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Communication

Selenium supplementation to chronic kidney disease patients on hemodialysis does not induce the synthesis of plasma glutathione peroxidase

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Background: Numerous authors have shown that selenium (Se) concentration and glutathione peroxidase (GSH-Px) activity in plasma of chronic kidney disease (CKD) patients are lower than in healthy subjects, but there are only few publications on the level of GSH-Px protein in those patients and no reports on the effect of Se supplementation to HD patients on the level of this enzyme. Subjects and Methods: Se concentration and GSH-Px protein level in plasma were measured in a group of 30 CKD patients on hemodialysis (HD) supplemented with 200 μg Se/day for 3 months, and 28 patients on HD administered with placebo. Se concentration was measured by graphite furnace atomic absorption spectrometry and plasma GSH-Px protein level by the sandwich ELISA method using polyclonal antibody specific for human plasma GSH-Px. Results: Se concentration in patients on placebo did not change throughout the 3-month study period, but increased significantly in Se supplemented group. Se supplementation to CKD patients on HD had no effect on the level of GSH-Px protein. Conclusions: The lack of GSH-Px protein in CKD patients on HD is not linked to Se deficiency since the level of this element increased after Se supplementation while enzyme protein level did not change. The damaged kidney of HD patients is unable to synthesize GSH-Px, even after induction with selenium.

Keywords: chronic kidney disease, glutathione peroxidase, hemodialysis, plasma, selenium supplementation

INTRODUCTION

There is increasing evidence of oxidative stress in chronic kidney disease (CKD) patients, particularly those on hemodialysis (HD) (Rico *et al.*, 2006). Selenium (Se) and some other trace elements play an important role in biological systems, being components of enzymes which participate in eliminating reactive oxygen species (ROS) (Zima *et al.*, 1998). Glutathione peroxidases (GSH-Pxs) are involved in ROS detoxification. They catalyze the reduction of hydrogen peroxide and a variety of organic hydroperoxides, using glutathione as the reducing agent. GSH-Pxs are implicated in protecting cell membrane lipids, proteins and DNA against oxidative stress and are widely believed to be major components of the human antioxidant defense (Papp *et al.*, 2007).

To date, five structurally and functionally distinct forms of GSH-Pxs have been identified in mammalian tissues and all are selenium-dependent (Kyriakopoulos & Behne, 2002; Brigelius-Flohe, 2006; Papp *et al.*, 2007). Two forms of GSH-Px have been identified in the blood (Yoshimura *et al.*, 1996): 1) cellular GSH-Px (GSH-Px 1) found in red blood cells, and 2) extracellular GSH-Px (GSH-Px 3) found in plasma. In healthy individuals, kidneys have the highest concentration of plasma GSH-Px mRNA, be-

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Abbreviations: CKD, chronic kidney disease; GSH-Px, glutathione peroxidase; HD, hemodialysis; ROS, reactive oxygen substances.

ing the main source of this enzyme (Chu *et al.*, 1992; Avissar *et al.*, 1994). Some authors (Zachara *et al.*, 2000) have shown that in patients on HD Se supplementation has no effect on plasma GSH-Px activity, while others (Saint-Georges *et al.*, 1989; Richard *et al.*, 1993) found that after Se administration the activity of this enzyme increases.

Analysing the GSH-Px protein concentration in plasma of CKD patients, Yoshimura *et al*. (1996) found only a trace amount of the enzyme, in some cases even below the level of determination. On the other hand, Yamamoto and coworkers (1995) have shown one third of the level found in healthy volunteers, while Roxborough and coworkers (1999) and Donica (2001) observed no differences between patients on HD and healthy controls. So far nobody has studied the effect of Se supplementation on GSH-Px protein level in CKD patients. Therefore, the aim of our study was to measure Se concentration and GSH-Px protein level in plasma of CKD patients on HD, supplemented with Se.

