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Acute administration of the organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one induces biochemical and hematological disorders in male rats

Denise dos Santos Lacerda[1], Vanessa de Oliveira Castro[1], Marcello Mascarenhas[1], Robson Brum Guerra[2], Caroline Dani[1], Adriana Coitinho[3], Rosane Gomez[3] and Cláudia Funchal[1]*

[1]*Centro Universitário Metodista IPA, Porto Alegre, Rio Grande do Sul, Brazil*
[2]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, Sertão, Rio Grande do Sul, Brazil*
[3]*Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

Organochalcogens are extensively produced and employed by industry and agriculture, and the risk of occupational and environmental toxicity to them has been poorly understood. Here, we investigated the acute effect of a new organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on biochemical and hematological parameters in male Wistar rats. The animals were treated with a single intraperitoneal injection of the organochalcogen at doses of 125, 250 or 500 $\mu g \cdot kg^{-1}$. After 60 min, the animals were sacrificed by decapitation, and the trunk blood was collected for determination of glucose, triglycerides, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase, lactate dehydrogenase, urea, creatinine, C-reactive protein, red blood cells, hematocrit, hemoglobin and white blood cells (WBC). Our results showed a reduction in cholesterol levels in all treated groups, an increase in ALT activity at doses of 250 and 500 $\mu g \cdot kg^{-1}$, a decrease of hemoglobin and an increase in WBC in animals that received 250 and 500 $\mu g \cdot kg^{-1}$ of the organoselenium. In addition, we observed an increase in neutrophil counts at 125 $\mu g \cdot kg^{-1}$ dose and a decrease at 500 $\mu g \cdot kg^{-1}$ dose. We also verified an increase in lymphocyte counts at the dose of 500 $\mu g \cdot kg^{-1}$. Thus, the present study shows that the acute treatment with this new organochalcogen causes biochemical changes and hematological disorders in male rats. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—organochalcogens; selenium; toxicity; biochemical parameters; hematological disorders

INTRODUCTION

The elements of the periodic table group 16, chalcogens are extensively produced and employed by the industry and agriculture.[1] Selenium (Se) is an essential micronutrient for biological functions. In the form of selenoproteins, it participates in cellular homeostasis and redox balance maintenance, as a component of antioxidant enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase.[2–4] Inorganic forms of Se such as sodium selenite (Na_2SeO_3) are present in low concentrations in some foods, and its deficiency can predispose to the development of some disorders such as cancer, cardiovascular disease and diabetes.[5] Some organic forms of Se exhibit antioxidant activity, and its action can be compared with the enzyme GPx.[6]

In the last years, studies identifying new biological properties of synthetic organic molecules of Se have gained a special attention.[7,8] Different pharmacological properties have been attributed to organochalcogens such as antioxidant activity,[6,9] anti-diabetogenic,[10,11] antinociceptive,[12] hepatoprotective[13] and anticonvulsant.[14] In contrast, other researchers have shown that organoselenium compounds can produce toxic effects on living organisms, both *in vitro* and *in vivo*.[15,16] Their beneficial pharmacological properties or toxical effects are related to their molecular structure and are dose dependent. In fact, structural changes in the organoselenium compounds can influence the pharmacokinetics and pharmacodynamics of these compounds and produce toxicity.[16] The toxicity of the organochalcogens seems to be related to the oxidation of proteins thiol groups, inhibiting enzyme activity by promoting a pro-oxidant condition, leading to toxicity and cell damage.[17]

In this context, Nogueira *et al*.,[18] described the neurotoxic effects of diphenyl diselenide in rats, measured by the lower latency of onset and higher scores of seizures episodes after its administration in rodents, showing that the brain is a potential target for toxicity of chemicals. The genotoxic and cytotoxic action of organochalcogens were demonstrated by Santos *et al*.,[4] showing a decrease in cell viability and an increase in fragmentation of DNA from leukocytes, caused by acute exposure of these cells to different organic compounds of Se.

*Correspondence to: Cláudia Funchal, Centro Universitário Metodista IPA Rua Cel. Joaquim Pedro Salgado, 80, 90420–060-Porto Alegre, RS, Brazil. E-mail: claudia.funchal@metodistadosul.edu.br

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Despite the growing use of organoselenium compounds in chemical and biochemical fields and the increasing risk of occupational and environmental human exposure to these elements, there has been little concern about their toxicity. Therefore, the aim of this study was to evaluate the acute effect of the new organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on some biochemical and hematological parameters in male rats.

