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Levels of Malondialdehyde and Antioxidant Enzymes in Plasma from Patients with End Stage Renal Disease

Mi Young Yeo, M.D. and Kyo Cheol Mun, M.D.

Department of Biochemistry,
Keimyung University School of Medicine and Institute for Medical Science,
Taegu, Korea

=Abstract=

The patients with end stage renal disease show several complications due to oxygen free radicals. There might be some alterations in damages related oxygen free radicals and antioxidant systems in patients with end stage renal disease. Malondialdehyde and three well-known antioxidant enzyme levels in plasma were measured before and after hemodialysis. Before dialysis, malondialdehyde level in the plasma from the patients with end stage renal disease was higher than those from healthy controls. Superoxide dismutase and glutathione peroxidase activities in patients were lower than those from healthy controls. Plasma catalase activity was similar with normal control value. After hemodialysis, malondialdehyde level was still higher. Superoxide dismutase and glutathione peroxidase activities were returned to normal level and catalase activity was increased after hemodialysis. Our results suggest that there is an overproduction of superoxide anion with an impairment in disposition of hydrogen peroxide in patients with end stage renal disease. These alterations may contribute the patients with end stage renal disease susceptible to oxidant damages. Although single hemodiaysis is not helpful for protection of the damage induced by oxygen free radicals, it is helpful for normalizing the activities of antioxidant enzymes, especially superoxide dismutase and glutathione peroxidase.

Key Words: Catalase, End stage renal disease, Glutathione peroxidase, Malondialdehyde, Superoxide dismutase

Introduction

Oxygen free radicals can be defined as oxygen molecules or molecular fragments that have an unpaired electron (Moslen, 1994; Punchard & Kelly, 1996). They are formed in all living organisms during physiological and pathophysiological metabolism, and cause cell and tissue damages due to their high chemical reactivity (Moslen, 1994; Punchard & Kelly, 1996). They can react with macromolecules including lipid, protein and DNA (Yagi, 1994). Peroxidation of lipids exposed to oxygen free radicals is

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responsible for damage to cells and tissues in vivo, where it may cause of cancer (Ames & Shigenaga, 1993; O'Brien, 1994), inflammatory disease (Leff, 1994), atherosclerosis (Reaven, 1994), heart disease (Ferrari, 1994), liver disease (Mun *et al.*, 1996) and kidney disease (Waz & Feld, 1994).

As in other organs, the kidney produces oxygen free radicals as a consequence of normal cellular metabolism, and kidney is a site of significant aerobic metabolism (Waz & Feld, 1994). In its role of maintaining fluid and electrolyte homeostasis, the kidney accounts for 10% of whole body oxygen consumption while making up less than 1% of total body mass (Waz & Feld, 1994). Glomerular cells, renal tubular epithelial cells from proximal, distal, and collecting segments, as well as interstitial, cells produce oxygen free radicals (Waz & Feld, 1994).

The patients with end stage renal disease show several complications such as artherosclerosis, anemia, increased susceptibility to infection by damage due to oxygen free radicals (Shurtz-Swirski, 1995; Fiorillo *et al.*, 1998; Mun *et al.*, 1998).

There might be some alterations in damages related oxygen free radicals and antioxidant systems in patients with end stage renal disease. In this paper, we measured the levels of malondialdehyde and three well-known antioxidant enzymes.

Materials and Methods

Patients

Twenty patients with end stage renal disease, mean age 43±14 years, were studied. The duration of hemodialysis was 34±12 years. No patient was taking vitamin C, vitamin E or transfusion within the last month. They were hemodialyzed using polymethyl methacrylate dialyzers (FILTRYZER, Toray, Tokyo, Japan). Blood flow was approximately 250 mL/min. The control group consisted of 14 healthy blood donors. Blood samples were taken from the arterial blood line before the start of dialysis and after hemodialysis. Plasma was separated by centrifugation.

Chemicals

Tris(hydroxymethyl) aminomethane, reduced glutathione, glutathione reductase, dimethylsulfoxide, NADPH, thiobarbituric acid, cytochrome c, catalase, glutathione peroxidase, superoxide dismutase, and bovine albumin were purchased from Sigma (U.S.A.). All other chemicals were of the highest commercially available purity.

