Endothelin-1, Big Endothelin-1, and Nitric Oxide in Patients With Chronic Renal Disease and Hypertension

Ivanka Mikulić,^{1*} József Petrik,² Krešimir Galešić,³ Željko Romić,³ Ivana Čepelak,² and Monika Zeljko-Tomić¹

¹Mostar University Hospital, Mostar, Bosnia and Herzegovina

²Department of Medical Biochemistry and Hematology, School of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

³Dubrava University Hospital, Zagreb, Croatia

The complex pathogenesis of chronic renal disease (CRD) depends on endothelin (ET) axis (ETs and ET receptors) and nitric oxide (NO) because of their vasoactive effects and their role in general modulation of vascular homeostasis. Various renal cells synthesize ETs and NO that play a significant role in renal hemodynamics as well as in water and salt excretion via urine. ET-1 is a strong vasoconstrictor. Besides its vasoactive effects, ET-1 modulates mitosis and apoptosis in a cell type-dependent manner, and may play an important role in CRD pathogenesis. The aims of this study were to emphasize the role and interactions of ET-1, Big ET-1, and NO in CRD. Concentrations of these vasoactive molecules were measured in plasma/serum and/or urine of 57 patients with diabetic nephropathy (subgroup 1), arterial hypertension (subgroup 2) or CRD with chronic renal insufficiency (subgroup 3), and in

healthy control subjects ($n = 18$). In comparison with control group, urine concentration of Big ET-1 was significantly increased (13.13 pmol/L vs. 11.34 pmol/L; $P < 0.001$) in CRD patients, whereas plasma and urine concentrations of ET-1 did not differ significantly. NO concentrations were also significantly increased in CRD patients (serum, 72.55 $\mu\text{mol/L}$; $P < 0.001$, and urine 141.74 $\mu\text{mol/L}$; $P < 0.05$) as compared to control group. Study results indicated that Big ET-1 and NO could be useful diagnostic parameters in CRD for their diagnostic sensitivity and diagnostic specificity (Big ET-1 in urine: 56.1 and 88.9%, and NO in serum: 66.7 and 83.3%, respectively). In addition, Big ET-1 may prove useful in the differential diagnosis of diabetic nephropathy (78.6% diagnostic sensitivity and 88.9% diagnostic specificity). *J. Clin. Lab. Anal.* 23:347–356, 2009. © 2009 Wiley-Liss, Inc.

Key words: endothelin-1; big endothelin-1; nitric oxide; chronic renal disease; hypertension

INTRODUCTION

Chronic renal diseases (CRD) pose a public health problem with a rising prevalence worldwide, while their association with cardiovascular disorders results in a steadily increasing morbidity and mortality (1). An important factor in CRD pathogenesis and progression is the development of hypertension, which is responsible for subsequent precipitated decline of renal function (2). The causes of the high prevalence of hypertension in both experimental CRD and patients with CRD include changes in the synthesis and release of various vasoactive mediators responsible for vascular tone regulation (3). The high prevalence of hypertension in

both experimental and clinical CRD can also partly be explained by changes in the synthesis and release of various vasoactive mediators responsible for vascular tone regulation. Among them, researchers' attention has

Grant sponsor: Ministry of Science, Education and Sports of the Republic of Croatia; Grant number: 006-1340227-1248.

*Correspondence to: Ivanka Mikulić, Master of Medical Biochemistry, Mostar University Hospital, Department of Laboratory Diagnostics, Bijeli Brijeg, 88000 Mostar, Bosnia and Herzegovina. E-mail: ivankacolak@yahoo.com

Received 28 November 2008; Accepted 15 April 2009

DOI 10.1002/jcla.20324

Published online in Wiley InterScience (www.interscience.wiley.com).

been focused on endothelin (ET) axis including ET-1 and ET receptors, and nitric oxide (NO), not only for their vasoactive properties but also for their role in the general modulation of vascular homeostasis (4).

ETs are a group of structurally similar 21-amino acid peptides (ET-1, ET-2, and ET-3) with a predominantly vasoconstrictive action, of which ET-1 has been most thoroughly investigated (5). ET-1 is synthesized from the inactive 38-amino acid precursor, Big ET-1, by the action of ET-converting enzyme, a membrane-bound neutral metalloproteinase (6). ET-1 exerts its action via ET_A and ET_B receptors, both localized on vascular smooth muscle cells and mediating their vasoconstrictive, proliferative, and hypertrophic action. ET_A are dominant vasoconstrictive receptors, whereas ET-1 can also influence the formation of NO via ET_B receptors, thus exerting its vasodilatory action (7). The role of ET-1 in the genesis and progression of cardiovascular (8,9) and pulmonary (10) diseases, cerebral ischemia, diabetes mellitus (11), arterial hypertension (12), and renal diseases (13,14) has been investigated in numerous studies. Thus, for example, the role of ET-1 and ET_B receptors in the pathogenesis of CRD was demonstrated by their increased *in situ* concentrations recorded by use of hybridization techniques in the kidneys of patients with glomerulonephritis (15). Considering arterial hypertension, the interest of researchers in the vasoactive properties of ET-1 has mostly focused on ET-1 inhibition with the ATII receptor blocking agents (16,17).

