Alteration in plasma antioxidant capacities in chronic renal failure and hemodialysis patients: a possible explanation for the increased cardiovascular risk in these patients

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Abstract

Objective: The high incidence of cardiovascular diseases in chronic renal failure (CRF) and hemodialyzed (HD) patients is now well established and the involvement of oxidative stress has been hypothesized in these phenomena. The aim of our study was to evaluate the level of oxidative stress in healthy controls (CTL) compared with CRF and HD patients before (pre-HD) and after (post-HD) the dialysis session, carried out on a high biocompatible polyacrylonitrile membrane AN69. Methods: Several indicators of the extracellular redox status were evaluated in plasma. The ascorbyl free radical (AFR) was directly measured using electron spin resonance spectroscopy (ESR) and expressed with respect to the vitamin C level to obtain a direct index of oxidative stress. Indirect plasma parameters such as vitamin E, thiol and uric acid levels were also quantified. The plasma antioxidant status (PAS) was evaluated by the allophycocyanin test. Nitric oxide (NO) stable-end metabolites: nitrites and nitrates (NOx), were measured in plasma. Results: In CRF patients, vitamin C and thiol levels were low, and the AFR/vitamin C ratio high compared with the CTL. On the other hand, PAS and uric acid levels were shown to be higher in CRF patients. After the dialysis session, vitamin C level decreased and AFR/vitamin C ratio increased. The thiol levels were shown to be increased, in return PAS and uric acid levels were significantly lower after the dialysis session. NOx levels rose during CRF, but were significantly decreased after the dialysis procedure. No differences in vitamin E status were observed between CTL, CRF and HD patients. Conclusion: Our study demonstrates that profound disturbances in the extracellular redox system occur during the course of chronic renal failure and hemodialysis, and may provide an explanation for the cardiovascular complications in these patients. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The high incidence of early cardiovascular disease in patients with chronic renal failure, either on dialysis or not, is well documented [1]. Ischemic heart diseases represent a major source of morbidity and remain one of the leading causes of death in these patients. The physiopathology of cardiovascular events in uremia is multifactorial, but accelerated atherosclerosis seems to play a central role in cardiovascular dysfunction. It is biologically plausible that oxidative stress contributes to the high prevalence of cardiovascular disease in these patients.

Over the past decade, many experimental studies have shown that reactive oxygen species (ROS) play a key role in the physiopathological pathways of a wide variety of clinical and experimental diseases. Evidence supports the hypothesis that ROS are involved in the pathophysiology of uremic complications. It has been observed that chronic renal failure (CRF) is a prooxidant state, characterized by increased levels of free radical oxidants relative to anti-
oxidants [2]. Moreover, oxidative stress could be exacerbated by maintenance hemodialysis sessions leading to neutrophil activation associated with ROS production [3–5].

In addition, other complications such as bleeding and hypotension during CRF and HD are possibly associated with a particular free radical, nitric oxide (NO). NO could also have deleterious effects via its reaction with superoxide, leading to the formation of a new powerful oxidant, the peroxynitrite anion. Several studies have indirectly reflected the presence of NO [6–8] in CRF and HD, and a possible role for NO in such pathological conditions is emerging.

Oxidative stress associated with the production of ROS could play a determining role in the development of cardiovascular events in CRF and HD patients. The present study was undertaken to investigate plasma antioxidant capacity in CRF patients, and the influence of hemodialysis sessions, performed with a highly compatible polyacrylonitrile membrane, on oxidative stress. In that way, we used several techniques including a direct index of oxidative stress: the ascorbyl free radical (AFR), detected by electron spin resonance (ESR) spectroscopy, and indirect measurements concerning extracellular antioxidants and NO metabolism.

2. Methods

2.1. Patients and sample collection

Sixteen patients with conservatively managed chronic renal failure (CRF) and 13 patients on maintenance hemodialysis (HD) were selected on the basis of their creatinine clearance and both groups were compared with 13 healthy controls (CTL). The study protocol was approved by the local Medical Ethics Committee, and informed consent was obtained from the patients. The characteristics and clinical data of the CRF and HD groups are summarized in Table 1.

