Oxidative Stress and Endothelial Function in Chronic Renal Failure

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Abstract. This study aimed to investigate the relationship between oxidative stress and endothelium-dependent vasodilation in patients with chronic renal failure (CRF). Thirty-seven patients with CRF underwent evaluation of endothelium-dependent vasodilation and endothelium-independent vasodilation by means of forearm blood flow measurements with venous occlusion plethysmography during local intra-arterial infusions of methacholine (evaluating endothelium-dependent vasodilation) and sodium nitroprusside (evaluating endothelium-independent vasodilation). Lag phase of lipoprotein fraction, and higher levels of diene conjugates, lipid hydroperoxide, and GSSG levels. The GSSG/GSH ratio was lower in patients with CRF. Endothelium-dependent vasodilation was positively correlated with total antioxidative activity (r = 0.41, P = 0.016), GSH (r = 0.44, P < 0.0098), and lag phase of LDL (r = 0.35, P = 0.036) and negatively correlated with GSSG (r = −0.40, P < 0.018), GSSG/GSH (r = −0.47, P = 0.0057), and diene conjugates (r = −0.53 P < 0.0015) in patients with CRF. These results show that an impaired endothelium vasodilation function and oxidative stress are related to each other in patients with CRF.

Cardiovascular disease (CVD) is the major cause of death in patients with renal insufficiency, accounting for 50% of all deaths in renal replacement therapy patients and in recipients of renal transplants. Mortality from CVD in patients with renal insufficiency is approximately 9% per year, which is about 30 times the risk in the general population (1).

Endothelial cell dysfunction is an early feature of vascular complication in different diseases such as diabetes (2), hypertension (3), hypercholesterolemia (4), and coronary heart disease (5,6). The precise mechanism inducing endothelial dysfunction is not clear, but several investigators have found correlations between endothelial dysfunction and oxidative stress markers. Peterson et al. (7) suggested that oxidative stress modulates endothelial function by regulating caveolae formation, endothelial nitric oxide (NO) synthase expression, and endothelial NO synthase-caveolin interactions. Ceriello et al. (8) hypothesized that the increase of glycosylated hemoglobin might result in an increase of superoxide anion generation and thus influence in NO action in diabetes. Henning and Chow (9) suggest that the lipid peroxidation (LP) products, lipid hydroperoxides (LOOH), directly injure endothelial cells and cause membrane malfunctions.

Accumulating evidence suggests that chronic renal failure (CRF) is associated with enhanced oxidative stress (10–13) as well as an impaired endothelial cell function (14–18), but to our knowledge, this has not previously been investigated simultaneously. This study was designed to investigate the relationship between markers of oxidative stress and endothelium-dependent and endothelium-independent vasodilation in patients with CRF.

Materials and Methods

Subjects

The study population consisted of 37 patients with chronic renal impairment (24 men and 13 women) (Table 1). All were outpatients recruited at the Renal Unit of the Medical Center, University Hospital, Uppsala, Sweden. The diagnoses of the patients with renal insufficiency were as follows: glomerulonephritis (n = 11), diabetic nephropathy (n = 7), pyelonephritis (n = 4), polycystic kidney (n = 4), nephrosclerosis (n = 4), hypoplastic kidneys (n = 2), amyloidosis (n = 2), and unknown (n = 3). The majority of the patients had mild to moderate CRF (creatinine clearance [mean ± SD] 25.1 ± 16.2 ml/min per 1.73 m²; serum creatinine 287 ± 168 μmol/L, serum urea 14.2 ± 7.1 mmol/L), and no patient was considered to need renal replacement therapy in the near future. The mean serum cholesterol concentration was 5.3 ± 1.9 mmol/L. The mean systolic BP was 160 ± 18.0 mmHg, and the diastolic BP was 88 ± 8.0 mmHg. Eight
patients had a history of CVD, and five patients had manifest peripher-
avascular disease. Thirty-one of the patients were on antihyper-
tensive therapy (14 patients were treated with angiotensin converting
enzyme inhibitors, 12 with calcium channel antagonists, and 11 with
β-blocking agents), 7 patients were on antidiabetes therapy, and 6
patients were on lipid-lowering therapy (statins).