Generally, NO has a major role in vascular biology. In addition to its marked vasodilatory action, NO inhibits platelet aggregation, monocyte adhesion, and smooth muscle cell proliferation, thus exerting anti-inflammatory, anti-proliferative, and anti-oxidative action (18). NO is synthesized from L-arginine by the catalytic action of nitric oxide synthases (NOS), a group of three isoenzymes: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Immunohistochemistry studies have shown all the three isoenzymes to be present in the kidney (19). Although NO is generally considered as a favorable factor in the regulation of vasomotor tone, immune response modulation, and neurotransmission, elevated concentrations of NO have a cytotoxic effect due to its reaction with reactive oxygen compounds, metal ions or thiol compounds (15,20). In renal tissue, NO has a major role in the regulation of intrarenal blood flow, glomerular hemodynamics, and sodium excretion (21).

ET-1 and NO are in part excreted by the kidneys and definitely play an important role in renal physiology and pathophysiology (22). Most studies performed *in vitro* and in different experimental models report on complex interaction between NO and ET-1 in various

cardiovascular disorders and functional kidney disorders (23–26). There are a number of studies investigating this interaction in samples from renal disease patients (13,15); however, quite frequently reporting contradictory results, depending on patient selection and characteristics of the analytical procedures employed (27,28).

In this study, serum and urine concentrations of ET-1 and NO were determined in a group of healthy control subjects and in patients with diabetic nephropathy, arterial hypertension, and CRD with renal insufficiency, in order to assess the role and interaction of the mentioned vasoactive molecules in patients with CRD and hypertension. The study also included determination of Big ET-1 in urine because of the short circulating ET-1 half-life and destruction of this peptide by neutral endopeptidases in proximal tubule and higher stability of the Big ET-1 peptide (5).

MATERIAL AND METHODS

Patient Population

Studies were performed in samples obtained from a group of patients ($N = 57$; 31 male and 26 female) with renal disease and arterial hypertension, treated at the Department of Nephrology, Dubrava University Hospital, Zagreb. All patients had pathologic urea and creatinine concentrations in serum (except for 11 patients with arterial hypertension, who showed normal findings of these analytes), and pathologic urea, creatinine, and protein concentrations in urine, which was, along with other clinical parameters, consistent with the diagnosis of CRD. Patients were showed different degrees of CKD (stages 2–4). CKD was defined according to the National Kidney Foundation guidelines.

The patient group was subsequently divided into three subgroups according to clinical picture, systolic/diastolic pressure, and other diagnostic work-up data: subgroup 1 ($n = 14$) diagnosed with diabetic nephropathy; subgroup 2 ($n = 14$) diagnosed with arterial hypertension (essential hypertension, renovascular, and renoparenchymal hypertension); and subgroup 3 ($n = 29$) with the diagnosis of CRD and associated chronic renal insufficiency (chronic glomerulonephritis, chronic interstitial nephritis, polycystic kidney disease, endemic nephropathy, obstructive uropathy, vasculitis). The control group consisted of 18 healthy volunteers (eight male and ten female).

Upon approval issued by the local Ethics Committee, all study subjects signed an informed consent for participation in the study, in line with the Helsinki Declaration.

Laboratory Methods

Serum and plasma samples (obtained with Na-citrate as anticoagulant) and morning void urine samples were stored at -20°C until analysis. Upon single thawing procedure, appropriate samples were used to determine the concentrations of ET-1 (plasma and urine), Big ET-1 (urine), and NO_2/NO_3 (serum and urine). Prior to nitrate and nitrite concentration measurement, serum samples were filtered through Millipore filters at $+4^{\circ}\text{C}$ to remove possible interferences and reduce possible false elevated concentrations of NO_2/NO_3 metabolites.

The concentrations of ET-1 in plasma and urine, and of Big ET-1 in urine were determined by ELISA method (27) using commercial test packages from Biomedica (Vienna). The spectrophotometric method used to determine NO_2/NO_3 concentration is based on enzymatic nitrate reduction to nitrites by nitrate reductase and determination of nitrites following Griess reaction with sulfanilamide and naphthylethylenediamine, with the absorption measured at a particular wavelength (28). The creatinine and protein concentrations were determined by routine methods on an Olympus AU 2700 analyzer using commercial Olympus (Ireland) reagents.

Statistical Analysis

Statistical analysis of the results was performed by the Statistica Version 5.5 software with the use of nonparametric and descriptive statistics. Data distribution was not normal; therefore, results were expressed as median with 25th and 75th percentile. The significance of between group differences was assessed by Mann–Whitney nonparametric test, and of between subgroup differences by Kruskal–Wallis test. Spearman coefficient

of correlation was used to analyze correlation between the study parameters, and diagnostic accuracy of particular parameters was estimated by use of Receiver operating characteristic (ROC) curves. Statistical significance was set at $P < 0.05$.

RESULTS

In this study we evaluated 57 patients with CRD and 18 healthy volunteers. Creatinine levels in serum and urinary protein excretion were significantly higher in CRD group than control group, as shown in Table 1. The concentrations of ET-1, Big ET-1, and NO_2/NO_3 were recorded in the group of 57 CRD patients and control group, and were also presented in Table 1.

A significant increase in comparison with the control group was found in patient serum and urine concentration of NO_2/NO_3 (by 99.15 and 115.85%, respectively; $P < 0.05$ for both), and a borderline significant increase in urine concentration of ET-1 and Big ET-1 (by 46.85%, $P < 0.07$ and 15.78%, $P < 0.0009$, respectively). Also, the urinary level of big ET-1 was found to be higher in the patient group (16%; $P < 0.0009$). Plasma concentration of ET-1 showed no significant difference between the patient and control groups. In the group of CRD patients as a whole, Spearman coefficient of correlation (R) between the measured biochemical parameters, and the same parameters in various samples showed some correlation only for the ET-1p/ NO_2/NO_3 s ($r = 0.31$; $P = 0.02$); ET-1p/Big ET-1u ($r = 0.37$; $P = 0.005$); and NO_2/NO_3 s/ NO_2/NO_3 u ($r = 0.38$; $P = 0.003$) ratios.