Thiobarbituric acid assay for malondialdehyde

The amount of malondialdehyde was measured by the thiobarbituric acid assay which is based on malondialdehyde reacts with thiobarbituric acid to give a red species absorbing at 535nm (Buege & Aust, 1978). In brief, the sample was mixed with a thiobarbituric acid reagent consisting of 0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 N hydrochloric acid. A standard (1,1,3,3- tetramethoxypropane) was run simultaneously. The reaction mixtures were placed in a boiling water bath for 15 minutes and centrifuged at 3000g for 5 minutes, after which the absorbance of the supernatant was read at 535 nm. Absorbance versus quantity of the standard was plotted on a graph, and the quan-

tity of the unknowns were read from this graph according to their absorbency.

Enzyme assays

Superoxide dismutase was assayed using alkaline dimethylsulfoxide as a superoxide anion-generating system in association with cytochrome c as a superoxide anion-indicating scavenger by the method of Hyland *et al.* (1983). 200 $\mu\ell$ of serum was added to 1 ml of 0.20 M potassium phosphate buffer (pH 8.6) containing 10-4 M EDTA and 2×10-5 M cytochrome c. Tubes were kept in an ice bath for 20 min. Then, 0.5 ml alkaline dimethylsulfoxide which contains 1% water and 5 mM NaOH, was added with stirring. Absorbance of reduced cytochrome c was determined at 550 nm against samples prepared under same conditions except that dimethylsulfoxide did not contain NaOH. One unit of its activity was defined as the amount which inhibited the reduction of cytochrome c by 50%.

Catalase activity was assayed using hydrogen peroxide as a substrate by the method of Nelson and Kiesow (1972). At 25℃, the decrease in absorbance was measured at 240 nm for 1 min after adding 20 $\mu\ell$ of serum into the 3.0 ml of 50 mM phoshate buffer, pH 7.0, containing 5 mM H2O2 and 0.1 mM EDTA. One unit of catalase was defined as equivalent to the elimination of 1 μM of H2O2 per min under the above condition.

Glutathione peroxidase activity was assayed by the method of St Clair and Chow (1996) in which the oxidation of glutathione is coupled to the glutathione reductase, thus promoting consumption of NADPH. 100$\mu\ell$ of serum was added to 875$\mu\ell$ of the reagent solution which contains 2mM EDTA, 1mM sodium azide, 1mM reduced glutathione, 0.2mM NADPH and 100 units of glutathione reductase in 50mM Tris-HCl buffer, pH 7.6. Twenty five $\mu\ell$ of hydrogen peroxide was added to start the reaction, and the final concentration of hydrogen peroxide was 0.25mM. The amount of NADPH oxidized was recorded spectrophotometically at 340nm.

Statistics

Values were expressed as mean ± S.E. Statistical evaluation of the difference between hemodialysis group and control group was performed with t-test, and of the difference between prehemodialysis group and posthemodialysis group was performed with paired t-test.

Results

Before dialysis, malondialdehyde level in the plasma from the patients with end stage renal disease (1.88 ± 0.22 nmol/ml) was higher than that from healthy controls (0.36 ± 0.10, $P<0.001$, Figure 1). Superoxide dismutase activity in the plasma from the patients with end stage renal disease (1.45 ± 0.32 unit/ml/min) was significantly lower than that from healthy controls (2.67 ± 0.46, $P<0.05$, Figure 2). Catalase activity in the plasma from the patients with end stage renal disease (4.85 ± 1.55 μmol/ml/min) was similar with normal control value (5.96 ± 0.59, Figure 3). Glutathione peroxidase activity in the plasma from the patients with end stage renal disease (36.30 ± 4.41 nmol/ml/min) was lower without significance than that from healthy con-