The group of healthy volunteers (49±9 years, eight males, five females) was recruited among the medical and research staffs.

The CRF group recruited patients with chronic renal insufficiency (15 ml/min/1.73 m² BS (body surface) ≤ creatinine clearance ≤ 30 ml/min/1.73 m² BS), who had never been dialyzed. The CRF group included patients (59±10 years, 10 males 6 females) in heterogeneous states. The etiology of their renal disease was nephrosclerosis (2), primary glomerulonephritis (4), polycystic disease (3), interstitial nephritis (4) and unknown in three cases. The patients received multidrug therapies including antihypertensive drugs with high dosages of loop diuretics (14/16 patients), but no antioxidant vitamin supplementation. Three patients were smokers. The CRF patients were hospitalized in the same Nephrology Unit for complications such as massive edematous or accelerated hypertensive states. Samples were collected after clinical stabilization, 5–7 days after admission in the Unit.

The HD group (61±11 years, six males, seven females) consisted of long-term dialysis patients (0 ml/min/1.73 m² BS ≤ creatinine clearance ≤ 5 ml/min/1.73 m² BS) with a mean dialytic course of 4.2 years. The patients were selected on the basis of their stable clinical condition over the past 3 months. The etiology of end-stage renal disease was chronic IgA nephropathy (1), primary glomerulonephritis (1), nephrosclerosis (1), polycystic disease (3), interstitial nephritis (5), and was unknown in two cases. The patients had 4–4.5 h unmodified dialysis procedures three times a week with a non reused AN 69 hollow fiber membrane (Hospal-Lyon, France). All the patients received bicarbonate dialysis, and the sessions were conducted under standard conditions in the same Unit, with sterile apyrogen delivered water. Blood and dialysate flow-rates were maintained at 250 ml/min and 500 ml/min, respectively. The final dialysate concentrations were: HCO₃⁻ 35 mM, Na⁺ 140 mM, K⁺ 1.5–2 mM, Ca²⁺ 1.5 mM. The patients received continuous infusion of heparin (10) or bolus of low molecular weight heparin (3). Blood pressure (BP) was monitored via a sphygmomanometer (Nippo Collin Electronics-Press Mate-BP 8800C, Japan), checked every 60 min during each dialysis session. Seven HD patients were hypertensive and received medical antihypertensive therapy: β-blockers (4), calcium inhibitor (2), ACE inhibitors (1), diuretics (1). All the patients received oral vitamin D and five patients folic acid supplementation. Two patients were smokers. Seven patients received subcutaneous erythropoietin three times a week and two received i.v. iron. Plasma samples for the study were collected from the arterial line of the arteriovenous fistula (11) or central venous catheter (2) just before and at the end of the hemodialysis procedure.

Diabetics, patients with dehydration, ischemic or infectious complications, and patients treated with vitamins C or E were excluded. Arterial blood samples (7 ml) were collected in standard tubes with lithium heparinate at 7 a.m. in fasting patients, and after the dialysis session for HD patients. The samples were immediately centrifuged, the plasma was removed, aliquoted and stored at −80°C until assayed.

2.2. Electron spin resonance spectroscopy measurements

ESR spectra were recorded on a Bruker (Wissembourg, France) ESP 300E-X band spectrometer. The plasma samples for ascorbyl free radical (AFR) measurements were introduced into an aqueous ESR quartz flat cell, and ESR spectra were recorded at room temperature using a TM₁₁₀ cavity. Spectrometer settings for the detection of AFR have been described elsewhere [9]. Relative radical concentrations (arbitrary units, AU) were determined by the measurement of line intensities on spectra recorded
with identical spectrometer settings. AFR was expressed as AU, and as AU relative to the vitamin C concentration in the plasma (expressed as mg/l).

