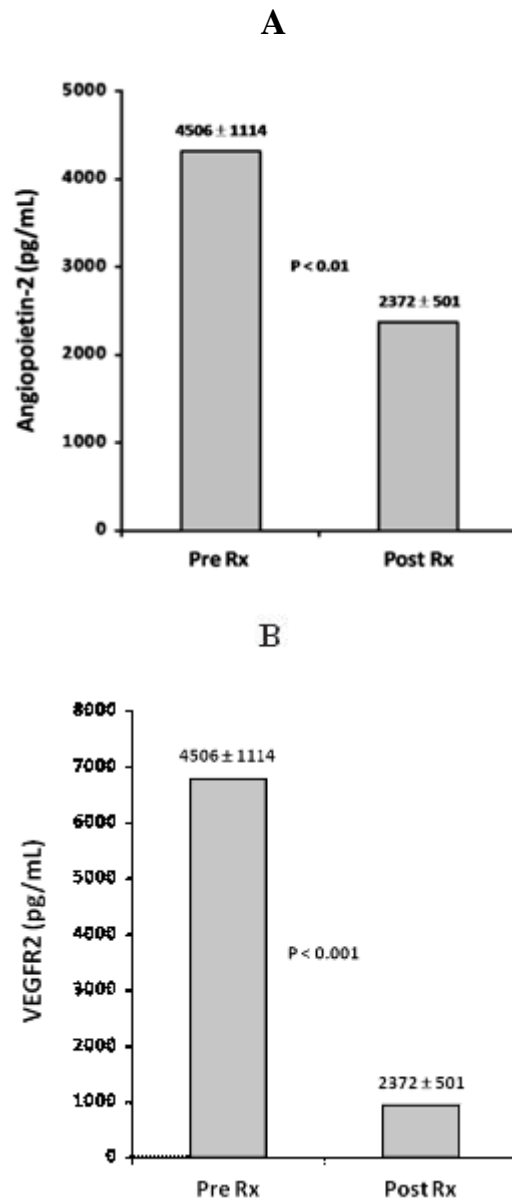


Fig. 2 Pre- and post-values of anti-angiogenic factors, angiotensin 2. (**A**) and VEGF-R2 (**B**), before and after treatment (Rx), showing a significant suppression in angiotensin-2 and VEGF-R2 after treatment.

Discussion

Impaired creatinine clearance observed in CKD patients is due to renal microvascular disease and to reduction in renal perfusion. This is secondary to hemodynamic maladjustment with preferential constriction at the efferent arteriole, and a reduction in peritubular capillary flow inducing chronic ischemic injury to the tubulointerstitial structure and eventually tubulointerstitial fibrosis [10]. Correction of the hemodynamic maladjustment with appropriate vasodilators at the early stage, under an environment

favorable for renal angiogenesis would improve renal perfusion as well as function. This view is supported by recent study in normoalbuminuric (early stage) type 2 diabetic nephropathy [5, 6]. The results of the mechanism of vascular repair in these CKD patients associated with mild impairment in renal function have also confirmed the mild impairment in vascular repair. In this study, following the treatment with vasodilators, the suppression of antiangiogenic factors, namely angiotensin-2, implies that the mechanism of vascular smooth muscle cell proliferation is likely to be

prohibited. The suppression of VEGF-R2 also prohibited the activation through the pathological VEGFR2 pathway of VEGF. In the presence of improved VEGF and VEGF-R1, the angiogenic factors would increase activation through the classical pathway (VEGF → VEGFR1), and would induce coupling of endothelial nitric oxide synthase (eNOS) and enhance further NO production. This activation would induce renal angiogenesis and adequate vascular repair. Improved vascular repair would respond adequately to vasodilators, and hence enhance renal microvascular perfusion and function. This study supports the therapeutic strategy towards restoration of renal function with appropriate vasodilators in early stage of CKD under environment favorable for renal angiogenesis and regeneration. This new therapeutic strategy would be able to effectively prevent the end-stage renal disease, and would substitute the present common therapeutic practice that fails to restore renal perfusion or function in late stage CKD.

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The authors have no conflict of interest to declare.

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