ROC curve showed determination of Big ET-1 concentration in urine and NO_2/NO_3 in serum to be of comparable diagnostic value in CRD patients, with diagnostic sensitivity of 56.1 and 66.7%, and diagnostic specificity of 88.9 and 83.3%, respectively (Fig. 1a,b).

TABLE 1. Clinical Characteristics of the Study Group

	Control ($n = 18$)	Patients group ($n = 57$)	Subgroup 1 ($n = 14$)	Subgroup 2 ($n = 14$)	Subgroup 3 ($n = 29$)
Age ^a (years)	40 (21–56)	51 (35–75)	59 (42–73)	49 (38–75)	51 (35–74)
Male (%)	44	54	57	57	52
Female (%)	56	46	43	43	48
Creatinine s ^a ($\mu\text{mol/L}$)	76 (59–94)	319* (56–1,450)	501* (57–771)	116* (56–872)	319* (68–1,450)
Proteins u ^a (g/L)	0.06 (0–1.06)	0.78* (0–5.70)	1.10* (0–5.70)	0.27 (0–1.84)	0.88* (0–3.83)
ET-1 p ^b (pmol/L)	0.73 (0.59–2.22)	0.87 (0.70–1.19)	1.02 (0.72–1.19)	0.72 (0.61–1.10)	0.92 (0.70–1.30)
ET-1 u ^b (pmol/L)	1.11 (0.83–1.41)	1.63 (0.89–2.32)	1.67 (1.11–2.19)	1.43 (0.38–3.51)	1.65 (0.90–2.53)
Big ET-1 u ^b (pmol/L)	11.34 (10.50–12.41)	13.13** (11.69–13.96)	13.42** (13.25–14.56)	12.53* (11.34–13.84)	12.77**** (11.60–13.87)
NO_2/NO_3 s ^b ($\mu\text{mol/L}$)	36.43 (29.08–47.75)	72.55* (35.43–190.48)	70.72* (33.06–206.33)	79.44 (32.45–227.45)	72.55** (44.54–162.93)
NO_2/NO_3 u ^b ($\mu\text{mol/L}$)	65.66 (44.39–114.80)	141.73* (73.47–221.71)	151.99 (50.20–231.73)	140.20 (69.80–195.92)	149.69* (80.66–221.71)

Subgroup 1, diabetic nephropathy; subgroup 2, arterial hypertension; subgroup 3, chronic renal insufficiency; s, serum; p, plasma; u, urine.

* $P < 0.05$ compared to Control; ** $P < 0.001$ compared to Control; *** $P < 0.05$ subgroup 1 compared to subgroup 3 (Mann–Whitney U Test);

**** $P < 0.01$ compared to Control.

^aData are expressed as median (min.–max.).

^bData are expressed as median (interquartile range).

Fig. 1. ROC curves for urine Big ET-1 (a) and serum NO₂/NO₃ (b) of CRD patients. a: Urinary Big ET-1 in patients with CRD vs. control group: AUC = 0.761; SE = 0.058; 95% CI = 0.649–0.852; cut off = 12.768 pmol/L for 56.1% sensitivity and 88.9% specificity. b: Serum NO₂/NO₃ in patients with CRD vs. control group: AUC = 0.753; SE = 0.059; 95% CI = 0.640–0.845; cut off = 50.204 pmol/L for 66.7% sensitivity and 83.3% specificity.

Fig. 2. Urinary Big ET-1 (a), serum NO₂/NO₃ (b), and urinary NO₂/NO₃ (c) in patient subgroups 1, 2, and 3, and control group. Subgroup 1: diabetic nephropathy; subgroup 2: arterial hypertension; subgroup 3: chronic renal insufficiency. Midline = median; square = 25th and 75th percentile; marginal lines = minimum and maximum; significance vs. control group ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$; ^dsubgroup 1 vs. subgroup 3 significance $P < 0.05$ (Mann–Whitney U test).

Figure 2a–c presents differences in absolute concentrations of the biochemical parameters that were statistically significant in a particular patient subgroup.

In comparison with the control group levels, urine concentration of Big ET-1 was significantly increased in subgroups 1, 2, and 3 ($P < 0.001$, $P < 0.05$, and $P < 0.01$,

respectively). Serum concentrations of NO_2/NO_3 were significantly elevated in subgroups 1 ($P < 0.05$) and 3 ($P < 0.001$), and borderline elevated in subgroup 2 ($P = 0.08$). The increase in urine concentration of NO_2/NO_3 reached borderline significance in subgroup 3 ($P < 0.05$).

There were no significant differences between plasma and urine concentrations of ET-1 measured in subgroups 1, 2, and 3, and those measured in control group (subgroup 1: ET-1p 1.02 (0.72–1.19) pmol/L vs. 0.73 (0.59–2.22) pmol/L; and ET-1u 1.67 (1.11–2.19) pmol/L vs. 1.11 (0.83–1.41) pmol/L; subgroup 2: ET-1p 0.72 (0.61–1.10) pmol/L; and ET-1u 1.43 (0.38–3.51) pmol/L; and subgroup 3: ET-1p 0.92 (0.70–1.30) pmol/L; and ET-1u 1.65 (0.90–2.53) pmol/L).