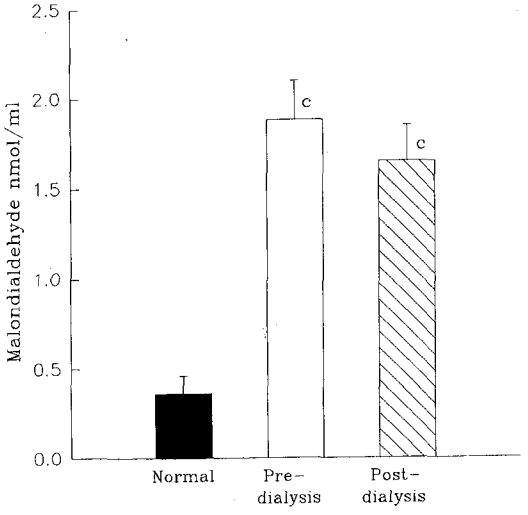

Figure 1. Malondialdehyde level in plasma with end stage renal disease. Significantly differnet from normal (c: P<0.001)

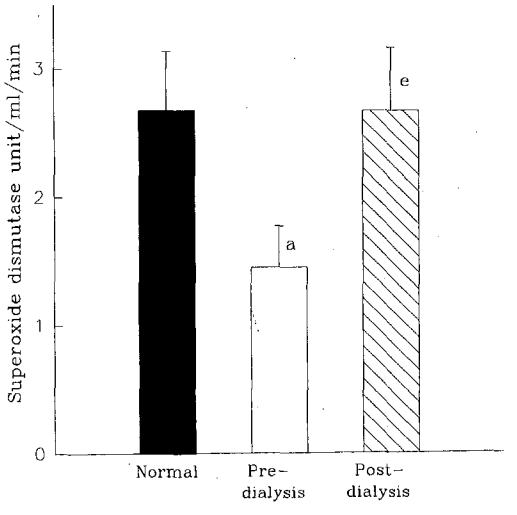

Figure 2. Superoxide dismutase activity in plasma with end stage renal disease. Significantly differnet form normal (a: P<0.05), and from predialysis (e: P<0.01)

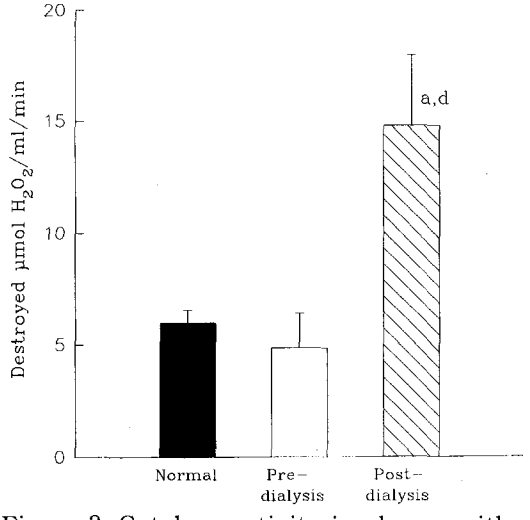

Figure 3. Catalase activity in plasma with end stage renal disease. Significantly different from normal (a: P<0.05), and from predialysis (d: P<0.05).

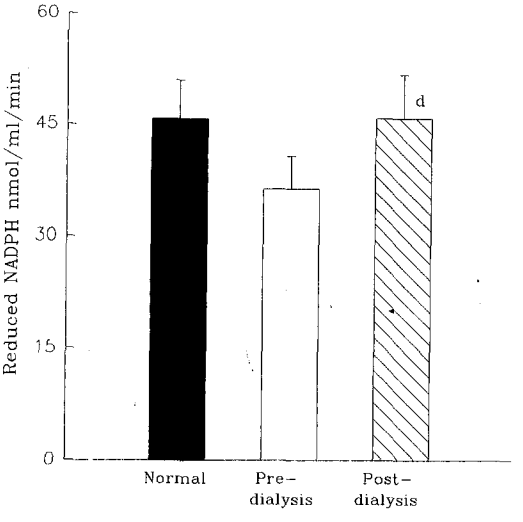

Figure 4. Glutahione peroxidase activity in plasma with end stage renal disese. Significantly different from predialysis (d: P<0.05).

trols (45.70 ± 5.21, Figure 4).

