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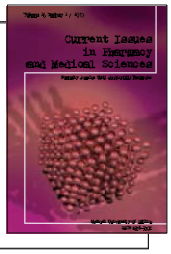

The effect of selenium supplementation in organic and inorganic form on oxidant balance in rat heart and femoral muscle

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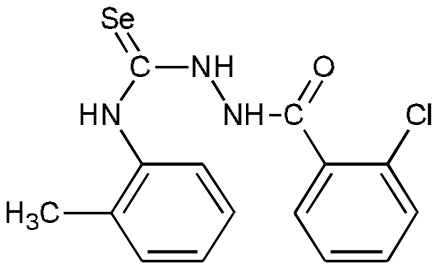
ABSTRACT

Total antioxidant status (TAS), activity of antioxidant enzymes - superoxide dismutase (SOD) and glutathione peroxidase (GPx), concentrations of non-enzymatic antioxidants - ascorbic acid (AA) and reduced glutathione (GSH) as well as concentration of lipid peroxidation marker - malonyldialdehyde (MDA) were determined in heart and femoral muscle of rats receiving different selecompounds (inorganic selenite and organic selenosemicarbazide of chain structure and selenazoline of ring structure). Chain selenosemicarbazide markedly decreased TAS values vs. control without Se-supplementation in heart. GPx was significantly depressed vs. control in heart of animals receiving organic selenium. Ring selenazoline decreased heart AA, whereas selenosemicarbazide increased heart GSH. Inorganic selenite diminished femoral muscle GSH. Selenium supplementation distinctly inhibited process of lipid peroxidation - MDA was decreased in Se-given animals, particularly in heart. As organic ring selenocompound depressed heart MDA to the highest degree, did not impair total antioxidant status and caused no disturbance of antioxidant barrier in femoral muscle, it could be suggested that further research may reveal possibilities of its application as a Se-supplement.

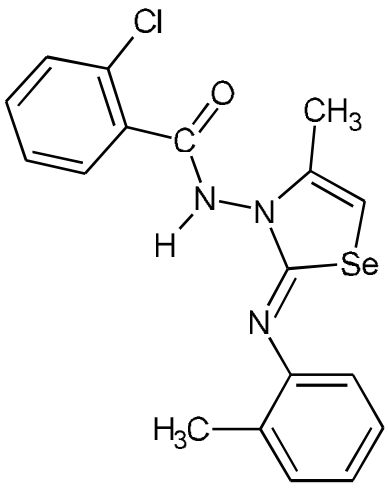
Keywords: selenium, rats, antioxidant barrier, lipid peroxidation

INTRODUCTION

Selenium belongs to essential trace bioelements and is necessary to correct functioning of organism. Its deficiency may result in diverse severe illnesses including heart disease [5, 11, 13]. The problem of selenium supplementation has been studyied during last years with the use of different substances both inorganic (sodium selenite or selenate) [1, 7, 8, 24] and organic [6, 19, 25] as well as Se-enriched natural products [8, 9, 18] but the question of the best form of supplementation remains unsolved. The main difficulties result from the narrow range between therapeutic and toxic dose of selenium [7] as well as from the dependence of its bioavailability on the form of supplementation [3]. One of the recent studies has revealed that both selenium deficiency and its modest supplementation can cause dysfunction to heart [13]. Another animal study has also displayed the fact that supplementation used in physiological state without selenium deficiency can be cardiotoxic [11].



compound A



compound B

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Many investigations have concerned relationships between selenium and oxidative parameters in organism [4, 6, 9, 16, 17, 19, 22, 25]. As a constituent of one of the main antioxidative enzymes (glutathione peroxidase) selenium is regarded to be an antioxidant [17]. Both glutathione peroxidase and other selenoproteins have been found to be involved into cardiovascular system functions [23]. Maintaining of proper oxidant balance plays a very important role in heart functioning [22].

The aim of the present study was to evaluate the influence of the two newly synthesized organic selenocompounds of different structure with that exerted by inorganic supplement sodium selenite, which is still used in clinical practice [21] and in research on effects of selenium supplementation [4, 7, 9], on oxidant processes in rat heart and femoral muscle tissue.

MATERIALS AND METHODS

Two selenoorganic compounds were synthesized in our department:

1) compound A: 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid (CAS Registry Number: 1374306-70-3) [14];
2) compound B: 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline (CAS Registry Number: 1279719-38-8) [15].