Calculation of coefficients of correlation for biochemical parameters within particular patient subgroups yielded a significant positive correlation coefficient between ET-1p and Big ET-1u in subgroup 1 ($r = 0.83$; $P = 0.0002$) (Fig. 3a) and a negative correlation coefficient

between NO_2/NO_3 s and ET-1u in subgroup 3 ($r = -0.42$; $P = 0.02$) (Fig. 3b).

Figure 4a,b shows ROC curves and respective values of urine Big ET-1 and urine NO_2/NO_3 in patient subgroup 1 (diabetic nephropathy), indicating a higher diagnostic value of urine Big ET-1 determination (diagnostic sensitivity 78.6% and diagnostic specificity 88.9%).

DISCUSSION

Study results indicated significant differences in urine concentration of Big ET-1 in all three patient subgroups (diabetic nephropathy, hypertension, and CRD with advanced renal insufficiency), serum concentration of NO in patients with diabetic nephropathy and CRD and urine concentration of NO in CRD patients, whereas differences in plasma and urine concentrations of ET-1 were less pronounced. A negative coefficient of correlation ($r = -0.42$) between the selected representatives of

Fig. 3. Correlation coefficients and regression curves for: (a) urinary Big ET-1 and plasma ET-1 in subgroup 1, diabetic nephropathy and (b) serum NO_2/NO_3 and urinary ET-1 in subgroup 3, chronic renal insufficiency.

Fig. 4. ROC curves for urinary Big ET-1 (a) and serum NO_2/NO_3 (b) in patient subgroup 1 (diabetic nephropathy). a: Urinary Big ET-1 (subgroup 1 vs. control group): AUC = 0.881; SE = 0.066; 95% CI = 0.717–0.967; cut off = 12.768 pmol/L for 78.6% sensitivity and 88.9% specificity. b: Serum NO_2/NO_3 (subgroup 1 vs. control group): AUC = 0.710; SE = 0.095; 95% CI = 0.524–0.856; cut off = 50.204 $\mu\text{mol/L}$ for 64.3% sensitivity and 83.3% specificity.

the vasodilatory (NO) and vasoconstrictive (ET-1) system was only recorded in the group of CRD patients.

The ET axis factors (ETs and ET receptors) and NO play a major role in the complex pathogenesis of CRDs, not only for their vasoactive properties but also for their role in the overall modulation of vascular homeostasis. Various renal cells synthesize NO and ETs, which influence renal hemodynamics as well as urinary salt and water excretion. Molecular interactions between the NO and ET systems have been described at different levels in *in vitro* and *in vivo* conditions, including acute regulation of particular protein function and signal conduction as well as receptor and converting enzyme modulation (11,29). NO and ET-1 act as mutual antagonists, functioning harmoniously and leading to a tightly regulated balance in healthy people (23,30,31) but not in a number of pathologic states. Results of previous studies investigating molecular interactions of these antagonist systems are variable and frequently contradictory, depending on the particular system representative under study, sample and method used on its determination, and type of renal dysfunction.

In this study, the results recorded in the overall, inhomogeneous group of patients with various renal function disorders ($n=57$) showed a significantly increased concentration of Big ET-1 ($P=0.0009$). ROC analysis indicated diagnostic sensitivity of 56.1% and diagnostic specificity of 88.9% for this analyte. However, plasma and urine concentration of ET-1 did not differ between the patient and control group, whereas difference in the urine concentration of ET-1 reached borderline significance ($P=0.076$). Circulating ET-1 is filtered through glomeruli and degraded by the action of neutral endopeptidases in the proximal tubule (32). However, the locally synthesized ET-1 mostly derives from distal tubular cells, where it is being secreted into the preurine fluid. So, normal plasma ET-1 concentration and increased urinary ET-1 excretion were observed in the study performed in a rat model with renal impairment (33). Results of our study showed the concentrations of Big ET-1 and ET-1 excreted in urine to be better indicators of renal impairment than plasma ET-1, which is consistent with other reports on ET-1 (34).

Our study demonstrated the activity of ET axis in functional kidney disorders and showed urinary Big ET-1 to be a more stable peptide than ET-1, and thus a better indicator of renal dysfunction. The increased renovascular synthesis of ET-1 is known to lead to glomerular lesion by increasing vasoconstriction (35), mesangial cell proliferation (36,) and glomerular permeability (37,38). Our inhomogeneous group of patients with various functional kidney disorders also

showed significantly increased serum and urine concentrations of NO metabolites (NO_2/NO_3) as compared with the control group, with a higher level of difference significance in serum than in urine ($P=0.001$ vs. $P=0.037$). ROC analysis of serum NO_2/NO_3 yielded a diagnostic sensitivity of 66.7% and diagnostic specificity of 83%. These changes were quite expected considering the known renal activity of NO as the main regulator of glomerular hemodynamics, tubular transport, tubuloglomerular feedback, and medullary blood flow (19,39), and its role in blood pressure, renal disease progression, inflammation, and atherosclerosis (20).