After hemodialysis, malondialdehyde level in the plasma from the patients with end stage renal disease (1.65 ± 0.20) was still higher than that from healthy controls (0.36 ± 0.10, P<0.001, Figure 1). Superoxide dismutase and glutathione peroxidase activities in the plasma from the patients

with end stage renal disease were normalized after hemodialysis (1.45 ± 0.32, P <0.01, Figure 2 and 45.70 ± 5.21, P<0.05, Figure 4). Catalase activity was increased after hemodialysis (14.76 ± 3.18, P<0.05, Figure 4).

Discussion

Identification and quantification of malondialdehyde gives an indirect index of oxidative injury which results in lipid peroxidation (Brown & Kelly, 1996). Malondialdehyde is the most abundant aldehyde arising from lipid peroxidation and its determination, by measurement of the coloured product formed upon reaction with thiobarbituric acid, is one of the most common assays used in lipid peroxidation studies (Buege & Aust, 1978; Brown & Kelly, 1996). Before dialysis, malondialdehyde level in the plasma from the patients with end stage renal disease was higher than that from healthy controls. This result indicates that damages by reactive oxygen species may occur in patients with end stage renal disease.

Oxygen free radicals are produced as a normal consequence of aerobic respiration, as a response to immunologic stimulation, and as a by-product of many oxidation reduction reactions in living organisms (Moslen, 1994; Punchard & Kelly, 1996). As in other organs, the kidney produces oxygen free radicals as a consequence of normal cellular metabolism (Waz & Feld, 1994). Electron transport, auto-oxidation of molecules such as thiols and catecholamines, the actions of enzymes such as xanthine oxidase and amino acid oxidases, and arachidonic acid metabolism all generate reactive oxygen molecules (Waz & Feld, 1994). However, the production of oxygen free radicals is, under most circumstances, balanced by intrinsic antioxidant defenses (Halliwell & Gutteridge, 1989; Murray, 1996; Babior, *et al.*, 1997). Any superoxide that enters the cytosol of the phagocytic cell is converted to hydrogen peroxide by the action of superoxide dismutase (Halliwell & Gutteridge, 1989; Murray, 1996; Babior, *et al.*, 1997). And hydrogen peroxide is disposed of by the action of catalase and glutathione peroxidase (Halliwell & Gutteridge, 1989; Murray, 1996; Babior, *et al.*, 1997). Like these, several enzymes are directly linked to the fate of the highly reactive oxygen metabolites. If there are some alterations in the activities of antioxidant enzymes, these alterations may contribute to the complications by reactive oxygen species in patients with end stage renal disease.

Before dialysis, superoxide dismutase activity in the plasma was significantly lower than that from healthy controls. Ichikawa *et al.* (1994) suggests that underproduction of superoxide dismutase leads to excess production of superoxide and reduced iron favoring hydroxyl radical formation. Our result suggest that there is an overproduction of superoxide anion in patients with end stage renal disease. The overproduction of superoxide anion may contribute the patients with end stage renal disease susceptible to oxidant damages.

Glutathione peroxidase, converts hydrogen peroxide to water with reduced glutathione as a substrate, was decreased in its activity before dialysis. Our result suggest that

there is an impairment in disposition of hydrogen peroxide. This impairment may also contribute the damages by hydrogen peroxide, and lead to the complications in patients with end stage renal disease.

After hemodialysis, malondialdehyde level in the plasma from the patients with end stage renal disease was still higher than that from healthy controls. Although, catalase activity was increased after hemodialysis. Superoxide dismutase and glutathione peroxidase activities in the plasma from the patients with end stage renal disease were normalized after hemodialysis. Our result indicate that single hemodiaysis is not helpful for protection of the damage induced by oxygen free radicals, but it is helpful for normalizing the activities of antioxidant enzymes, especially superoxide dismutase and glutathione peroxidase. There will be some factors, except antioxidant enzymes, which protects the damages induced by oxygen free radicals in patients with end stage renal disease.

Summary

The alterations in damages related oxygen free radicals and antioxidant systems in patients with end stage renal disease were studied. The patients showed some alterations in the levels of malondialdehyde and three well-known antioxidant enzyme before and after hemodialysis. These results suggest that there is an overproduction of superoxide anion with an impairment in disposition of hydrogen peroxide in patients with end stage renal disease. These alterations may contribute the patients with end stage renal disease susceptible to oxidant damages.References

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