Cyclooxygenase inhibition improves endothelium-dependent vasodilation in patients with chronic renal failure

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Abstract

Background. Some studies have demonstrated beneficial effects of L-arginine as a substrate for nitric oxide synthesis, and diclofenac as an inhibitor of cyclooxygenase (COX)-derived vasoconstrictive agents on vascular responses in humans during several pathological conditions. The aim of the present study was to investigate the acute effects of L-arginine and diclofenac on endothelium-dependent vasodilatation (EDV) and endothelium-independent vasodilatation (EIDV) in patients with chronic renal failure (CRF).

Methods. Effects of L-arginine and diclofenac on EDV and EIDV were measured in 15 patients with CRF and in 15 healthy controls by means of forearm blood flow measurements with venous occlusion plethysmography during local intra-arterial infusions of methacholine (2 and 4 μg/min evaluating EDV) and sodium nitroprusside (5 and 10 μg/min evaluating EIDV).

Results. L-Arginine infusion increased methacholine-induced vasodilatation both in patients with CRF and healthy controls. Diclofenac infusion increased methacholine-induced vasodilatation only in patients with CRF. There was no significant change in nitroprusside-induced vasodilatation after L-arginine and diclofenac infusions both in patients with CRF and healthy controls.

Conclusions. These results suggest that COX inhibition reduces the levels of a prostanoid-derived vasoconstrictive agent contributing to the impaired EDV in patients with CRF, while in this age group L-arginine improves EDV regardless of renal function.

Keywords: chronic renal failure; cyclooxygenase inhibition; endothelium; L-arginine

Introduction

The vascular endothelium produces a variety of biologically active substances that participate in the regulation of vascular relaxation and contraction. It is generally believed that nitric oxide (NO) is the principal compound being constantly released from the endothelium in order to maintain a proper vascular tone. NO is produced from the conversion of the semi-essential amino acid L-arginine to L-citrulline by NO synthase in endothelial cells [1].

It is well recognized that chronic renal failure (CRF) is associated with impaired endothelium-dependent vasodilatation (EDV) [2]. The basis for this is however unknown. Furthermore, Blum and co-workers [3] found that CRF is associated with NO deficiency and they suggested that L-arginine supplementation may improve renal function.

Two studies have demonstrated beneficial effects of L-arginine on vascular responses in vivo, both in experimental animals and in humans with several pathological conditions, including hypercholesterolemia and diabetes [4,5]. Oral feeding of L-arginine appears to have beneficial effects on atherosclerosis development in cholesterol-fed rabbits [6], and decreases proteinuria in diabetic rats [7]. L-Arginine was also found to correct renal failure-associated endothelial dysfunction in patients undergoing haemodialysis [8].

Lüscher and co-workers [9] demonstrated in experiments in spontaneously hypertensive rats that inhibition of cyclooxygenase (COX) by indomethacin improved EDV, suggesting that endothelium-derived contracting factor (EDCF) is a prostanoid-derived factor, possibly thromboxane A2 or prostaglandin H2. Furthermore, Taddei and co-workers [10] showed that a prostanoid-derived constrictive substance probably contributes to a defective EDV in hypertensive patients, as indomethacin restored EDV in these patients.

Thus, in order to further investigate the mechanism behind the impaired EDV in CRF, the present study examined the acute effects of L-arginine, as a substrate for NO synthesis, and diclofenac as an inhibitor of COX-derived vasoconstrictive agents, on EDV in patients with CRF and in healthy controls.

Subjects and methods

The study population consisted of 15 patients (Table 1) with renal impairment. All were out-patients recruited from the
Renal Unit of the Department of Medical Sciences, University Hospital, Uppsala, Sweden. The diagnoses of the renal patients were: glomerulonephritis (n = 5), diabetic nephropathy (n = 3), nephrosclerosis (n = 3), polycystic kidney (n = 2) and amyloidosis (n = 2). The majority of the patients had a mild to moderate CRF and no patient was considered to need renal replacement therapy in the near future (Table 1).