The control population for evaluation of the oxidative stress mark-
ers was recruited from the Blood Center at the University Hospital,
Uppsala (Table 2). Another control population for evaluation of the
endothelium function was recruited from the general population in
Uppsala (Table 1). Routine analyses showed that controls had normal
renal function and no disorders of lipid metabolism. Subjects with a
history of metabolic or other serious concomitant disease were ex-
cluded. Both patients and controls were nonsmokers. Informed con-
sent was obtained from each subject.

Markers of Oxidative Stress

The procedures met the criteria and principles described previously
(19), with some minor modifications of a technical character. Blood
samples were obtained from v. cubitalis and stored frozen at −195°C
until analyzed. All measurements of LP and antioxidant markers were
performed in triplicate. LP products were measured in serum and
samples were treated with antioxidant butylated hydroxytoluene
(BHT) twice, immediately after obtaining and before adding the test
reagents to suppress artifactual changes during handling and assay
procedures. All reagents were purchased from Sigma Chemical Co.
(St. Louis, MO).

Lipid Peroxidation Markers. LP markers comprised diene con-
jugates (DC), LOOH, thiobarbituric acid (TBA), and TBA reactive
substances. The first stage of LP consists of the molecular rearrange-
ment of the double bonds in polyunsaturated fatty acid residues of
lipids, which leads to the formation of DC. DC levels were measured
according to previously described methods (20). Briefly, samples were
incubated at 37°C for 25 min, 0.25% BHT and lipids were
extracted by heptane/isopropanol (1:1), and samples were acidified by
5 M hydrochloric acid and extracted by heptane. After centrifugation
lag phase. The lag phase can be estimated as the point of intersection
of 1 mM 5,5’dithiobis-(2-nitrobenzoic acid). The change in OD was
measured as described earlier (20), assessing the ability of the test
sample to inhibit linolenic acid peroxidation. Previously, we estab-
lished a good correspondence with the Randox total antioxidative
status method. Briefly, standard linolenic acid in isotonic saline
(0.4 ml), sodium dodecyl sulfate (0.015 ml), and serum (0.030 ml
1:3:3 in isotonic saline) were incubated in the presence of 0.2 mM
iron at 37°C for 60 min. BHT was added, and samples were treated
with acetate buffer (pH 3.5), heated with TBA solution, and
assessed for TBA reactive substances. The results were expressed
as percentage of inhibition of linolenic acid peroxidation induced
by serum samples.

Glutathione was measured by an enzymatic method (21), modified
by Griffith (22), described by Bhat et al. (23), and slightly modified
by us. Protein was removed from 0.3 ml of heparinized whole blood
by adding an equal volume of a 10% solution of metaphosphoric acid
in water, leaving the mixture at room temperature, and then centri-
fuging it (4°C, 1200 g, 10 min). The supernatant was carefully
collected and stored at −20°C. The sample was divided into two parts
for measurement of total amount of GSH (TGSH; TGSH or GSH plus
oxidized GSH [GSSG]) and GSSG. To assay TGSH or GSSG, the
supernatant was mixed with 0.895 ml of 0.2 M sodium phosphate
buffer (pH 7.5) containing 0.01 M ethylenediaminetetraacetic acid
and with 0.5 ml the same buffer containing 0.5 U GSH-reductase
and 0.3 mM NADPH. The reaction was initiated by the addition of 0.1 ml
of 1 mM 5,5’dithiobis-(2-nitrobenzoic acid). The change in OD was
measured at 412 nm after 10 min and quantitated by comparison with
standard curve.

