Oxidant/Antioxidant Status of Erythrocytes from Patients with Chronic Renal Failure: Effects of Hemodialysis

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Abstract
Objective: It has been suggested that oxidative processes may be increased in patients with chronic renal failure (CRF), and that this is a possible factor contributing to the development of anemia and atherosclerosis, characteristic complications of CRF. The aim of this study was to investigate erythrocyte oxidant/antioxidant status in patients with CRF and to elucidate possible effects of hemodialysis on erythrocyte antioxidant system. Methods: Fasting blood samples were obtained from 33 patients with CRF and from 12 healthy controls. Of the patients, 17 subjects were under regular hemodialysis. Values of the activities of antioxidant enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and antioxidant potential, non-enzymatic superoxide radical scavenger activity (NSSA) and levels of thiobarbituric acid reagent substances (TBARS) were measured in the erythrocytes from both patients and controls. Results: Antioxidant potential and NSSA values were found to be significantly decreased, while TBARS levels were increased in the erythrocytes of patients. SOD activity was found to be unchanged, but GSH-Px and CAT activities were significantly lower in the patient group. Moreover, the erythrocyte TBARS level in the hemodialysis group was higher than in the controls and nonhemodialysis patients. Conclusion: The results suggest that antioxidant potential is reduced due to impaired antioxidant system in erythrocytes from patients with CRF and that oxidant stress causes significant peroxidation. Hemodialysis is determined to further increase oxidative reactions. These changes seem to contribute to the occurrence of some complications of CRF. Therefore, it has been suggested that antioxidant supplementation may give beneficial results for these patients.

Introduction

It has long been known that anemia [1] and cardiovascular diseases [2] are characteristic complications of chronic renal failure (CRF) and there is accumulating evidence indicating that oxidative reactions may play a part in these events [3, 4].

Recent data suggest that oxidative processes may be increased in patients with CRF [3–5]. It has been reported that the enzymatic antioxidant system is impaired in erythrocytes from patients with CRF [6, 7] and that the resulting oxidant load may play a role in the development of some complications of CRF [8, 9]. It has been reported that oxidative damage due to excessive free radical production is increased in uremic patients, and that this is a
possible factor contributing to the development of anemia and atherosclerosis [10]. Other studies have revealed increased protein oxidation in the erythrocyte membranes from chronic hemodialysis patients [11].

The study presented here aims to investigate the erythrocyte antioxidant system and the oxidant/antioxidant status of patients with CRF and to elucidate possible molecular mechanisms leading to CRF-related complications, such as anemia and atherosclerosis.

Materials and Methods

Thirty-three patients with CRF and 12 healthy volunteers participated in the study. Seventeen of the patients were under regular hemodialysis for 1–4 years (mean ± SD: 2.6 ± 1.2). The ages of the patients ranged from 38 to 70 years (mean ± SD: 51.4 ± 8.5) and those of the controls from 35 to 56 years (mean ± SD: 47.8 ± 5.6). The duration of complaints resulting from renal failure ranged from 1 to 10 years (mean ± SD: 4.6 ± 2.7). Diagnosis of CRF was made by clinical, laboratory and radiological examinations.

Fasting blood samples were put in anticoagulated tubes and erythrocytes were prepared as described previously [12]. In the erythrocyte hemolysate, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured [13–15]. The SOD activity method is based on the measurement of absorbance increase at 560 nm due to reduction of nitroblue tetrazolium (NBT) to NBTH2. One unit of SOD activity is defined as the amount of enzyme protein causing a 50% inhibition in the NBT salt reduction rate. The GSH-Px activity method is based on the measurement of absorbance decrease at 340 nm due to consumption of NADPH, and that of CAT is based on the measurement of absorbance decrease due to H2O2 consumption at 240 nm. The GSH-Px and CAT activities were given in international units per milliliter erythrocyte sediment (IU/ml).