The experiment was carried out on adolescent male Wistar rats. After three days of acclimatization the animals were randomly divided into four groups (ten animals each):

- group I (control with no selenium supplementation) – treated with saline,
- group II – treated with sodium selenite,
- group III – treated with 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid (A),
- group IV – treated with 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline (B).

At the beginning of the experiment the weights of rats were included within a range of 110-150 g. Sodium selenite was given in a form of water solution. Organic compounds A and B given to groups III and IV were suspended in the emulsion composed of oil, arabic gum and water in the following proportion 2:1:1.5. The administration was performed by stomach tube. Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg of Se/g of b.w. once a day for a period of 10 days. Body weights of animals were measured every day before Se administration and the appropriate amount of selenium compound was calculated for each animal. Rats had free access to standard feed LSM and drinking water. After the end of the experiment animals were sacrificed under pentothal narcosis and the samples of heart and femoral muscle tissue were collected.

Ten per cent (w/v) tissue homogenates were prepared in 0.1 mol/L Tris – HCl buffer, pH = 7.4. Supernatants were obtained by centrifugation at 5000 x g for 30 min. The following substances were determined in homogenates: total antioxidant status (TAS), activity of antioxidant enzymes - superoxide dismutase (SOD) and glutathione peroxidase (GPx), concentrations of non-enzymatic antioxidants – ascorbic acid (AA) and reduced glutathione (GSH) as well as concentration of lipid peroxidation marker – malonyldialdehyde (MDA). TAS was measured using diagnostic kit produced by RANDOX and expressed in mmol/g of protein. SOD and GPx activities were determined using diagnostic kits RANSOD and RANSEL produced by RANDOX and expressed in U/mg of protein and U/g of protein, respectively. Reduced glutathione concentration was determined using BIOXYTECH® GSH-400™ kit produced by OxisResearch™ and expressed in g of GSH/mg of protein. Ascorbic acid concentration was determined using modified Kyaw method [20] and expressed in mol of AA/g of protein. Malonyldialdehyde concentration was determined using Ledwożyw et al. method [10] and expressed in nmol of MDA/mg of protein. Protein was measured using method of Bradford [2]. The assays were performed with use of spectrophotometer SPECORD M40 (Zeiss Jena).

Statistical analysis was performed using ANOVA test. Comparisons between control and Se-supplemented groups as well as between individual Se-supplemented groups were made using the Tukey's HSD test or Dunnett's T3 test. Values were considered significant with $p<0.05$.

The research or review article may include 15 at the most, and in case of short reports or introductory reports – 5 references. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by Local Ethical Commission of Medical University of Lublin, acceptance no. 65/AM/2004.

RESULTS

Heart TAS values were significantly decreased in rats receiving the chain organoselenium compound (group III) in comparison with all other groups. Inorganic selenite and cyclic selanazoline practically did not change heart TAS vs. control without Se-administration. Heart SOD activity was not altered vs. control and decreased in group III (selenosemicarbazide) vs. group II. Heart GPx was significantly diminished in groups receiving organic selenium (III and IV) vs. both control and group II given inorganic selenite. Heart AA concentration was markedly decreased in group IV receiving cyclic organic selena-

zoline vs. control and group III. GSH was enhanced in well-marked way in animals given chain selenosemicarbazide (group III) vs. control and group II and depressed in group IV (selenazoline) vs. group III (selenosemicarbazide). Heart MDA was significantly decreased in all Se-supplemented groups vs. control.

In femoral muscle TAS values, activities of GPx and SOD as well as AA concentrations did not show significant differences. GSH concentration was decreased in group II (inorganic selenite) vs. control and enhanced in group IV (selenazoline) vs. groups II and III. MDA concentration was markedly diminished in group III given organic chain selenosemicarbazide vs. control.

All the presented outcomes are presented in Fig.1.