Upon dividing the overall group of patients into more homogeneous subgroups according to clinical and other diagnostic parameters, the diagnostic role of these analytes turned more distinctive in terms of differential diagnosis of diabetic nephropathy, arterial hypertension, and CRD with advanced renal insufficiency. Urine concentration of Big ET-1 was significantly elevated in all three subgroups, while ROC analysis showed urine concentration of Big ET-1 to be of highest, i.e., very good diagnostic value in patients with diabetic nephropathy (78.6% diagnostic sensitivity and 88.9% diagnostic specificity). Urine and serum concentrations of ET-1 showed a rather low diagnostic value in all three patient subgroups. Serum concentration of NO_2/NO_3 was also significantly elevated in the subgroup of patients with diabetic nephropathy (64.3% diagnostic sensitivity and 83.3% diagnostic specificity), whereas increased urinary concentration of these metabolites was only recorded in the subgroup of CRD patients with advanced renal insufficiency. In the subgroup of patients with arterial hypertension, only urinary concentration of Big ET-1 showed a significant increase ($P<0.05$) in comparison with the control group.

There are reports on ET axis activation in diabetic patients (40). Elevated plasma and urine concentrations of ET-1 were found in diabetic patients with microalbuminuria (41–44), while Zojka et al. believe that tubular cell exposure to high-molecular mass protein such as albumins could be one of the potential mechanisms involved in ET-1 activation (45). In our study, serum and urine ET-1 concentrations did not differ significantly between patients with diabetic nephropathy and healthy controls, whereas urine concentration of Big ET-1 was significantly increased in the former ($P<0.001$). Thus, our results appear to confirm the activation of ET axis in patients with diabetic nephropathy, with Big ET-1 being superior to ET-1 concentration as a more sensitive indicator of pathobiochemical alterations. The rather poorly defined differences in ET-1 concentration may have been due to the inclusion of both type 1 and type 2 diabetes patients in this study group.

Endothelial dysfunction is known to be an important pathophysiologic marker of micro- and macrovascular complications of diabetes mellitus, including diabetic nephropathy (19). Hyperglycemia, hypertension, and activation of the intrarenal renin-angiotensin system contribute to the development and progression of diabetic renal disease, and all these three factors influence intrarenal synthesis of NO (19). A number of studies investigated impaired intrarenal synthesis of NO, however, frequently with relatively contradictory and inconsistent results (11,19).

These differences could in part be explained by different study design, e.g., in vivo and in vitro studies, use of different analytical procedures of NO measurement, assessment of NO system activity by metabolite determination, NOS activity or intrarenal presence of all three NOS isoenzymes (46) or determination of different NOS isoenzymes (19,47).

Our finding of the significantly elevated serum concentration of NO_2/NO_3 in diabetic nephropathy patients is consistent with the studies demonstrating that early diabetic nephropathy is associated with an increased NO synthesis, which primarily results from eNOS (48) and maybe nNOS activity. Such an increased NO synthesis is the likely cause of intraglomerular hemodynamic alterations in early diabetic renal disease, which lead to microalbuminuria and hyperfiltration (49). Studies in streptozotocin-induced diabetic rats demonstrated an increased urinary excretion of NO_2/NO_3 metabolites as a measure of NO synthesis, which is also associated with hyperfiltration in diabetes (50,51). We can only presume that changes in serum and urinary concentration of NO_2/NO_3 , reported by various authors, reflect the duration and grade of pathologic kidney lesions in diabetic patients.

Hypertension is both the cause and complication of CRD (3). Because of their vasoconstrictive action, ETs are also involved in the pathogenesis of hypertension (14). The increased concentration of ET-1 in patients with hypertension, reported from a number of studies (12,52), may reflect either its increased synthesis or reduced clearance (53). Thus, circulating ET-1 concentration does not appear to be a reliable indicator of the ET-system involvement in the pathology of hypertension in humans. In 1999, Schiffrin demonstrated that the concentration of immunoreactive ET-1 in plasma, which quite poorly reflects tissue synthesis of ET-1, was not elevated in essential hypertension, with the exception of Afro-Americans (54). In 2004, Hirai showed that there was no association between plasma ET-1 and blood pressure, thus confirming that the increased concentration of ET-1 was not associated with hypertension but correlated with creatinine concentration, i.e., with renal function (55). In this study, only a

significant increase in urine concentration of Big ET-1 and no changes in serum and urine ET-1 concentrations were recorded in the group of patients with arterial hypertension (subgroup 2), which is consistent with the report of Hirai et al. (55). We presume that the increase in Big ET-1 concentration is an indicator of initial nephropathy consequential to hypertension. Although many authors describe decreased NO_2/NO_3 concentrations in patients with hypertension (56), we observed no significant changes in the concentrations of these metabolites. This discrepancy may be due to the patient group variation according to antihypertensive therapy, primarily use of ACE inhibitors, which increase NO synthesis (57).

Many authors report on elevated plasma concentrations of immunoreactive Big ET-1 and ET-1 in patients with renal disease (58,59). As elevated ET concentrations can induce vasoconstriction, plasma ET-1 may play a major role in the development or genesis of hypertension in CRD. Indeed, there is a positive correlation between plasma ET concentrations and blood pressure in hemodialysis patients (60,61). In addition to their hemodynamic action, ETs also contribute to progressive structural tubular lesion in CRD. In rat with partial nephrectomy, ET-1 excretion increases significantly, in parallel with the development of glomerulosclerosis (62–64), while a significantly increased urinary excretion of ET-1 was also demonstrated in CRD patients (65). Our results did not indicate significantly increased urine or serum concentration of ET-1 in CRD patients with chronic renal insufficiency (subgroup 3), including various diagnoses (chronic glomerulonephritis, chronic interstitial nephritis, polycystic kidney disease, endemic nephropathy, obstructive uropathy, and vasculitis). However, this patient subgroup had a significantly elevated urine concentration of Big ET-1, pointing to ET axis involvement in the respective pathogenesis. The unaltered ET-1 concentration probably reflected the grade of renal insufficiency, which was still inadequate to influence ET-1 concentration. Studies in experimental animals have shown that urinary excretion of ET-1 increases with the duration and correlates with the signs of renal lesion (66). We believe that the elevated urine concentration of Big ET-1 in this patient subgroup suggested it to be a more sensitive indicator of local ET system activation than ET-1.