The antioxidant potential (AOP) assay was performed using the method of Durak et al. [16] which is mainly based on the determination of thiobarbituric acid reagent substances (TBARS) levels before and after exposure to superoxide radicals produced by the xanthine/xanthine oxidase system. In the reaction medium enriched with fish oil, samples (supernatant obtained after centrifugation) were exposed to superoxide radicals (O2·) produced by the xanthine/xanthine oxidase system for 1 h. Fish oil was used because it is a polyunsaturated oil, and polyunsaturated fatty acids are very sensitive to free radical attacks. It is known that if there is an inability of the cell to eliminate free radicals, unsaturated fatty acids will be easily attacked and cause lipid peroxidation followed by an increase in TBRAS levels. Therefore, AOP levels of the reaction medium (nmol/g tissue) were measured before (blank) and after (sample) O2·radical attack. The difference between both values was inversely proportional to the AOP of the erythrocyte sediment (ml erythrocyte sediment-h/nmol).

The nonenzymatic superoxide radical scavenger activity (NSSA) assay was made as described previously [17], and the TBARS assay was carried out using the thiobarbituric acid method [18]. In the NSSA assay, proteins including SOD are first precipitated using tri-chloroacetic acid solution 20% (w/v), and then NSSA assay is performed in the upper clear solution without protein as is SOD activity measurement. Using this method, total nonenzymatic (non-SOD) superoxide radical scavenger activity was to be established. One unit of NSSA was defined as the antioxidant activity causing a 50% inhibition in the NBT salt reduction rate and expressed in the erythrocyte sediment (U/ml). As to the TBARS analysis, although the TBARS analysis has some disadvantages such as indicating reactions with some peroxidation products other than those of lipids, the intensity of color of the red pigment formed in the TBARS reaction is so great that the method offers great sensitivity in detection and measurement of lipid auto-oxidation. TBARS values were given in nanomoles per milliliter erythrocyte sediment. Blood urea and creatinine levels (mg/dl) were measured in a routine biochemistry laboratory.

The Student’s t test and the Mann-Whitney U test were used for statistical analyses.

Results

Results are given in table 1. As seen from the table, erythrocyte SOD activity remained unchanged, while GSH-Px and CAT activities were significantly lower in the patient groups. Consequently, AOP and NSSA values were decreased, but TBARS levels were significantly increased in the patient groups. In the hemodialysis group, the TBARS levels and CAT activities were found to be higher compared with nonhemodialysis patient group. With regard to other parameters, there were no meaningful differences between the patient groups.

Discussion

The results of the present study demonstrate that the erythrocyte antioxidant system is impaired and antioxidant potential is reduced in the erythrocytes derived from patients with CRF, the results of which are oxidant stress and accelerated peroxidation reactions. As a result, total (reduced AOP), nonenzymatic (reduced NSSA) and enzymatic (decreased enzyme activities) antioxidant systems seem to be impaired in the erythrocytes from patients. In addition, the study shows that hemodialysis induces peroxidation reactions.

It seems that the reduced antioxidant potential due to impaired antioxidant system leads to oxidant stress and peroxidation reactions in the erythrocytes of patients. Impaired antioxidant system may arise from several factors including uremia [10], lowered concentrations of some antioxidant substances like vitamins E, C and glutathione etc. [19–21] and inhibition of some free radical enzymes [6, 20, 22–25]. Reduced GSH-Px and CAT activities, on the other hand, may result from reduced...
Table 1. Mean ± SD values of SOD, GSH-Px, CAT activities, AOP, NSSA and TBARS levels in the erythrocytes from patients and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD U/ml</th>
<th>GSH-Px IU/ml</th>
<th>CAT IU/ml</th>
<th>AOP ml-h/nmol</th>
<th>NSSA U/ml</th>
<th>TBARS nmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 12)</td>
<td>172.2 ± 13.1</td>
<td>7.18 ± 0.97</td>
<td>46.055 ± 6.440</td>
<td>2.71 ± 0.35</td>
<td>23.9 ± 4.3</td>
<td>328.9 ± 30.4</td>
</tr>
<tr>
<td>II (n = 16)</td>
<td>180.0 ± 23.0</td>
<td>6.65 ± 0.68</td>
<td>38.987 ± 5.861</td>
<td>2.39 ± 0.32</td>
<td>18.8 ± 4.5</td>
<td>367.9 ± 29.9</td>
</tr>
<tr>
<td>III (n = 17)</td>
<td>166.3 ± 24.1</td>
<td>6.17 ± 0.69</td>
<td>41.449 ± 5.440</td>
<td>2.36 ± 0.23</td>
<td>16.9 ± 3.4</td>
<td>412.8 ± 22.2</td>
</tr>
</tbody>
</table>