2.3. Metabolic measurements

Protein plasma levels (g/l) were evaluated with the spectrophotometric method according to Lowry et al. [10] using the Folin reagent. Correction for hemoconcentration after hemodialysis session was calculated with the following equation, where $C_{\text{pre-HD}}$ is the protein concentration before HD and $C_{\text{post-HD}}$ is the protein concentration after HD:

Corrected value = measured value × ($C_{\text{pre-HD}}/C_{\text{post-HD}}$)

Total plasma antioxidant status (PAS) was determined using the method described by Cao et al. [11]. This technique is based on the ability of plasma components to protect an indicator protein, allophycocyanine (APC, 37.5 nM), whose fluorescence is altered when it is oxidized. The oxidative stress is induced in vitro by a peroxyl radical generator, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH 3 mM). The results are expressed by measuring the net protection provided by 1 μM Trolox. The APC fluorescence was measured at 37°C at 652 nm after excitation at 598 nm, every minute until the end of the oxidation reaction. The results were expressed in ORAC (oxygen radical absorbing capacity) units, where 1 ORAC unit represents the net protection provided by 1 μM Trolox.

Total plasma vitamin C concentration (mg/l) was measured spectrophotometrically with the method described by Roe and Kuether [12]. Plasma vitamin E (α-tocopherol) concentrations were quantified by HPLC assay, according to the method of Burton et al. [13]. Vitamin E concentrations were expressed as mg/ml and as mg per mmol of total cholesterol. Total plasma cholesterol concentration was determined by an enzymatic method (Sigma Diagnostics, France).

Thiol group (μM) measurements in plasma were assayed according to Tietze’s method [14] using the Ellman reagent. Uric acid levels (mg/l) were determined by the Sigma enzymatic procedure (Sigma Diagnostics) using uricase and peroxidase. Nitrites and nitrates (NO$_2$) plasma concentrations (μM) were measured via a procedure previously described by Lecour et al. [15], based on the Greiss reaction [16].

2.4. Statistical analysis

All data were presented as mean±SEM. The data were compared using a one-way analysis of variance. If appropriate, it was followed by a Tukey test. Correlations were evaluated with the Pearson test. A P value <0.05 was considered significant.

3. Results

The clinical characteristics of the CTL, CRF and HD patients are shown in Table 1. The three groups were age and sex matched. Blood pressure was higher in CRF patients than in HD patients (systolic blood pressure: 156.6 ±7.5 vs. 128.9±4.4 mmHg, P<0.05). CRF patients were anemic with a hemoglobin level lower than in HD patients (9.0±0.5 g/dl vs. 10.5±0.5 g/dl, P<0.05). Creatininemia was lower in CRF patients compared with HD patients before the dialysis session (64.2 mg/l±9.9 vs. 98.3±6.6 mg/l, P<0.05).

No adverse clinical event occurred during the dialysis session. BP declined slowly and linearly as a function of time, and without significant variations during HD procedure. No significant difference concerning weight, blood pressure and frequency was found between the values before and after the dialysis session. Proteins were significantly increased after the dialysis session (67.3±2.6 g/l pre-HD vs. 78.9±3.5 g/l post-HD, P<0.05). Creatininemia decreased after the HD session (98.3±6.6 mg/l pre-HD vs. 36.0±3.0 mg/l post-HD, P<0.05).

Table 2 presents the serum oxidative stress parameter levels in healthy controls (CTL), patients with CRF and HD patients before (pre-HD) and after a HD session (post-HD). In the CRF patients, the vitamin C level was lower compared with the CTL (13.9±2.5 vs. 7.9±1.2 mg/l, P<0.05). The CRF patients had higher AFR/vitamin C ratio than the CTL, but the modification was not statistically significant (0.59±0.11 vs. 0.82±0.12 AU/mg/l, NS). Total thiol plasma levels were reduced in CRF (588±28 vs. 442±22 mM, P<0.05). The levels of vitamin E adjusted to cholesterol did not present any variation with CRF (4.66±0.27 vs. 5.46±0.24 μmol/mmol, NS). PAS,