Changes in NO synthesis play a crucial role in the pathogenesis of CRD (67,68). There are many reports on a decreased NO synthesis in CRD patients (69). The activity of eNOS is considered to be modified by angiotensin II, which stimulates rapid and transient activation of endothelial NAD(P)H oxidase, followed by metabolic reactions that eventually result in tetra-

hydrobiopterin, eNOS cofactor, deficiency (70,71). One of the possible explanations is that it is not NO synthesis but NO bioavailability that is being reduced, which is consistent with the concepts postulated by some authors (72). Namely, NO exerts both antioxidant and oxidant actions, the latter depending on NO reaction with reactive oxygen radicals, thus forming numerous reactive nitric radicals, potent oxidants; NO and O_2^- produce peroxynitrites ($ONOO^-$), while myeloperoxidase can synthesize NO_x from NO^{2-} (reaction product of NO and H_2O_2) (73).

In our study we recorded increased serum and urine concentrations of NO_2/NO_3 metabolites in CRD patients with chronic renal insufficiency (subgroup 3), which may have resulted from the enhanced activity of iNOS isoenzymes, while the progression of renal insufficiency may have been due to the reduced NO bioavailability rather than reduced NO synthesis.

Our study results showed the concentration of Big ET-1 as a marker of ET system activity to be significantly elevated in urine of all three patient subgroups, and NO_2/NO_3 concentration in serum of patients with diabetic nephropathy and in urine of CRD patients with advanced renal insufficiency. Accordingly, diabetic nephropathy could be identified by determination of urinary concentration of Big ET-1, with a 78.6% diagnostic sensitivity and 88.9% diagnostic specificity, and by determination of serum concentration of NO_2/NO_3 , with a 64.3% diagnostic sensitivity and 83.3% diagnostic specificity. Mutual interaction, i.e., negative correlation between the markers of vasodilatory and vasoconstrictive system, was only recorded in the group of CRD patients with advanced renal insufficiency ($r = -0.42$).

In spite of these conclusions based on the results obtained in selected patient groups, additional studies in larger and homogeneous groups of patients according to underlying pathogenesis, histopathology, disease duration, and therapy received are needed to elucidate the role of ET and NO systems in renal disease and associated hypertension. Determination of the concentration of ET receptors (37,74,75) along with Big ET-1 and the activity and action of particular NOS isoenzymes and their polymorphisms, reflecting on NO synthesis, will add to the value of future studies in the field.

REFERENCES

- Potter GS, Johnson RJ, Fink GD. Role of endothelin in hypertension of experimental chronic renal failure. *Hypertension* 1997;6:1578–1584.
- Oparil S, Zaman MA, Calhoun DA. Pathogenesis of hypertension. *Ann Intern Med* 2003;139:761–776.
- Jaber BL, Madias NE. Progression of chronic kidney disease: Can it be prevented or arrested? *Am J Med* 2005;118:1323–1330.
- Parmar MS. Chronic renal disease. *BMJ* 2002;325:85–90.
- Rabelink TJ, Kaasiager KAH, Stroes ESG, Koomans HA. Endothelin in renal pathophysiology: From experimental to therapeutic application. *Kidney Int* 1996;50:1827–1833.
- Emoto N, Yanagisawa M. Endothelin-converting enzyme-2 is a membrane-bound, phosphoramidone-sensitive metalloproteinase with acidic pH optimum. *J Biol Chem* 1995;270:15262–15268.
- Hoffman A, Abassi ZA, Brodsky S, Ramadan R, Winaver J. Mechanisms of big endothelin-1-induced diuresis and natriuresis: Role of ET_B receptors. *Hypertension* 2000;35:732–739.
- Rabelink TJ, Stroes ESG, Bouter KP, Morrison P. Endothelin blockers and renal protection: A new strategy to prevent end-organ damage in cardiovascular disease? *Cardiovasc Res* 1998;39:543–549.
- Sharma R, Coats AJS, Anker SD. The role of inflammatory mediators in chronic heart failure: Cytokines, nitric oxide, and endothelin-1. *Int J Cardiol* 2000;72:175–186.
- Sofia M, Maniscalco M, Celentano L, et al. Abnormalities of renal endothelin during acute exacerbation in chronic obstructive pulmonary disease. *Pulm Pharmacol Ther* 2001;14:321–327.
- Mather KJ, Lteif A, Steinberg HO, Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. *Diabetes* 2004;53:2060–2066.
- Pollock DM. Renal endothelin in hypertension. *Curr Opin Nephrol Hypertens* 2000;9:157–164.
- Hand MF, Haynes WG, Webb DJ. Reduced endogenous endothelin-1-mediated vascular tone in chronic renal failure. *Kidney Int* 1999;55:613–620.
- Goddard J, Johnston NR, Hand MF, et al. Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure. *Circulation* 2004;109:1186–1193.
- Merta M, Reiterova J, Ryšava R, et al. Role of endothelin and nitric oxide in the pathogenesis of arterial hypertension in autosomal dominant polycystic kidney disease. *Physiol Res* 2003;52:433–437.
- Benigni A, Remuzzi G. How renal cytokines and growth factors contribute to renal disease progression. *Am J Kidney Dis* 2001;37:21–24.
- Zoja C, Benigni A, Camozzi D, et al. Combining lisinopril and L-arginine slows disease progression and reduces endothelin-1 in passive Heymann nephritis. *Kidney Int* 2003;64:857–863.
- Ignarro JJ, Cirino G, Casini A, et al. Nitric oxide as a signaling molecule in the vascular system. *J Cardiovasc Pharmacol* 1999;34:879–876.
- Sharma SP. Nitric oxide and the kidney. *Indian J Nephrol* 2004;14:77–84.
- Klahr S. The role of nitric oxide in hypertension and renal disease progression. *Nephrol Dial Transplant* 2001;16:60–62.
- MacAllister R, Vallance P. Nitric oxide in essential and renal hypertension. *J Am Soc Nephrol* 1994;5:1057–1065.
- Seccia TM, Maiolino G, Pessina AC, Rossi GP. The molecular basis of the interplay between endothelin-1 and nitric oxide and its relevance for atherosclerosis and arterial and pulmonary hypertension. *Vasc Dis Prevention* 2005;2:53–66.
- Cardillo C, Kilcoyne CM, Cannon III RO, Panza JA. Interactions between nitric oxide and endothelin in the regulation of vascular tone of human resistance vessels in vivo. *Hypertension* 2000;35:1237–1241.
- Liu Z, Wildhirt SM, Weismuller S, et al. Nitric oxide and endothelin in the development of cardiac allograft vasculopathy. *Atherosclerosis* 1998;140:1–14.
- Goligorsky MS, Budzikowski AS, Tsukahara H, Noiri E. Cooperation between endothelin and nitric oxide in promoting

