Increased renal excretion of endothelin-1 in nephrotic patients

J. Vlachojannis¹, S. Tsakas¹, C. Petropoulou¹ and P. Kurz²

¹Department of Internal Medicine-Nephrology, University Hospital, Patras, Greece; and ²KfH-Diasysezentrum, Frankfurt, Germany

Abstract

Background. Renal function is influenced by direct and indirect action of endothelins. They reduce renal blood flow and glomerular filtration. The aim of the present study was to determine plasma and urinary endothelin-1 (ET-1) in two major categories of renal patients and to compare them with normal subjects.

Methods. Endothelin-1 was measured in the plasma and urine of patients with chronic renal disease and reduced glomerular filtration rate (GFR), and in patients with proteinuria due to glomerular dysfunction with unaffected GFR. A group of healthy subjects was used as a reference.

Results. Plasma endothelin-1 was increased in all patients to 60 ± 13 pg/ml independent of GFR compared to 29 ± 5 pg/ml in normal subjects (P < 0.001). The endothelin-1 load was decreased to 1190 ± 450 pg/ml/1.73 m² in patients with reduced GFR, compared to 2780 ± 690 pg/ml/1.73 m² of normal subjects, whereas in patients with glomerular damage and normal GFR, it was increased to 5480 ± 1910 pg/ml/1.73 m² (P < 0.01). ET-1 was found to be excreted and reabsorbed by the renal tubules by the same mechanisms as sodium and potassium, because its secretion fraction changes in parallel to those of the above ions. The excreted endothelin increased to 730 ± 420 and 710 ± 250 pg/ml/1.73 m² (P < 0.01) in the two categories of patients respectively, compared to 290 ± 100 pg/ml/1.73 m² in the normal group. The excretion fraction of patients with normal GFR was similar to normal subjects, while it appeared to increase in patients with reduced GFR (P < 0.01).

Conclusions. In the development of renal disease the plasma endothelin concentration is independent of the renal filtration capability and endothelin may be involved in functional and anatomical changes of the kidney as a causal factor or resulting from the renal disease.

Key words: chronic renal failure; endothelin-1; proteinuria

Introduction

Endothelins (ET-1, ET-2, and ET-3) are a recently isolated and characterized group of potent vasoconstrictors [1]. Their main vascular effect is temporal vasodilatation followed by sustained vasoconstriction [2]. Endothelins are 21 amino-acid peptides and are classified as endothelium-derived contracting factors—EDCF [3]. Although the pathophysiological influence of ET-1 on renal insufficiency (acute and chronic) has not yet been defined, it is assumed to be of primary significance [4]. Endothelins stimulate proliferation of vascular smooth muscle and glomerular mesangial cells [5, 6]. Because of their mitogenic properties [7–9], they may cause structural changes of the renal architecture.

Endothelial cells, neurons and renal cells are the main sites of endothelin synthesis. Vascular smooth muscle cells also produce endothelins under certain conditions. Their release is stimulated by hypoxia and/or ischaemia, angiotensin II, vasopressin, TGF-beta, insulin, thrombin, several cytokines, as well as by some nephrotoxic drugs (radiocontrast agents, cyclosporin, amphotericin and OKT-3) [10]. Endothelin production is inhibited by nitric oxide (NO) and ANP via a cGMP-dependent mechanism [11, 12]. The half-life of plasma endothelin-1 is approximately 4–7 min due to its removal by lungs, kidneys and liver [13,14] and not because of a specific enzymatic hydrolysis in blood.

