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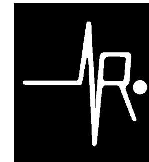

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Original Contribution

Optimization of selenium status by a single intraperitoneal injection of Se in Se-deficient rat: Possible application to burned patient treatment

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Abstract

In order to investigate the efficiency of a single selenium (Se) administration in restoring selenium status, Se and antioxidant enzymes were studied in an animal model of Se depletion. In Se-depleted animals receiving or not a single parenteral administration of Se, plasma, red blood cell (RBC), and tissue Se levels were measured concurrently with glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities. The oxidative stress was assessed by thiobarbituric acid-reactive species (TBARs), total thiol groups, glutathione, and tocopherol measurements. Our study showed that Se depletion with alterations in the antioxidant defense system (Se and GPx activity decreases) led to an increase of lipid peroxidation, a decrease of the plasma vitamin E level, and SOD activation. Sodium selenite injection resulted after 24 h in an optimal plasma Se level and a reactivation of GPx activity. In liver, brain, and kidney, Se levels in injected animals were higher than those in reference animals. However, this single administration of Se failed to decrease free radical damage induced by Se depletion. Therefore, in burned patients who exhibit an altered Se status despite a daily usually restricted Se supplementation, the early administration of a consistent Se amount to improve the GPx activity should be of great interest in preventing the impairment of the antioxidant status.
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Keywords: Selenium; Antioxidant enzymes; Se supplementation

Introduction

Under physiological conditions, the continuous production of free radicals and reactive oxygen species in aerobic organisms is constantly neutralized by a variety of antioxidant systems consisting of enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and numerous nonenzymatic antioxidants including tocopherol and vitamin C. Among antioxidant micronutrients, selenium (Se) is one of the most important regarding its anti-inflammatory effects [1] and its structural function in antioxidant selenoenzymes which are involved in the removal of free radicals or their products [2]. Alterations of Se status in man [3–5] and in animals [6] have been widely described, suggesting a large involvement of this trace element in the defense against injury.

In accordance with Bertin-Maghit's results [7], we observed in a precedent study performed in the French Army Burn Centre that all burned patients remained depleted in Se for 1 month despite a daily consistent supplementation (100 μg day^{-1} of Se) [8]. It is now well documented that burn injury induces an early inflammatory reaction [9,10] and an oxidative stress in animals [11,12] and humans [13] and there is increasing evidence that the

Abbreviations: SOD, superoxide dismutase; GPx, glutathione peroxidase; Se, selenium; RBC, red blood cell; TBARs, thiobarbituric acid-reactive species; tBOOH, *tert*-butyl-hydroperoxide; TBA, thiobarbituric acid; DTNB, bis-5,5′-dithiobis-2-nitrobenzoic acid; Mops, 4-morpholinepropanesulfonic acid.

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increased production or ineffective scavenging of reactive oxygen species may play a crucial role in determining tissue injury. The role of antioxidant status against free radical deleterious effects is crucial and a recent study [7] in burned patients reported alterations in antioxidant micronutrient status, antioxidant enzyme activities, and endogenous antioxidant defenses.

Given the well-documented selenium depletion in burned patients and the lack of data regarding the efficient doses needed to reach a recommended selenium status, the objective of the present study was to measure in a standardized Se-depleted animal model allowing blood and tissue investigations, the early evolution of selenium, and antioxidant status in relation to lipid peroxidation when a single parenteral supply of Se was administrated.

Materials and methods

Animal model

The study conformed to the guidelines established by the National Institutes of Health and it was carefully handled according to a technical protocol reviewed and approved by the ethical committee on research animal care of the French Army Medical Research Centre.

Male Wistar rats weighing 125 ± 10 g, purchased from Charles River Laboratories (Les Oncins, France), were randomized in three groups and handled daily in order to get them used to the experimenters and to avoid later manipulation stress. All animals were housed individually in thermoformed polystyrene cages in conformity with standards accredited by the French Minister of Agriculture and Environment. The cages were located in a room with a 12-h/12-h light–dark schedule and a pressurized filter air barrier. The constant temperature was controlled to 21 ± 1°C for the duration of the experiment with a relative humidity of 55%. In addition, in order to limit the risk of infection, the wood shaving litter and the water were autoclaved, the food was sterilized by gamma irradiation and the access to the locale was only restricted to allowed people. Animals received ad libitum in group 1 a pelleted standard diet from SAFE (Epinay sur Orge, France) with a normal level of selenium (190 ng g^{-1}) or in groups 2 and 3 a selenium-depleted diet (39 ng g^{-1}) from SDS (Saint Gratien, France). Except selenium levels, analysis of the formulation data of the two diets allowed us to expect a similar assimilation of the nutrients in the two groups. The diets were given for 5 weeks before the measurements of different biological parameters to allow the stabilization of the selenium status. During the study, the animals were examined daily for changes in body weight, behavior, and food intake. Twenty-four hours before sacrifice, animals from group 3 received a single intraperitoneal injection of sodium selenite (0.2 mg kg^{-1} of Se).

Sample collection

The blood collection was performed under halothane anesthesia and animals were killed by section of major chest vessels before tissue sampling.

Plasma and red blood cell (RBC) samples

Blood was collected by caudalis cava vena puncture using S-monovette syringes (Sarstedt, Germany) with lithium-heparin or EDTA anticoagulant. For glutathione measurement, 200 μl of whole blood collected with lithium-heparin was mixed with 1.8 ml of metaphosphoric acid (MPA 6%) and after centrifugation for 10 min at 3000g at 4°C, the supernatant was removed and stored at −80°C until analysis. For other plasmatic parameters, after whole blood centrifugation for 20 min at 2500*g* at room temperature, plasma was removed and stored at −80°C until analysis. After rinsing, 450 mg RBC pellets (400 μl) was lysed with 3.6 ml of Tris-HCl buffer (5 mM, pH 7.4) and stored at −80°C for the analysis of erythrocytic parameters.

Tissue samples

Immediately after blood collection and animal sacrifice, liver, kidney, femoral quadriceps, and brain samples were removed, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until analysis. Briefly, 500 mg of tissue samples (liver, kidney, femoral quadriceps) or one cerebral hemisphere was extracted in 5 ml of buffer (Tris-NaOH 10 mM, diethylenetriamine-pentaacetic acid (DTPA) 1 mM, phenylmethanesulfonyl fluoride (PMSF) 1 mM, pH 7.4) and centrifuged for 10 min at 3000*g* at 4°C. First, 750 μl of supernatant was collected and stored at −80°C for thiobarbituric acid-reactive species (TBARs) measurement and 100 μl of supernatant was added to 100 μl of NaOH 1 N for 2 h at room temperature and then stored at −20°C for total proteins determination. After a second centrifugation for 20min at 12,000*g* at 4°C, the supernatant was aliquoted and stored as follows for specific determinations : 200 μl of supernatant at −20°C for soluble proteins, 800 μl at −80°C for GPx activity, and 800 μl at −80°C for SOD activity.

Biological parameters

Selenium (Se)

Samples preparation and measurement were performed as previously reported by Ducros and co-workers [14–16]. Briefly, a defined amount of enriched ^{76}Se in the form of selenite, used as internal standard, was first added to the sample. After mineralization (HNO_3 and H_2O_2) and reduction of all selenium forms into selenite (Se^{4+}) by HCl, a chelate was formed with 4-nitro-ortho-phenylenediamine (Se-NPD) and extracted with chloroform. After chloroform evaporation, the dry residue was dissolved in

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