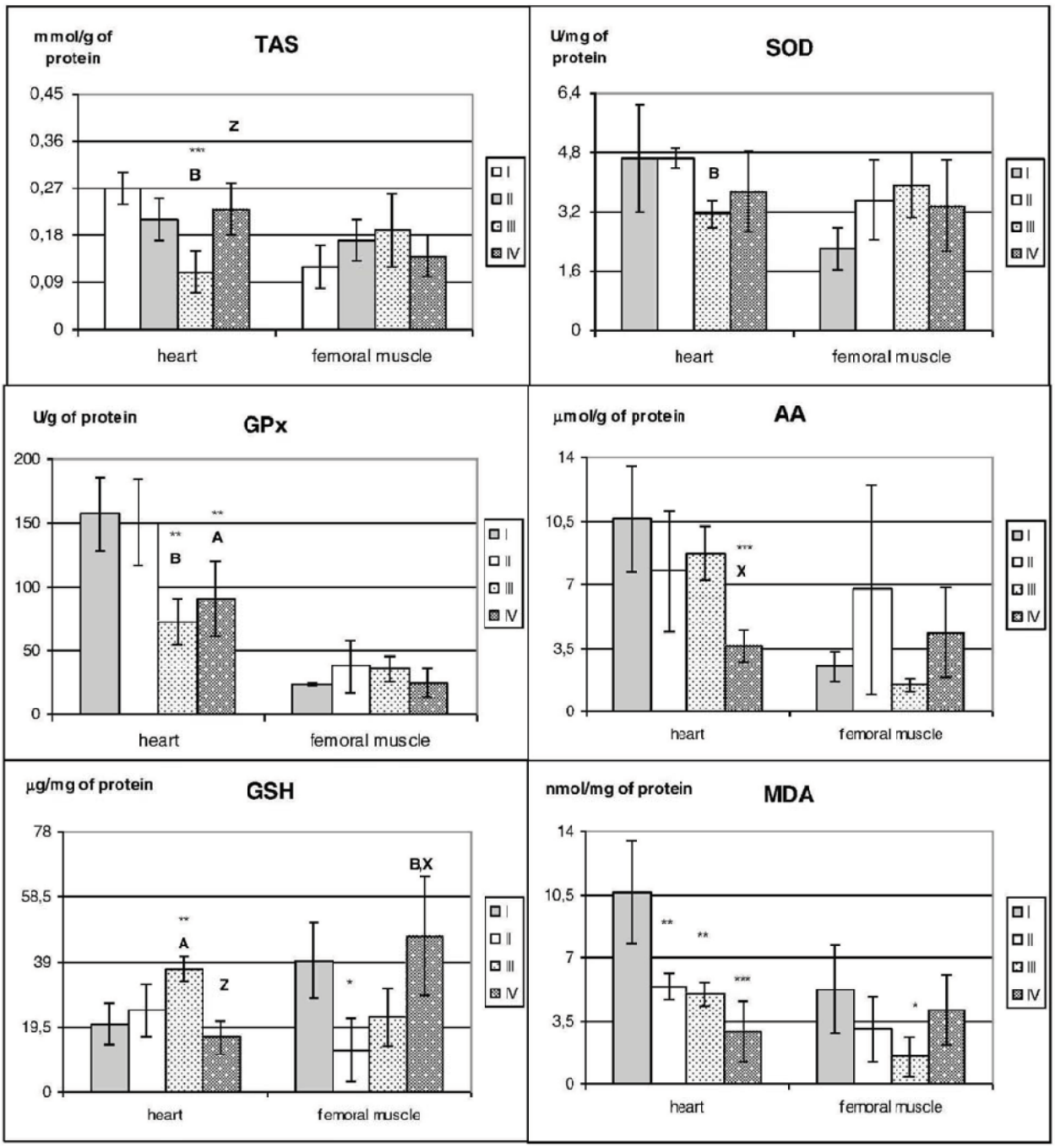

Fig. 1. The oxidant parameters (TAS – total antioxidant status, SOD – superoxide dismutase, GPx – glutathione peroxidase, AA – ascorbic acid, GSH – reduced glutathione, MDA – malonyldialdehyde) in heart and femoral muscle of rats receiving different forms of selenium supplementation. Rats were rendomly divided into four groups and intragastrically treated with: saline (group I); sodium selenite (group II), 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid (group III) and 3-(2-chlorobenzoylamino-) -2-(o-tolylimino-)-4-methyl-4-selenazoline (group IV).

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs. group I

^{A} $p<0.05$; ^{B} $p<0.01$; ^{C} $p<0.001$ vs. group II

^{X} $p<0.05$; ^{Y} $p<0.01$; ^{Z} $p<0.001$ vs. group III

DISCUSSION

The present experiment revealed that the influence of selenium supplementation on oxidant balance depends on both the form of the used supplement and the studied tissue. The results obtained by other scientist are partially consistent, but some discrepancies were also reported.