- endothelial cell migration and angiogenesis. *Clin Exp Pharmacol Physiol* 1999;26:269–271.
26. Aiello S, Remuzzi G, Noris M. Nitric oxide/endothelin balance after nephron reduction. *Kidney Int* 1998;53:63–67.
 27. Rossi GP, Seccia TM, Albertin G, Pessina AC. Measurement of endothelin: Clinical and research use. *Ann Clin Biochem* 2000;37: 608–626.
 28. Editorial. Nitric oxide determinations: Much ado about NO-thing? *Clin Chem* 1997;43:553–556.
 29. Goligorsky MS, Tsukahara H, Magazine H, Andersen TT, Malik AB, Bahou WF. Termination of endothelin signaling: Role of nitric oxide. *J Cell Physiol* 1994;158:485–494.
 30. Verhaar MC, Strachan FE, Newby DE, et al. Endothelin A-receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation* 1998;97:752–756.
 31. Ahlborg G, Lundberg JM. Nitric oxide–endothelin-1 interactions in humans. *J Appl Physiol* 1997;82:1593–1600.
 32. Abassi ZA, Klein H, Golomb E, et al. Urinary endothelin: A possible biological marker of renal damage. *Am J Hypertens* 1993;6:1046–1054.
 33. Brown M, Chou SJ, Porush JG. Endothelins and kidney disease. *Nephron* 1996;73:375–382.
 34. Abassi ZA, Tate JE, Golomb E, et al. Role of neutral endopeptidase in the metabolism of endothelin. *Hypertension* 1992;20:89–95.
 35. King AJ, Brenner BM, Anderson S. Endothelin: A potent renal and systemic vasoconstrictor peptide. *Am J Physiol* 1989;256: 1051–1058.
 36. Simonsom MS, Wan S, Menne P. Endothelin stimulates phospholipase C, Na^+/H^+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J Clin Invest* 1989;83:708–712.
 37. Benigni A, Zoja C, Corna D, et al. Specific endothelin subtype A receptor antagonist protects against injury in renal disease progression. *Kidney Int* 1993;44:440–444.
 38. De Mattia G, Cassone-Faldetta M, Bellini C, et al. Role of plasma and urinary endothelin-1 in early diabetic and hypertensive nephropathy. *Am J Hypertens* 1998;11:983–988.
 39. Schnackenberg C, Patel AR, Kirchner KA, Granger JP. Nitric oxide, the kidney and hypertension. *Clin Exp Pharmacol Physiol* 1997;24:600–606.
 40. Rabelink TJ, Bakris GL. The renin-angiotensin system in diabetic nephropathy: The endothelial connection. *Miner Electrolyte Metab* 1998;24:381–388.
 41. Takahashi K, Gbatei MA, Lam HC, O'Halloran DJ, Bloom SR. Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia* 1990;33:306–310.
 42. Tarquini B, Perfetto F, Tarquini R, Cornelissen G, Halberg F. Endothelin-1 chronome indicates diabetic and vascular disease chronorisk. *Peptides* 1997;18:119–132.
 43. Lee YL, Shin SJ, Tsai JH. Increased urinary endothelin-1-like immunoreactivity in NIDDM patients with albuminuria. *Diabetes Care* 1994;17:263–266.
 44. Sharma K, Ziyadeh FN, Alzahabi B, et al. Increased renal production of transforming growth factor- β in patients with type II diabetes. *Diabetes* 1997;46:854–859.
 45. Zoja C, Morigi M, Figliuzzi M, et al. Proximal tubular cell synthesis and secretion of endothelin-1 on challenge with albumin and other proteins. *Am J Kidney Dis* 1995;26:934–941.
 46. Komers R, Anderson S. Paradoxes of nitric oxide in the diabetic kidney. *Am J Physiol Renal Physiol* 2003;284:1121–1137.
 47. Kone BC. Localization and regulation of nitric oxide synthase isoforms in the kidney. *Semin Nephrol* 1999;19:230–241.
 48. Sugimoto H, Shikata K, Matsuda M, et al. Increased expression of endothelial cell nitric oxide synthase (ecNOS) in afferent and glomerular endothelial cells is involved in glomerular hyperfiltration of diabetic nephropathy. *Diabetologia* 1998;41:1426–1434.
 49. Craven PA, DeRubertis FR, Melhem M. Nitric oxide in diabetic nephropathy. *Kidney Int* 1997;60:46–53.
 50. Tolins JP, Shultz PJ, Raij L, Brown DM, Mauer SM. Abnormal renal hemodynamic response to reduced renal perfusion pressure in diabetic rats: Role of NO. *Am J Physiol* 1993;265:886–895.
 51. Bank N, Aynedjian HS. Role of EDRF (nitric oxide) in diabetic renal hyperfiltration. *Kidney Int* 1993;43:1306–1312.
 52. Schrijvers BF, De Vriese AS, Flyvbjerg A. From hyperglycemia to diabetic kidney disease: The role of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. *Endocr Rev* 2004;25:971–1010.
 53. Webb ML, Meek TD. Inhibitors of endothelin. *Med Res Rev* 1997;17:17–67.
 54. Schiffrin EL. Vascular endothelin in hypertension. *Vasc Pharmacol* 2005;43:19–29.
 55. Hirai Y, Adachi H, Fujiura Y, Hiratsuka A, Enomoto M, Imazumi T. Plasma endothelin-1 level is related to renal function and smoking status but not to blood pressure: An epidemiological study. *J Hypertens* 2004;22:713–718.
 56. Ito A, Tsao PS, Adimoolam S, et al. Novel mechanism for endothelial dysfunction. Dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 1999;99:3092–3095.
 57. Bataineh A, Raij L. Angiotensin II, nitric oxide, and end-organ damage in hypertension. *Kidney Int* 1998;54:14–19.
 58. Koyama H, Tabata T, Nishizawa Y, et al. Plasma endothelin levels in patients with uraemia. *Lancet* 1989;1:991–992.
 59. Warrens AN, Cassidy MJD, Takahashi K, Gbatei MA, Bloom SR. Endothelin in renal failure. *Nephrol Dial Transplant* 1990;5:418–422.
 60. Miyauchi T, Sugishita Y, Yamaguchi I, et al. Plasma concentrations of endothelin-1 and endothelin-3 are altered differently in various pathophysiological conditions in humans. *J Cardiovasc Pharmacol* 1991;17:394–397.
 61. Web DJ, Cockcroft JR. Plasma immunoreactive endothelin in uraemia. *Lancet* 1989;1:1211.
 62. Benigni A, Perico N, Gaspari F, et al. Increased renal endothelin production in rats with reduced renal mass. *Am J Physiol* 1991;260:331–339.
 63. Brooks DP, Contino LC, Storer B, Ohlstein E. Increased endothelin excretion in rats with renal failure induced by partial nephrectomy. *Br J Pharmacol* 1991;104:987–989.
 64. Orisio S, Benigni A, Bruzzi I, et al. Renal endothelin gene expression is increased in remnant kidney and correlates with disease progression. *Kidney Int* 1993;43:354–358.
 65. Ohta K, Hirata Y, Shichiri M, et al. Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. *Kidney Int* 1991;39:307–311.
 66. Dumont Y, D'Amours M, Lebel M, Larivière R. Supplementation with a low dose of L-arginine reduces blood pressure and endothelin-1 production in hypertensive uraemic rats. *Nephrol Dial Transplant* 2001;16:746–754.
 67. Moeslinger T, Spickermann PG. Urea-induced inducible nitric oxide synthase inhibition and macrophage proliferation. *Kidney Int* 2001;78:2–8.
 68. Aiello S, Noris M, Remuzzi G. Nitric oxide/L-arginine in uremia. *Miner Electrolyte Metab* 1999;25:384–390.
 69. Schmidt RJ, Baylis C. Total nitric oxide production is low in patients with chronic renal disease. *Kidney Int* 2000;58: 1261–1266.