The content of GSH was calculated as the difference between
TGSH and GSSG. The resistance of LPF to copper-catalyzed oxida-
tion (lag phase of LPF) was estimated as described by Ristimäe et al.
(20). Briefly, a non-HDL fraction was isolated by a dextran-magne-
sium precipitation method (24). Peroxidation of LPF solutions in
phosphate-buffered saline (2 mg protein/ml) was initiated with Cu2+
(0.45 mM), and the ability of this fraction to oxidize was evaluated by
measuring DC at different time intervals of incubation at 37°C. The
change of DC absorption as a function of time reflects the process of
LDL oxidation, and the oxidation resistance is defined as the length of
lag phase. The lag phase can be estimated as the point of intersection
between the tangents to the lag time and the propagation phase.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Renal Patients</th>
<th>n</th>
<th>Controls</th>
<th>n</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>69.1 ± 11</td>
<td>37</td>
<td>62 ± 9</td>
<td>37</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>24/13</td>
<td>37</td>
<td>23/14</td>
<td>37</td>
<td>NS</td>
</tr>
<tr>
<td>Resting FBF</td>
<td>6.2 ± 1.9</td>
<td>37</td>
<td>5.9 ± 2.1</td>
<td>37</td>
<td>NS</td>
</tr>
<tr>
<td>FBF at MCh 2 μg/min</td>
<td>11.3 ± 4.0</td>
<td>37</td>
<td>14.8 ± 3.8</td>
<td>37</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>FBF at MCh 4 μg/min</td>
<td>14.7 ± 3.9</td>
<td>37</td>
<td>18.9 ± 4.2</td>
<td>37</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>FBF at SNP 5 μg/min</td>
<td>12.0 ± 4.1</td>
<td>37</td>
<td>13.3 ± 3.6</td>
<td>37</td>
<td>NS</td>
</tr>
<tr>
<td>FBF at SNP 10 μg/min</td>
<td>15.8 ± 4.7</td>
<td>37</td>
<td>16.3 ± 4.1</td>
<td>37</td>
<td>NS</td>
</tr>
</tbody>
</table>

* FBF, forearm blood flow; MCh, methacholine; SNP, sodium nitroprusside. Values are expressed as means ± SD.
Measurement of Endothelium-Dependent Vasodilation and Endothelium-Independent Vasodilation

Endothelium-dependent vasodilation was measured by venous occlusion plethysmography (25,26). During the blood flow measurements, the subjects were supine in a quiet room maintained at a temperature of 21 to 23°C. An arterial cannula was inserted into the brachial artery of one arm for regional infusions of methacholine and sodium nitroprusside. A mercury-in-SILASTIC strain gauge, connected to a calibrated plethysmograph, was placed at the upper third of the forearm, which rested comfortably slightly above the level of the heart.

Venous occlusion was achieved by a BP cuff applied proximal to the elbow and inflated to 40 mmHg by a rapid cuff inflator. Approximately 4 inflations per minute for about 7 s each were performed. After measurements of resting forearm blood flow, local infusion of methacholine (2 and 4 μg/min) was performed. This muscarinic receptor agonist has been shown to increase the forearm release of nitrite and nitrate, the breakdown products of NO, more than 10-fold in healthy volunteers (27).

To control for the mechanical properties of the vascular bed in the skeletal muscle, the exogenous NO-donor nitroprusside (5 and 10 μg/min) was infused. The vasoactive drug infusions were given during 5 min for each dose at a rate of 1 ml/min, with a 20-min washout period between the drugs. The order of the vasodilations was randomized. Endothelium-dependent vasodilation was defined as forearm blood flow obtained at the highest dose of methacholine (4 μg/min) minus resting forearm blood flow divided by resting forearm blood flow. Endothelium-independent vasodilation was defined as forearm blood flow obtained at the highest dose of nitroprusside (10 μg/min) minus resting forearm blood flow divided by resting forearm blood flow. The coefficient of variation for the forearm blood flow measurements at rest and during methacholine and nitroprusside infusions has been found to be less than 10% in our hands (28).