The control population (n = 15, Table 1) was recruited from the general population in Uppsala. Routine analyses showed that all controls had a normal renal function and no lipid metabolism disorders. No one with a history of any metabolic or other serious concomitant disease was included in the study. All patients and controls were non-smokers. All patients were on antihypertensive therapy (eight patients in the study. All patients and controls were non-smokers. Antihypertensive therapy (%) 100 0

<table>
<thead>
<tr>
<th>Laboratory variables</th>
<th>Renal patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>0.4210</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>69.7 ± 11.3</td>
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</tr>
<tr>
<td>Sex (male/female)</td>
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<td>9/6</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>146 ± 17</td>
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<td>Diastolic blood pressure (mmHg)</td>
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<td>79 ± 8</td>
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</tr>
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<td>Serum triglycerides (mmol/l)</td>
<td>2.4 ± 2.3</td>
<td>1.4 ± 0.7</td>
<td>0.1493</td>
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<td>Serum cholesterol (µmol/l)</td>
<td>6.0 ± 1.2</td>
<td>5.5 ± 1.1</td>
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<tr>
<td>Serum creatinine (µmol/l)</td>
<td>287 ± 143</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>15.1 ± 8.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Antihypertensive therapy (%)</td>
<td>100</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

After a measurement of resting FBF with venous occlusion plethysmography, local infusion of methacholine (2 and 4 µg/min) was performed.

To control the mechanical properties of the vascular bed in the skeletal muscle the exogenous NO-donor sodium nitroprusside (5 and 10 µg/min) was infused. The vasoactive drug infusions were given during 5 min for each dose, with a 20-min washout period between the drugs. The order of the vasodilatations was randomized. The increase in FBF after methacholine or nitroprusside infusion was taken as a measure of EDV or EIDV, respectively. The performing of venous occlusion plethysmography measurements was blinded. The coefficient of variation for the FBF measurements at rest and during methacholine nitroprusside infusions has been found to be <10% in our hands [12].

**Laboratory variables**

Measurements of serum creatinine and urea were performed using routine methods of the clinical chemistry laboratory. Twenty-four-hour urine collections were sampled, then acidified to a pH < 2 before laboratory measurements. Triglyceride and cholesterol concentrations in serum were measured after an overnight fast by enzymatic methods, using IL Test Cholesterol Trinder’s Method 181618-80 and IL Test Enzymatic-Colorimetric Method 181709-00 for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, MA).

The study protocol was approved by the local ethics committee and informed consent was obtained from all participants.

**Statistical analysis**

Numerical data are presented as mean values ± SD, while graphically depicted data are given as mean values ± SEM (Figures 2 and 3) and mean values ± SD (Figure 4). Differences between groups were calculated by factorial ANOVA while changes due to L-arginine or diclofenac administration were evaluated by ANOVA for repeated measurements. P < 0.05 was regarded as significant.
Results

Infusion of methacholine increased FBF in both groups: in renal patients from $4.5 \pm 0.8$ (SD) to $13.8 \pm 3.1$ ml/min/100 ml tissue at the highest dose and in controls from $4.3 \pm 1.0$ to $16.6 \pm 3.6$ ml/min/100 ml tissue (Figure 2). As compared with controls, FBF was significantly lower during methacholine infusion in patients with renal failure ($P < 0.03$).

Infusion of nitroprusside caused an increase in the FBF in renal patients from $4.5 \pm 0.8$ to $13.9 \pm 3.2$ ml/min/100 ml tissue at the highest dose and from $4.3 \pm 1.0$ to $15.3 \pm 3.5$ ml/min/100 ml tissue in control subjects (no significant difference between groups).

The infusion of L-arginine (40 and 80 mg/h) increased FBF in both groups: in renal patients from $4.5 \pm 0.8$ to $7.4 \pm 1.6$ ml/min/100 ml tissue ($P < 0.001$) at the highest dose and in controls from $4.3 \pm 1.0$ to $5.8 \pm 1.4$ ml/min/100 ml tissue ($P = 0.003$) (Figure 3). The increase in FBF was significantly higher in renal patients ($P = 0.005$).

Infusion of L-arginine (10 mg/h) increased resting FBF both in patients with renal failure (from $4.5 \pm 0.8$ to $5.3 \pm 1.4$ ml/min/100 ml tissue, $P = 0.004$) and in healthy controls (from $4.3 \pm 1.0$ to $4.9 \pm 0.9$ ml/min/100 ml tissue, $P = 0.03$). Similarly, diclofenac infusions increased resting FBF in both groups: in patients with renal failure from $4.5 \pm 0.8$ to $5.9 \pm 1.2$ ml/min/100 ml tissue ($P = 0.004$) and in controls from $4.3 \pm 1.0$ to $4.9 \pm 0.9$ ml/min/100 ml tissue ($P = 0.01$) (Figure 4).

L-Arginine infusion (10 mg/h) resulted in a significant increase in FBF during methacholine infusion at the highest dose in renal patients from $13.8 \pm 2.1$ to $15.0 \pm 3.1$ ml/min/100 ml tissue ($P = 0.007$) and in controls from $16.6 \pm 3.0$ to $18.9 \pm 3.2$ ml/min/100 ml tissue ($P = 0.01$) (Figure 4).