MATERIAL AND METHODS

Patients and controls. A 3-month, randomized double-blind, placebo-controlled trial was carried out. The study groups consisted of: 1) 30 patients (mean age, 61.0±11.6 years) in the end-stage of CKD, treated with regular HD for 24.7±20.1 months and supplemented with 200 μg Se/day (as Se-rich yeast, Pharma Nord, Bioselenium, Denmark) and 2) 28 patients (mean age, 56.0±12.0 years), administered with placebo (bakers yeast, Pharma Nord). The patients were dialyzed 3 times a week for 4 h. 3) The results were compared with 52 healthy volunteers (mean age 51.0±8.7 years).

Methods. Blood samples were drawn from all patients before HD session into vacutainer tubes containing lithium heparin as anticoagulant. After centrifugation (+4°C, 5000 r.p.m., 10 min), the plasma was harvested and stored at –20°C until analysis. Creatinine concentration was determined by routine laboratory method using Jaffy reaction (a kit produced by Cormay, Lublin, Poland). Plasma Se concentration was determined by graphite furnace atomic absorption spectrometry according to the method of Neve and coworkers (1987) using a Unicam 989 QZ Solaar apparatus and the values were expressed as nanogram per millilitre. The accuracy of the method was checked with serum reference material (Nycomed, Oslo, Norway, batch No. 605113). The mean Se level of reference plasma was 78.0 ng/ml, while that obtained in our laboratory was 77.4±5.0 ng/ml. Plasma GSH-Px protein level was measured by the sandwich ELISA method (Calbiochem, Glutathione Peroxidase Elisa Kit, No. 353918; www.emdbiosciences.com/pathways) using polyclonal antibody specific for human plasma GSH-Px. For color reaction, covalently linked streptavidin, alkaline phosphatase (AP) and AP substrate, *p*-nitrophenylphosphate, were used. Plasma samples were diluted 200-fold in sample diluting buffer immediately before analysis. Purified human plasma GSH-Px was used as a standard. Samples with low or high protein values were analyzed 2 or 3 times. The study protocol was approved by the Institute of Occupational Medicine Ethics Commission for Medical Research No. 18/2003. The nature and purpose of the study was explained to the participants and their written consent was obtained.

Statistical analysis. Comparisons of the levels under study at three time points (before the study, one month and three months after study) were made by multivariate analysis of variance (Morrison, 1990). When significant differences were obtained between the groups, the differences were tested at all time points. The tests were based on Shapiro-Wilks' statistics, significance being set at 0.05. All statistics were conducted using the STATA 9 package.

RESULTS AND DISCUSSION

Mean plasma Se concentration in both groups of HD patients (+ Se and placebo taken together) before the study was 41.9±10.6 ng/ml and this value was significantly lower ($P<0.0001$) than in the control group (52.2±11.0 ng/ml). After 1 and 3 months of Se supplementation to HD patients, plasma element concentration increased significantly as compared with the initial value, to 102.4±28.8 and 132±47.5 ng/ml, respectively ($P<0.0001$). In HD placebo group the Se concentration did not change (Fig. 1). Plasma

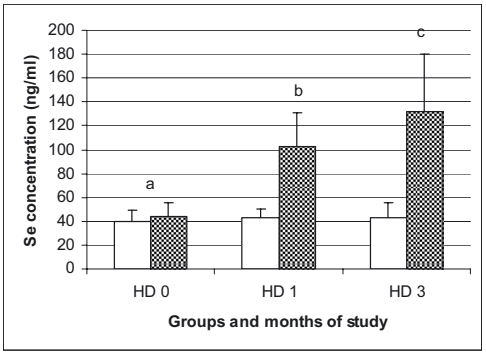

Figure 1. Plasma selenium concentration in healthy subjects and in CKD patients on HD supplemented with selenium and placebo.
CKD patients on hemodialysis at the beginning of the study (HD 0) and after 1 and 3 months (HD 1 and HD 3, respectively) of Se or placebo supplementation. HD patients: white columns = placebo, filled columns = + Se. Statistics: a, $P<0.0001$ *vs* controls; b, $P<0.0001$ *vs* placebo group and initial values (HD 0), and c, $P<0.01$ *vs* HD 1.