Laboratory Variables

Measurements of serum creatinine were performed on a free diet by routine methods of the Clinical Chemistry Laboratory. Twenty-four-hour urine collections were sampled, then acidified to pH <2 before laboratory measurements were made. Excretion of albumin was measured on a free diet by routine methods.

Table 2. Basic characteristics and oxidative stress parameters in patients with chronic renal failure and in healthy controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Renal Patients</th>
<th>Controls</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>69.1 ± 11</td>
<td>58.5 ± 7</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>24/13</td>
<td>40/21</td>
<td>NS</td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>53.2 ± 19.2</td>
<td>60.0 ± 14.0</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>TBA activity (MDA eqs)</td>
<td>1.59 ± 0.38</td>
<td>1.60 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>DC (μM)</td>
<td>43.2 ± 11.6</td>
<td>38.6 ± 5.7</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>TAA (%)</td>
<td>41.4 ± 5.4</td>
<td>39.7 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>LOOH (nmol/mL)</td>
<td>2.1 ± 1.9</td>
<td>0.89 ± 0.76</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>GSSG (μg/mL)</td>
<td>103.3 ± 8.7</td>
<td>33.9 ± 13.8</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>GSH (μg/mL)</td>
<td>201.1 ± 53.2</td>
<td>241.9 ± 33.9</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>GSSG/GSH</td>
<td>0.59 ± 0.41</td>
<td>0.14 ± 0.05</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

* Lag time, resistance of lipoprotein fraction of oxidation; TBA, thiobarbituric acid; MDA, malondialdehyde; DC, diene conjugates; TAA, total antioxidant activity; TAS, total antioxidant status; LOOH, lipid hydroperoxide; GSSG, oxidized glutathione; GSH, reduced glutathione. Values are expressed as means ± SD.

Oxidative Stress

Oxidative stress markers in the patients with renal failure and healthy controls are shown in Table 2. It was found that the lag phase (lag time) of LPF to oxidation, known as marker of antiatherogeneity, was longer in healthy controls compared with patients with renal insufficiency. Levels of DC and LOOH (as
markers of LP) were significantly higher in the patients with renal insufficiency (43.2 ± 11.6 μM versus 38.6 ± 5.7 μM and 2.1 ± 1.9 nmol/ml versus 0.89 ± 0.76 nmol/ml, respectively).

Patients with renal failure were characterized by significantly higher levels of GSH redox ratio (ratio of oxidized and reduced GSH) compared with healthy controls (0.59 ± 0.41 versus 0.14 ± 0.05) (Table 2). Multiple regression analysis was performed to assess the role of other factors (age, gender, antihypertensive treatment, lipid-lowering treatment, vascular diseases, diabetes) to oxidative stress in patients with CRF. These factors did not contribute significantly in this patient group with the multivariate model used. There were no differences in total antioxidant activity or TBA reactivity between patients with renal insufficiency and healthy controls (Table 2).

**Endothelial Function and Oxidative Stress**

There was a positive correlation between serum creatinine and GSSG concentrations \( (r = 0.38; P < 0.05) \). There were also positive correlations between endothelium-dependent vasodilation and TAA \( (r = 0.41, P = 0.016) \), lag phase of LPF \( (r = 0.35, P = 0.036) \) (Figure 1), and GSH levels \( (r = 0.44, P = 0.0098) \) in patients with renal insufficiency. These findings indicate that the patients with high endothelium-dependent vasodilation had higher levels of TAA and higher levels of reduced GSH as a central cellular antioxidant. Patients with renal insufficiency with better endothelium-dependent vasodilation had longer lag phase of LPF.