Infusion of L-arginine (10 mg/min) did not result in a significant change in FBF during nitroprusside infusion in renal patients and in controls (Figure 5).

Diclofenac infusion increased FBF significantly during methacholine infusion at the highest dose from $13.8 \pm 2.1$ to $15.5 \pm 2.4$ ml/min/100 ml tissue in patients with renal failure ($P < 0.05$). There was no significant change in FBF during methacholine infusion in healthy controls (from $16.6 \pm 3.0$ to $16.9 \pm 0.9$ ml/min/100 ml tissue) (Figure 4). Infusion of diclofenac did not increase FBF during nitroprusside infusions in any of the groups (Figure 5).

Discussion

The present study showed that low-dose intra-arterial infusion of L-arginine resulted in significant improvement in EDV both in patients with CRF and in healthy
controls, while COX inhibition improved EDV in renal patients only, suggesting that a prostanoid-derived vasoconstricting factor contributes to the impairment in EDV, as demonstrated in the CRF patients.

A prostanoid-derived contracting factor has been found to be co-released by the endothelium during muscarinic-receptor stimulation in hypertensive patients [13] and in spontaneously hypertensive rats [9]. This can be disclosed by inhibition of the COX pathway, as used in the present study. Such EDCF has also been described to be acting in elderly subjects [13] and in hypercholesterolaemic rats [14]. As the patients with CRF in the present study showed a higher blood pressure, this might in part explain the beneficial effect of COX inhibition on EDV. Another mechanism whereby COX inhibition could improve EDV is by a decreased rate of lipid peroxidation. Lipid peroxidation products might inactivate NO by the formation of ONOO⁻. Recently, we were able to show that the oxidative status in CRF was closely related to EDV [15]. However, as this mechanism also would affect exogenously given NO, and no effect on FBF during nitroprusside infusion was seen, this mechanism seems less likely. It could be seen that although COX inhibition improved EDV, it was not normalized. This implies that other mechanisms are involved in the impairment in EDV seen in CRF.

It is known that high blood pressure might contribute to endothelial dysfunction. In the present study the patients with CRF had significantly higher systolic blood pressure compared with controls. However, our previous study [2] was able to demonstrate in large patient groups that EDV was impaired irrespective of high blood pressure.

COX inhibition increased resting FBF in both CRF and controls suggesting that during resting conditions a tonic effect of an EDCF is present in this age group. In end-stage renal failure there is accumulation of methylated analogs of l-arginine, such as asymmetrical dimethyl arginine (ADMA). These substrates are known as competitive inhibitors of NO synthase [16]. Therefore, we administrated l-arginine, the precursor of NO, to patients with CRF and to the healthy controls to examine if the responses on methacholine and nitroprusside improved. The low dose of l-arginine (10 mg/min) resulted in an increase in resting FBF, as well as in EDV, in both renal patients and in controls with no significant difference between the groups, suggesting that lack of l-arginine, or competition by ADMA, do not play a role exclusively for CRF patients, but might be a general factor in this age group. Chauhan and co-workers [17] have also reported selective impairment of EDV with ageing and that administration of l-arginine can restore endothelial function.

It is well recognized that CRF is associated with impaired EDV [2,3]. The basis for this is however unknown. l-Arginine infusion at higher doses resulted in an increase in FBF both in patients with CRF and in healthy controls, being more pronounced in patients with CRF. This finding supports the idea that patients with CRF have low l-arginine levels resulting in an impaired EDV and that increased concentrations of l-arginine would lead to an improvement in NO production. However, two recent studies demonstrated the normal plasma l-arginine levels in patients with CRF [18,19] and acute administration did not improve arterial endothelial function in CRF [20]. Despite these facts the concentrations of l-arginine might be different between patients and healthy controls on the site of synthesis of l-arginine (in the endothelial cell).

In summary, this study demonstrated that intrarterial infusion of l-arginine augmented methacholine-induced vasodilation both in patients with CRF and in elderly controls, while the COX inhibitor diclofenac augmented methacholine-induced vasodilatation in renal patients only. These results may suggest that COX inhibition reduces the levels of a prostanoid-derived vasoconstrictive agent contributing to the impaired EDV in patients with CRF, while in this age group l-arginine improves EDV regardless of renal function. However, these results do not indicate that diclofenac treatment is good in the renal patients because it may cause an iatrogenic renal failure. This study aimed to clarify pathophysiological mechanisms in patients with CRF.

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References

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