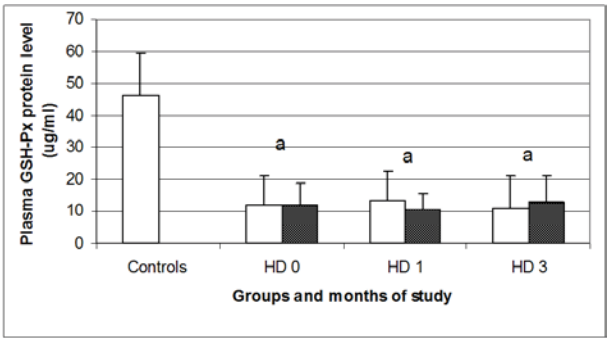

Figure 2. Plasma GSH-Px protein level in healthy subjects and in CKD patients on HD supplemented with selenium and placebo.
CKD patients on hemodialysis at the beginning of the study (HD 0) and after 1 and 3 months (HD 1 and HD 3, respectively) of Se or placebo supplementation. HD patients: white columns = placebo, filled columns = + Se. Statistics: a, $P<0.0001$ *vs* controls.

GSH-Px protein level in both HD groups at the beginning of the study was 11.4±6.7 μg/ml and was 4.2 times lower as compared with healthy controls (48.4±12.3 μg/ml; Fig. 2) ($P<0.0001$). During the 3-month period of the study, in both groups of HD patients (supplemented with Se and administered with placebo), the plasma GSH-Px protein level did not change significantly. Creatinine concentration in HD patients at the beginning of the study was 9.93±2.91 mg/dL and during the 3-month period of the study it did not change significantly in the Se supplemented group, or in the placebo group.

The correlations between some parameters of dialyzed patients at the beginning of the study (HD 0; both groups taken together) were as follows: Se concentration: GSH-Px protein level, r = 0.052; creatinine concentration: GSH-Px protein level, r = –0.110.

In CKD patients, plasma GSH-Px, an enzyme that is predominantly synthesized in the kidney (Chu *et al.*, 1992; Avissar *et al.*, 1994) plays an extremely important role. Some researchers (Ceballos-Picot *et al.*, 1996; Yoshimura *et al.*, 1996; Zachara *et al.*, 2006) have shown that plasma GSH-Px activity in CKD patients decreases along with the progression of the disease. We obtained similar results in our previous studies (Zachara *et al.*, 2000; 2004a; 2004b). So far only a few papers have been published on plasma GSH-Px protein level in CKD patients (Yamamoto *et al.*, 1995; Yoshimura *et al.*, 1996; Roxborough *et al.*, 1999; Donica, 2001; Nishioka *et al.*, 2001) and their results are inconsistent. Although Roxborough and coworkers (1999) found that plasma GSH-Px activity in HD patients was lower by 40% as compared with healthy people ($P<0.001$), they did not observe any difference in plasma GSH-Px protein levels in those patients. The authors have shown that plasma GSH-Px protein level is the same in HD patients as in controls and remains unaltered during HD session. On the other hand, Nishioka *et al*. (2001) have shown that plasma GSH-Px protein level in hemodialyzed patients is 18.7% of that found in healthy volunteers (5.4 *vs* 28.8 μg/ml) while Yamamoto and coworkers (1995) found 32% of that obtained in controls (5.7 *vs* 17.8 μg/ml). The results of our study, in percentage terms, are similar to the data published by Yamamoto and coworkers (1995). The authors believe that a lower level of plasma GSH-Px protein may reflect its decreased synthesis in the damaged kidney tissue. Quite different are the results presented by Yoshimura and coworkers (1996), who showed that in nondialyzed CKD patients with low plasma GSH-Px activity, plasma GSH-Px protein level was strongly reduced or even undetectable. These authors assumed that the reduced plasma GSH-Px protein level was due to an impaired synthesis of the enzyme in the kidney or its enhanced catabolism. Since in their patients plasma Se concentration was within the normal range, they proposed that the low plasma GSH-Px activity was not due to low Se concentration, as some authors think (Richard *et al.*, 1993), but to the decreased concentration of GSH-Px protein in the plasma.