Endothelins reduce renal blood flow (RBF) and glomerular filtration rate (GFR) [10] because of the evoked vasoconstriction in both afferent and efferent arterioles. Infusion of ET-1 in humans increases blood pressure, causes sodium retention and decreases urine flow [15,16]. Strictly in vitro effects of endothelin-1 include inhibition of renin release and stimulation of aldosterone, vasopressin, and ANP release [10]. ET-1 levels do not correlate with proteinuria [17], but prepro-ET-1 was shown to be related with urinary protein excretion [18]. Phosphoramidon, an inhibitor of the endothelin converting enzyme, has a protective action in ischaemic acute renal failure, suppressing tubular sodium wasting and proteinuria [19]. Recent studies in experimental models revealed increased ET-1
production and elevated ET-1 concentration at the renal cortex following surgical reduction of the renal mass [7, 20]. Despite a reduction in GFR, the amount of excreted ET-1 and plasma ET-1 concentration is increased in patients with chronic renal failure [21, 22]. Although ET-1 is removed by the kidney, it has been suggested that urinary ET-1 reflects its renal synthesis rather than its removal from the circulation [20].

To date, sufficient evidence for the role of endotoxins in the cause and/or the development of renal disease is lacking. Therefore we determined the ET-1 concentration in plasma and urine of patients with chronic renal disease and reduced GFR, and in patients with nephrotic syndrome due to glomerular dysfunction with normal GFR.

Subjects and methods

Subjects

Endothelin-1 plasma and urinary levels were measured in nine normal subjects (five males and four females), from 24 to 39 years of age with an average age of 30 years. Urinary and plasma endothelin-1 were also determined in eight patients (five males and three females), from 20 to 58 years of age with an average age of 35 years, with nephrotic syndrome (protein loss: 3.2 ± 1.8 gr/24h), but with no reduction of the glomerular filtration rate (GFR 99 ± 16 ml/min/1.73 m²). An additional group of fifteen patients (eight males and seven females), with chronic renal insufficiency (GFR 20 ± 7 ml/min/1.73 m²), from 30 to 50 years of age with an average age of 38 years was examined.

Sample preparation

Patients were placed in the supine position for 20 min before blood for plasma ET-1 was collected in ice cold tubes containing K₂EDTA. After centrifugation for 10 min at 1500 g and 4 °C, plasma was removed and stored at −20 °C until assay. An aliquot from a 3-h urine collection was also stored for urinary ET-1 measurement. This short time period was chosen since the 24-h urine collection was not convenient for most of the individuals. Pilot measurements had been performed in eight 3-h and 24-h urine collection and the amount of ET-1 in pg/min was the same.

Endothelin-1 measurement

After extraction from Amprep C2 columns, plasma and urine samples were assayed for endothelin-1 using Amersham’s RPA5559 kit. Urine samples were diluted 1:10 prior to extraction. The ET-1 concentration of undiluted and 1:100 diluted urine samples were too high or too low respectively to be calculated by the standard curve. The measurement of ET-1 was based on the competition between unlabelled ET-1 and a fixed quantity of [¹²⁵I]-labelled ET-3 for a limited number of binding sites on an ET-1 specific antibody. The cross-reactivity of the above antibody with ET-1 is 100%; ET-2, 144%; ET-3, 52%, and big ET-1, 0.4%. A sample of known ET-1 content was measured in triplicate. The average recovery was 40 ± 3% of the initial concentration of the ET-1, as some was partly lost in the C2 column extraction. All measurements were corrected to 100%.

Biochemical determinations

Plasma and urinary creatinine were determined with a picric acid assay. Sodium and potassium were estimated with an Eppendorf flamephotometer. Protein concentration in urine samples was determined according to Bradford [23] with a modified solution containing 6.7 mg% Coomassie G250 in 3.1% ethanol, 5.7% H₂PO₄, and O.D. recorded at 595 nm.

Calculations

The equations, according to which the magnitudes of the renal function were calculated, are:

(a) ET-1 load (filtered load of ET-1 presented to the proximal tubule) = ET-1 plasma concentration × Glomerular filtration rate

(b) ET-1 loss (excreted amount of ET-1 per min) = ET-1 urinary concentration × Urine flow rate

(c) ET-1 excretion fraction (fractional excretion rate of ET-1) = (ET-1 clearance: glomerular filtration rate) × 100

Statistical analysis

The results are expressed as means ± SD. Differences between each one of the patient groups and the group of healthy subjects were determined by comparison of their mean values using Student’s t test. A P value < 0.05 was considered to be significant.