We also found inverse correlations between endothelium-dependent vasodilation and DC levels \( (r = -0.53, P = 0.0015) \) (Figure 1), GSSG \( (r = -0.40, P = 0.018) \), and the GSSG/GSH ratio \( (r = -0.47, P = 0.0057) \). These relationships indicate that the patients with higher levels of the LP products (DC) had a higher degree of impaired endothelium-dependent vasodilation and patients with better endothelium-dependent vasodilation had lower GSSG/GSH ratios. Endothelium-independent vasodilation was also significantly correlated with DC levels \( (r = -0.46, P = 0.0067) \)—that is, patients with higher levels of LP products (conjugated dienes) had a more impaired endothelium-independent vasodilation.

Stepwise multiple regression analyses were performed to assess the role of other factors (age, gender, BP, antihypertensive treatment, lipid-lowering treatment, vascular diseases, diabetes) as potential cofounders in the relationship between oxidative stress and endothelium-dependent vasodilation. With this model, it could not be demonstrated that the classical risk factors contributed significantly in this aspect. The relationship between endothelium-dependent vasodilation and markers of oxidative stress remained significant.

**Discussion**

This study demonstrated a relationship between markers of oxidative stress and endothelium-dependent vasodilation in patients with CRF in that the elevated LP products and decreased level of antioxidants may impair endothelium-dependent vasodilation. Endothelial dysfunction could be the result of either a diminished endothelial capacity to synthesize and release NO or an increased inactivation of NO after its synthesis (29). Because the superoxide ion rapidly reacts with NO to form peroxynitrite, ONOO⁻, a compound with diminished vasodilatory properties, free radical generation and oxidative stress have been proposed as putative mechanisms of endothelial dysfunction.

Healthy people are protected against free radicals by several defense mechanisms. Reduced GSH is the most important intracellular scavengers of free radicals (19). GSH serves as a reductant in oxidation reactions resulting in the formation of...
GSSG. Thereby decreased GSH levels and increased GSSG levels may reflect depletion of the antioxidant reserve (19). Increased levels of GSSG and decreased levels of GSH have been found in patients with CRF (10,30,31).

The results of this study show a close positive relationship between endothelium-dependent vasodilation and GSH and a negative relationship between endothelium-dependent vasodilation and GSSG (or redox ratio), indicating the important role of GSH in the regulation of endothelial dysfunction in patients with CRF. The free radical attack on cell membrane–bound polyunsaturated fatty acids results in formation of LP products such as DC, LOOH, and malondialdehyde. High levels of DC, LOOH, and malondialdehyde (the latter is expressed as TBA activity) are considered to be markers of systemic oxidative stress (19).

Studies of predialysis patients have demonstrated a relationship between LP and the degree of renal failure (11). In this study, a significant inverse correlation was found not only between DC levels and endothelium-dependent vasodilation but also between DC levels and endothelium-independent vasodilation. These findings indicate that endothelial cell function and vascular smooth muscle cell function may be modulated by LP.

The role of lipoprotein oxidation in atherosclerosis is well established. A decreased resistance to oxidation of LDL (shortened lag phase) indicates an atherogenic nature of LDL (32,33) and is associated with an increased risk of atherosclerosis (34). The study presented here demonstrated a significant correlation between the lag phase of LPF and endothelium-dependent vasodilation in patients with CRF, a patient group known to experience rapid atherosclerosis. This means that the shorter the lag phase, the higher the propensity to produce severely oxidized LDL. This is associated with reduced endothelium-dependent vasodilation in a patient group known to have an increased risk for atherosclerosis, including cardiac and peripheral vascular disease.

It is known that vitamin E is able to enhance the resistance of LDL oxidation. Boaz et al. (35) demonstrated in a double-blind, placebo-controlled, randomized trial that supplementation with 800 IU/d vitamin E reduces composite CVD and myocardial infarction in patients receiving hemodialysis with prevalent CVD, further supporting the important role of oxidative stress for the development of cardiovascular disorders in this patient group.

The present findings allow us to conclude that patients with CRF have increased levels of oxidative stress markers and impaired endothelial cell function. The degree of oxidative stress is related to endothelial dysfunction. These factors may be important with respect to the high morbidity and mortality of CVD found in patients with CRF.

**Acknowledgments**

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**References**