In the present study heart GPx was not affected by inorganic Se and decreased by organic one in comparison with control without Se-supplementation. Similarly, Venardos et al. observed that in rat hearts that were subjected to ischemia-reperfusion selenite dietary supplementation did not change GPx activity [24]. Soudani et al. also reported no influence of Na_2SeO_3 on rat heart GPx [22]. Orun et al. observed that sodium selenite diminished heart GPx in rainbow trout. Interestingly, in fish additionally exposed to chromium or cadmium selenite caused distinct GPx increase [17]. Sodium selenite supplementation significantly increased GPx in heart of sheep, but the period of the experiment was very long (2 years) and the reference group was fed Se-deficient diet [7].

In the present experiment muscle GPx was unchanged, regardless of the used form of selenium. The same result was reported by O'Grady et al. who observed no effect of organic selenium on GPx activity in bovine muscle [16]. No effect of dietary selenium on pheasant chicks breast tissue GPx was also found [9]. Selenomethionine did not exert any effect on skeletal muscle GPx in broiler chicks [6]. On the contrary, dietary selenium, both organic and inorganic, enhanced muscle GPx in pigs [25]. Juniper et al. also observed increase in skeletal muscle tissue GPx of beef cattle supplemented with selenized enriched yeast but the period of experiment was considerably longer (112 days) [8]. The same results were obtained in pigs – dietary Se-yeast 16-week-administration significantly enhanced GPx in skeletal muscle [18].

The present research revealed that selenium administration affected neither heart nor muscle SOD vs. control. Similar outcomes were displayed in heart of fish given sodium selenite [17]. Dietary selenite also showed no influence on heart SOD in rats [22], whereas dietary selenomethionine administered to broiler chicks did not affect skeletal muscle SOD [6].

In the present study heart and muscle ascorbic acid was not significantly altered by selenium administration, except for decrease caused by ring selenazoline in heart. Similarly, Prigol et al. observed either no changes or depression of AA in skeletal muscle of mice receiving diphenyl diselenide [19]. Inorganic selenite given via diet did not affect vitamin C level in rats' heart [22]. The experiment carried out by Bertinato et al. on guinea pigs fed Se-deficient diet and receiving marginal amounts of AA revealed that Se-supplementation in inorganic form (sodium selenate) resulted in increase in total vitamin C in heart and skeletal muscle [1], but the period of the experiment was longer (5 or 12 weeks).

Our experiment revealed that selenium supplementation markedly decreased heart MDA vs. control. These results are consistent with those reported by Cui et al. Dietary selenium deficiency significantly increased heart MDA in mice [5]. Soudani et al. observed no impact of selenite on heart MDA in healthy rats, but in those additionally exposed to potassium chromate significant decrease was shown [22]. The same results were obtained by Orun et al. in fish heart [17].

In the present study, MDA in muscle was insignificantly lower in rats receiving chain organoselenium compound than in those given inorganic selenite. A similar effect was obtained in the experiment performed by Marković et al. on broilers receiving selenium in two forms: selenite and selenized yeast and additionally supplemented with vitamin E. In inorganic selenite group breast muscle MDA was higher after 21 and 42 days of supplementation [12]. Both selenite and selenosemicarbazide A decreased muscle MDA vs. control, although only the latter significantly. Similarly, dietary selenomethionine and selenite diminished muscle MDA in pigs [25]. Selenium in organic form (diphenyl diselenide) caused significant decrease in TBARS level in skeletal muscle of mice [19]. However, the experiment performed on pheasant chicks revealed no influence of selenite or selenized-enriched yeast on breast tissue TBARS [9]. In fish receiving vitamin E, the increase in Se-content in diet resulted in no changes or decrease in muscle MDA [4]. Organic selenium (selenomethionine) was found to diminish MDA in skeletal muscle of broiler chicks undergoing heat stress, whereas in birds kept at normal conditions no significant changes were noted [6].

Organic ring selenocompound B 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline depressed heart MDA to the highest degree, did not impair total antioxidant status and caused no disturbance of antioxidant barrier in femoral muscle. Having regarded these outcomes, it could be suggested that further research may reveal possibilities of its application as a supplement in pathological states connected with selenium deficiency.

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