Results

ET-1 plasma level determination

Endothelin-1 concentration was determined in the plasma of subjects in the three groups tested. Measurements revealed that plasma ET-1 levels were raised by 100% in both groups of patients, compared with normal subjects (Table 1). The causes of the proteinuria in the patients of group B were: (a) acute glomerulonephritis (n = 4), (b) minimal-changes disease (n = 2), and (c) unknown (n = 2). The causes of the CRF in the patients of group C were: (a) diabetic nephropathy (n = 5), (b) glomerulonephritis (n = 5), (c) chronic pyelonephritis (n = 2), (d) nephrosclerosis (n = 2), and (e) haemolytic uraemic syndrome (n = 1). Patients with proteinuria and normal GFR (group B) showed increased ET-1 plasma levels to 54.5 ± 13.5 pg/ml, compared with 29 ± 5 pg/ml in normal subjects (P < 0.001). ET-1 plasma concentration was also raised to 60 ± 13 pg/ml in patients with chronic renal insufficiency. This fact led us to conclude that ET-1 plasma concentration is associated with renal disease in a general way and not with the reduction of the glomerular filtration. Statistical analysis revealed that there is no linear correlation between ET-1 plasma concentration and GFR (data not shown). Since it has been noted that endothelin-1 plasma levels are increased in cases with proteinuria and normal GFR, a question is raised whether protein excretion and elevated ET-1 plasma levels are directly correlated to each other. However, statistical analysis revealed no linear correlation.
ET-1 renal load and urine excretion

As expected, the ET-1 load decreased to 1190 ± 450 pg/min, approximately 50% (P < 0.01), in patients with reduced GFR, while in the group with glomerular damage and physiological GFR, the ET-1 load was increased to 5480 ± 1910 pg/min, approximately twofold (P < 0.01) (Table 2), compared to 2780 ± 690 pg/min of normal subjects. The urine flow rates for the three tested groups were A, 1.38 ± 0.29 ml/min; B, 1.45 ± 0.22 ml/min, and C, 1.41 ± 0.31 ml/min. The amount of the excreted ET-1 in the urine increased in patients with low GFR by the same amount as in patients with normal GFR and proteinuria (P < 0.05) to 710 ± 250 and 730 ± 420 pg/min respectively. Normal values of ET-1 excretion were estimated to be 290 ± 100 pg/min. The remainder between the ET-1 renal load and urine loss is an indicator of its tubular reabsorption and/or its degradation. Patients with proteinuria and normal GFR rates revealed a twofold (P < 0.01) increase in the amount of the reabsorbed and/or degraded ET-1 along the renal tubules compared to normal subjects. The respective amount of ET-1 in nephrotic patients with reduced GFR is even lower, compared with the two other groups (P < 0.01), approximately 1/5 of normal values (Table 2).

The estimation of the ET-1 clearance did not reveal any difference (P > 0.1) among the tested groups (Table 1) and appears not to be affected by renal dysfunction.

ET-1 excretion fraction

The excretion fraction of endothelin between patients with proteinuria and normal GFR (group B) and normal subjects (group A), 12 ± 3.6% and 10.2 ± 3.2% respectively, showed no significant statistical difference (Table 1). On the other hand, the ET-1 excretion fraction was raised to 64 ± 27% in patients with low rates of glomerular filtration, which was statistically significant compared to groups A and B (P < 0.001). Furthermore, the excretion fraction of ET-1 appears to have changed in parallel with the sodium and potassium excretion fractions in all three groups (Table 3). Patients with reduced GFR had an increased (P < 0.001) excretion fraction of ET-1 (64 ± 27%), as well as 3.3 ± 1.8% for sodium and 48 ± 18% for potassium. In patients with proteinuria and normal GFR, all three fractions (ET-1, 11 ± 3.6%; Na, 0.4 ± 0.2%; K, 11 ± 6%) were similar to those of normal subjects (ET-1, 10.2 ± 3.2%; Na, 0.6 ± 0.3%; K, 8 ± 2.2%).