Table 1

| Clinical characteristics of chronic renal failure (CRF) and hemodialysis patients before (pre-HD) and after (post-HD) the dialysis session |
|-------------------|------------------|------------------|
|                   | CRF              | HD               |
| Age (years)       | 59±10            | 61±11            |
| Sex               | 10M/6F           | 6M/7F            |
| Weight (kg)       | 66.0±5.4         | 71.7±5.0         |
| Blood pressure (mmHg) | 156.6±7.5         | 128.9±4.4        |
| Systolic          | 84.8±5.8         | 72.1±3.2         |
| Diastolic         | 84.2±9.1         | 76.1±3.8         |
| Frequency (beats/min) | 64.2±9.9         | 98.3±6.6         |
| Creatininemia (mg/l) | 9.0±0.5          | 10.5±0.5         |
| Proteins (g/l)    | 71.8±3.2         | 67.3±2.6         |

P<0.05 vs. CTL.

P<0.05 pre-HD vs. post-HD.
as well as uric acid levels, were higher in CRF patients than in CTL (1.87±0.01 vs. 2.20±0.02 ORAC, P<0.05 and 30.2±3.4 vs. 71.1±4.7 mg/l, P<0.05, respectively). As for NOx, their levels increased with CRF (54.5±3.1 vs. 115.4±15.8 µM, P<0.05).

No significant modification of the studied parameters was found between CRF and chronic HD patients before treatment (pre-HD), except for uric acid level which was lower in the HD group compared with the CRF patients (71.1±4.7 vs. 55.5±3.2 mg/l, P<0.05).

The hemodialysis procedure induced a decrease in the plasma vitamin C level (8.4±1.0 before vs. 4.4±0.5 mg/l after dialysis, P<0.05), associated with an increased AFR/vitamin C ratio (0.62±0.10 before vs. 0.95±0.06 AU/mg/l after dialysis, P<0.05). The vitamin E concentration was not modified by hemodialysis. PAS declined during the hemodialysis procedure (2.20±0.01 before vs. 1.99±0.02 ORAC after dialysis, P<0.05). Similar results were obtained for uric acid (55.5±3.2 before vs. 14.5±1.6 mg/l after treatment, P<0.05). In the post-HD samples, thiol concentration values were higher compared with pre-HD values (481±13 vs. 651±24 mM, P<0.005), but the results became non significant when corrected for hemoconcentration (481±13 pre-HD vs. 556±22 µM post-HD, NS). Hemoconcentration adjustment relative to the plasma protein level after hemodialysis did not change the interpretation of the other results and the values are not presented. The vitamin E concentrations adjusted to cholesterol did not show any variation during HD. NOx levels however, showed a significant decrease after hemodialysis (127.3±16.6 before vs. 61.0±5.7 µM after treatment, P<0.05).

Finally, uric acid and antioxidant levels were globally correlated (Fig. 1) in CTL, CRF, pre-HD and post-HD patients (r=0.4635, P<0.05).

4. Discussion

Our data indicate that chronic renal failure was characterized by a prooxidant state in plasma, demonstrated both by a depletion of antioxidants and by the presence of the products of free radical actions. Our results are in accordance with previous studies [17–19]. We found significant changes in the plasma antioxidant capacity of patients with chronic renal failure. In our study, the AFR/vitamin C ratio showed an increase in CRF patients that did not reach significance. AFR detected by ESR is derived directly from the oxidation of vitamin C. In normal conditions the levels balance each other out, whereas when an oxidative stress occurs, the equilibrium is shifted to the radical form. Therefore the AFR/vitamin C ratio can be considered as a reliable noninvasive marker of free radical oxidation [9]. We demonstrated a simultaneous decrease of plasma vitamin C levels, consistent with the consumption of this major-soluble antioxidant, and in accordance with previous reports [20]. As previously reported elsewhere [21,22], total thiol levels were decreased in CRF patients compared to CTL.