Our data seem to support the conjecture of Yoshimura and coworkers (1996). We believe this may be the case owing to the fact that in our patients, after 3 months of Se supplementation, the element concentration in plasma was 3 times higher than at the beginning of the study, but GSH-Px protein level, which was extremely low at the start of the study, did not undergo any change. This may indicate that in the end-stage of CKD, the damaged renal tubules are unable to synthesize this enzyme, even after supplementation with Se.

The novelty of our study is the lack of effect of Se supplementation on the GSH-Px protein level in plasma of CKD patients on HD. In healthy subjects with low blood Se concentration Se supplementation increases the activity of plasma GSH-Px (Xia *et al.*, 1992). The activity kept increasing in direct proportion to the amount of Se supplied until it reached its optimal value (Saint-Georges *et al.*, 1989).

The estimates of the level of GSH-Px protein in plasma of healthy persons determined with the enzyme-linked immunosorbent assay (ELISA) reported by different authors are highly divergent. Roxborough and coworkers (1999), Yamamoto and coworkers (1995) and quite recently Jacobson and coworkers (2006) obtained almost identical results (15.2, 17.8 and 18.5 μg/ml, respectively), but their values were 2.5 to 3 times lower than ours (48.4 μg/ml). Nishioka and coworkers (2001) found 28.8 μg/ml, while McGill and coworkers (2003) obtained 63 μg/ml in healthy subjects. Surprisingly, a very high level of plasma GSH-Px protein (6.04 mg/ml) was re-

ported in Poland by Donica (2001). Despite the very high value of this enzyme, this author, similarly as Roxborough *et al*. (1999), reported that the level of GSH-Px protein in patients on HD (5.97 mg/ml) did not differ from that found in healthy persons.

Our results on the effect of Se supplementation to HD patients on GSH-Px protein level are the first ones published to date. Along with the kidney, GSH-Px 3 is also synthesized, in small amounts, in the liver, lung, heart, breast, intestine, brain, skeletal muscle and placenta, from which it is secreted into the extracellular fluid (Chu *et al.*, 1992; Avissar *et al.*, 1994). Nevertheless, the plasma GSH-Px level largely depends on renal function (Nishioka *et al.*, 2001). Consequently it comes as no surprise that the protein level of this enzyme is low in CKD patients, and that this becomes increasingly pronounced with the progress of the disease, reaching a maximum in the end-stage of the disease. Our patients showed high creatinine concentrations ranging from 5.4 and 16.6 mg/dL, yet there was no significant relationship between creatinine level and GSH-Px protein level. Nor did we observe any relationship between Se concentration and GSH-Px protein level.

As was mentioned above, Se supplementation in healthy subjects induces GSH-Px synthesis in the tissues. With that in mind we set out to determine the effect of Se supplementation on GSH-Px 3 protein in patients with CKD on HD. A lack of stimulation of GSH-Px 3 synthesis in the kidneys after Se supplementation does not mean that other glutathione peroxidases are not synthesized in other tissues. Se supplementation may thus have a beneficial effect on the body's antioxidative defense system by inducing GSH-Px synthesis in other tissues. Indeed, some authors (Nishioka *et al.*, 2001) suggest that CKD patients should be given selenium already in the early stages of the disease.

In summary, our data show that Se supplementation to CKD patients on HD has no effect on the level of plasma GSH-Px protein.

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