Discussion

Renal function is influenced by direct and indirect action of endothelins. They reduce renal blood flow and glomerular filtration. This is due mainly to vaso-constriction of both afferent and efferent arterioles [10]. ET-1 increases blood pressure and decreases urine flow [15,16]. Our results indicate that ET-1 plasma concentration seems to be related to functional and anatomical changes of the kidney. ET-1 plasma levels were increased in patients with chronic renal insufficiency, thus confirming relevant literature [21,22]. The most important finding was that ET-1 was also raised in the plasma of patients with proteinuria and normal GFR. This finding is of great interest since these patients were normotensive (BP 110–120/70–80 mmHg). Questions therefore arise regarding the reason for this increase in spite of the normal values of GFR and blood pressure.

We suggest that the increased ET-1 in the plasma of normotensive patients with proteinuria may be

<table>
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<tr>
<th>Group A (n=9)</th>
<th>Group B (n=8)</th>
<th>Group C (n=15)</th>
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<tbody>
<tr>
<td>ET-1 load (pg/min/1.73 m²)</td>
<td>ET-1 loss (pg/min/1.73 m²)</td>
<td>ET-1 (load-loss) (pg/min/1.73 m²)</td>
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<tr>
<td>2780 ± 690</td>
<td>290 ± 100</td>
<td>2490 ± 610</td>
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<tr>
<td>5480 ± 1910*</td>
<td>730 ± 420*</td>
<td>4760 ± 1560*</td>
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<tr>
<td>1190 ± 450*</td>
<td>710 ± 250*</td>
<td>480 ± 320*</td>
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*Statistically different (P < 0.01) compared to group A.
essential to balance existing hypotension that usually follows a low-plasma-protein state in nephrotic syndrome. However, this assumption, or a more evident explanation needs further investigation. The increase in endothelin-1 excretion (approximately 150%) in patients with proteinuria and normal GFR and in patients with chronic renal insufficiency suggests possible renal synthesis of ET-1, especially in the latter group (C) where ET-1 load is approximately half of that of the normal group (A). In the former group (B), the ET-1 load and ET-1 plasma concentration were increased by twice the value of the control group, indicating possible enhancement of extra-renal ET-1 synthesis. In addition, increased amounts of reabsorbed ET-1 were observed in patients with proteinuria and normal GFR, while the other group with low GFR, showed decreased reabsorption of ET-1.

The fact that both groups of patients and normal subjects showed the same ET-1 clearance in conjunction with increased excretion of ET-1 supports the idea of a possible renal synthesis of endothelin-1. However, total ET-1 clearance was not determined, since endothelin-1 is not cleared exclusively by the kidneys, but is also removed by the lungs and the liver [13,14]. Furthermore, some portion of the excreted ET-1 is of renal origin.

ET-1 is associated with sodium excretion [24,25]. It has been shown that ET-1 increases sodium excretion in the isolated perfused kidney despite the decline in GFR [26]. In the present study, it was shown that in patients (group C) with chronic renal failure (GFR 20 ± 7 ml/min/1.73 m²), where plasma ET-1 is increased to 60 ± 13 pg/ml, there is also an increase in sodium excretion fraction. Changes in the excretion fraction of ET-1 seemed to follow the same changes in pattern of sodium and potassium, respectively. This conclusion not only confirms that augmented ET-1 plasma levels are in agreement with increased sodium excretion in vivo, but also indicates that endothelin-1 is excreted and reabsorbed by the same mechanisms as sodium and potassium ions in the renal tubules.

Given the above, we may assume that renal dysfunction, such as a minimal-change glomerulonephritis, evokes an increase in circulating ET-1. The elevated endothelin-1 plasma levels are followed by the reduction of GFR and the resulting increased excretion of ET-1, sodium and potassium. Beyond this hypothesis, it still remains to be clarified whether enhanced ET-1 synthesis in glomerular disease contributes to the process of progressive deterioration of renal function, either as a causal agent or as a result of renal dysfunction.

References


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