Our observations demonstrate that despite the use of
polyacrylonitrile high biocompatible membranes, the dialysis procedure was associated with an oxidative stress. Some reports have demonstrated that the hemodialysis procedure was responsible for inflammation and neutrophil activation [4,5], the latter being associated with free radical production [3,23]. Our study showed that oxidative stress parameters were affected by the dialysis session, with a significant increase of the AFR/vitamin C ratio, and a decrease of vitamin C. These observations are in accordance with a recent report, which considered the evolution of other metabolites derived from ascorbic acid oxidation during hemodialysis [24]. The decrease of plasma concentrations of vitamin C observed after the dialysis session could be attributed to the consumption, or more likely to the filtration of this low-molecular-weight compound through the membrane. On the other hand, the lipophilic vitamin E, which is not filtered by the membrane, did not seem to play a role in the regulation of redox state of the extracellular space during CRF and HD. Nevertheless, total thiol levels were increased with the dialysis session. This phenomenon is probably partly due to hemoconcentration as suggested by the results adjusted to the protein levels. The rise in total thiol levels after dialysis is in accordance with the increased plasma thiol glutathione demonstrated by Epperlein et al. [18]. The exact mechanism underlying blood thiol levels in dialysis patients remains to be determined.

PAS was shown to be high in CRF or pre-HD patients and decreased after the dialysis procedure. Identical results were reported in a study using a chemiluminescence assay in CRF patients [25]. In contrast, previous investigations on PAS have shown conflicting results. These discrepancies may be related to differences in assay methodology (detection technique and free radical generating system employed) [26], or to the use of a non-specific method such as the evaluation of TBARS levels [27]. Our results showed a parallel progressive trend in both uric acid and PAS levels. The importance of uric acid is still debated as an effective antioxidant in vivo. Studies have shown a beneficial antioxidant effect of this molecule on organs such as the myocardium [28]. On the other hand, there is still a tendency to regard uric acid as a risk factor with respect to coronary heart disease and atherosclerosis. Our results concerning PAS in uremic and HD patients must therefore be interpreted with caution.

The function of NO in CRF and HD has been the subject of controversial reports; NO could be implicated in hypotension, bleeding and tissue damage associated with uremia and hemodialysis [6]. In our study we showed that stable-end NO metabolite plasma levels (nitrites and nitrates) were increased during CRF and maintenance hemodialysis. Post-dialysis concentrations of NOX were significantly lower than pre-dialysis concentrations, probably due to its elimination during the dialysis session prevailing production, and returned to high levels between the sessions. These results are consistent with observations from other studies [6,29] suggesting that uremia and long-term hemodialysis are associated with chronic stimulation of NO production. The role of NO in hemodialysis hypotension is still debated. Some reports demonstrated a correlation between NO level, pre-hemodialysis blood pressure of patients and dialysis hypotensive episodes during the dialysis session [29,30]. NO production could occur early during the hemodialysis procedure due to shear stress, heparin or blood–membrane interactions [7,8]. In addition, leukocyte activation by the dialysis membrane results in the release of high levels of cytokines, which can increase the NO release via the expression of inducible NO synthase. Important concentrations of NO can induce vasodilation, but could also be involved in tissue damage. Actually, the reaction between NO and superoxide, released for example from activated leucocytes, yields peroxynitrite [31] which is a potent oxidant that can attack a wide variety of biological tissues, and could play a role in the pathogenesis of diseases such as atherosclerosis [32].

Our study leads to the conclusion that profound disturbances in extracellular redox systems occur in the course of chronic renal failure, with or without hemodialysis. Although no differences have been found between CRF and pre-HD patients, we have demonstrated that oxidative stress was exacerbated in the setting of the hemodialysis procedure, even when conducted with a high biocompatible membrane. It would therefore appear reasonable to imagine that repetitive free radical aggressions during maintenance hemodialysis could be capable of causing peroxidation of the membrane and initiate endothelial injury, responsible for long-term cardiovascular complications in these patients. The use of antioxidant therapies in CRF and HD patients may be particularly relevant in limiting these disorders.

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References