Mann-Whitney U test

<table>
<thead>
<tr>
<th></th>
<th>I–II</th>
<th>I–III</th>
<th>II–III</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.0005</td>
<td>p &lt; 0.005</td>
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<tr>
<td>NS</td>
<td>p &lt; 0.005</td>
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<tr>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.025</td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
</table>

I = Control group; II = nondialysed CRF group; III = dialysed CRF group; NS = nonsignificant (p > 0.05).

The units are as defined in the 'Materials and Methods' section.

synthesis or inhibition of the enzymes by some inhibitor substances accumulated in the blood of patients with CRF [26]. Deficiency of some trace elements including selenium (Se) might be one of the other factors responsible for the reduced enzyme activities in patients with CRF [22, 26, 27].

Further potential sources of oxidant stress in dialysis patients are, in particular, activation of leukocytes [28] and iron overload [29]. It has been established that hemodialysis itself accelerates lipid peroxidation in blood [30]. It has also been reported that hemodialysis causes a broad pattern of tissue injury in patients on regular hemodialysis [27]. Ross et al. [19] have argued that hemodialysis patients are at increased risk from oxidative stress due to glutathione deficiency in whole blood and erythrocytes. Jackson et al. [9] reported that depletion of some antioxidants leads to accelerated atherogenesis in hemodialysis patients. In general, our results are in accord with these evaluations. Shortened red blood cell survival [31] and changed erythrocyte membrane fluidity [32] may be the results of oxidant stress due to an impaired antioxidant system in the erythrocytes from patients with CRF. These changes may contribute to the occurrence of some complications of CRF like anemia and atherosclerosis etc. [6, 23–25, 32–34].

The findings that when erythrocytes from patients with CRF were given to normal people, the erythrocytes had normal survival times and when erythrocytes from normal people were given to the patients with CRF, they had shortened survival times [35, 36] suggest that primary factors responsible for the shortened erythrocyte survival were present in the circulation. Inhibitor substances accumulating in uremia [31], increased serum neuraminidase activity [37], changed erythrocyte membrane fluidity [38], impaired Na-K pump system in the erythrocytes [39] and increased purine/pyrimidine content in uremic hemolysates [40] have all been suggested to contribute to the hemolysis in patients with CRF. In addition to reduced antioxidant potential, artifacts occurring during dialysis, several uremic metabolites [31], oxidative stress due to hexose monophosphate shunt inhibition [23, 24] and reduced ATP/ADP ratio due to oxidative metabolism [40] may all contribute to the increased toxic-free radical production in the erythrocytes. Similarly, the metabolites of some drugs and urea [31, 41], exposure to some toxic trace elements like aluminium, silicon etc. during dialysis [34] may also be additional factors leading to oxidant stress and peroxidation reactions in the hemodialysis patients.

Apart from the observation that enzymatic (decreased enzyme activities) antioxidant systems are impaired in the erythrocytes of patients, this study also shows that nonenzymatic (reduced NSSA) antioxidant defense and AOP of the erythrocytes are reduced, which is a new finding in patients with CRF.

Conclusion

Our results demonstrate that oxidant/antioxidant equilibrium is changed in the erythrocytes in patients with CRF, and that erythrocytes and possibly some other tissue cells are exposed to great oxidant stress, the results of which are accelerated peroxidation reactions and cellular aberrations. It is therefore suggested that antioxidant supplementation may help CRF patients to cope with oxidant stress and prevent peroxidation reactions leading to cellular dysfunction.

Oxidant Stress in Hemodialysis Patients
References