70. Li H, Wallerath T, Münzel T, Förstermann U. Regulation of endothelial-type NO synthase expression in pathophysiology and in response to drugs. *Nitric Oxide* 2002;7:149–164.
71. Chalupsky K, Hua C. Endothelial dihydrofolate reductase: Critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *PNAS* 2005;25: 9056–9061.
72. Passauer J, Bussemaker E, Range U, Plug M, Gross P. Evidence in vivo showing increase of baseline nitric oxide generation and impairment of endothelium-dependent vasodilatation in normotensive patients on chronic hemodialysis. *J Am Soc Nephrol* 2000;11:1726–1734.
73. Hazen SL, Hazen SL, Zhang R, Shen Z. Formation of nitric oxide derived oxidants by myeloperoxidase in monocytes: Pathways for monocyte mediated protein nitration and lipid peroxidation in vivo. *Circ Res* 1999;85:950–958.
74. Karet F, Kuc R, Davenport A. Novel ligands BQ123 and BQ3020 characterize endothelin receptor subtypes ET_A and ET_B in human kidney. *Kidney Int* 1993;44:36–42.
75. Nambi P, Pullen M, Wu HL, Aiyar N, Ohlstein EH, Edwards RM. Identification of endothelin receptor subtypes in human renal cortex and medulla using subtype-selective ligands. *Endocrinology* 1992;131:1081–1086.