MATERIALS AND METHODS

Chemicals

3-Methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one was synthesized according to Silveira *et al*. (2002). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that the compound obtained presented analytical and spectroscopic data in full agreement with its assigned structure. Stock solutions of the organochalcogen were prepared in dimethylsulfoxide (DMSO) just before use. The final concentration of DMSO was 0·1%, and it did not modify any parameter tested. Biochemical commercial kits were obtained from Labtest, Diagnostica S.A., (Minas Gerais, Brazil). All other chemicals were of analytical grade and were purchased from local suppliers.

Animals

Forty adult male Wistar rats (~300 g, 90 days old) were obtained from our own breeding colony. They were maintained at 22 ± 2 C, on a 12-h light/12-h dark cycle, with free access to food and water. The 'Principles of laboratory animal care' (National Institutes of Health publication no. 80–23, revised 1996) were followed in all experiments, and our research protocol was approved by the Ethical Committee for Animal Experimentation of Centro Universitário Metodista IPA. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Acute in vivo treatment

The animals were treated intraperitoneally with a single dose of 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one (125, 250 or 500 $\mu g \cdot kg^{-1}$ of body weight). Control rats were randomly chosen and received saline solution (0·9% NaCl) in the same volumes. Rats were sacrificed 60 min after the injection. The doses of the organochalcogen were chosen on the basis of previous studies from our group and pilot experiments.

Biochemical parameters

After 60 min of the drug administration, the animals were euthanized by decapitation and, the trunk blood was collected in tubes without any anticoagulant (serum) or in tubes containing ethylenediaminetetraacetic acid for hematological parameters. Serum was obtained by centrifugation at 1000 *g* for 10 min (hemolysed serum was discarded). Glucose, triglycerides and cholesterol were used as biochemical markers. Hepatic function was analysed using alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities as markers of toxicity. Renal function was analysed by determining urea and creatinine. C-reactive protein (CRP) was used as an inflammatory process marker. All assays were carried out using commercial kits (Labtest, Diagnostica S.A., Minas Gerais, Brazil).

Hematological parameters

Red blood cells (RBC), hematocrit, hemoglobin and white blood cells (WBC) were determined with an automated counter (ADVIA 60). WBC differentiation and erythrocyte morphology evaluation were performed by the May–Grunwald/Giemsa staining method in fresh blood films.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by the Tukey test. Values of $P < 0{\cdot}05$ were considered to be significant. All analyses were carried out using the Statistical Package for Social Sciences (SPSS) software.

RESULTS

Biochemical parameters

Treatment of rats with the organoselenium caused a reduction on the cholesterol levels in all treated groups and was able to increase ALT activity at doses of 250 and 500 $\mu g \cdot kg^{-1}$ when compared with the control group (Table 1). No significant differences were obtained in glucose, triglycerides, creatinine, urea, AST and LDH levels (Table 1). Moreover, no alterations were observed in CRP levels (Table 1).

Hematology

Results illustrated in Tables 2 and 3 showed significant changes in red and white blood cells of male rats exposed acutely to different doses 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one when compared with the control groups. Hemoglobin was reduced at doses of 250 and 500 $\mu g \cdot kg^{-1}$, whereas RBC and hematocrit were not changed by the organochalcogen (Table 2). On the other hand, WBC counts significantly increased at doses of 250 and 500 $\mu g \cdot kg^{-1}$ (Table 3), and neutrophil counts increased at dose of 125 $\mu g \cdot kg^{-1}$ and decreased at dose of 500 $\mu g \cdot kg^{-1}$. We also observed an increased in lymphocyte counts at the dose of 500 $\mu g \cdot kg^{-1}$ (Table 3). Monocyte, eosinophil and basophil counts were not altered by the acute organoselenium treatment (Table 3).

DISCUSSION

In recent decades, there has been progress in understanding the importance of Se on physiological functions. However, the optimal functional concentration as well as the appropriate form of supplementation of this compound is still

Table 1. Effect of acute treatment with different doses of the organoselenium 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on biochemical parameters of rats

	Control	125 μg·kg^{-1}	250 μg·kg^{-1}	500 μg·kg^{-1}
Glucose (mg·dl^{-1})	142·71 ± 3·48	136·73 ± 2·95	132·72 ± 2·55	134·24 ± 3·16
Cholesterol (mg·dl^{-1})	184·41 ± 15·04	121·91 ± 11·04*	121·73 ± 15·26*	123·47 ± 16·19*
Triglycerides (mg·dl^{-1})	81·37 ± 2·60	100·45 ± 3·76	96·36 ± 5·76	89·24 ± 4·35
Creatinine (mg·dl^{-1})	3·42 ± 0·15	3·02 ± 0·19	3·01 ± 0·15	3·29 ± 0·24
Urea (mg·dl^{-1})	61·11 ± 2·80	60·25 ± 5·96	58·80 ± 5·33	62·14 ± 3·27
Alanine aminotransferase (U·l^{-1})	37·11 ± 5·60	64·44 ± 6·59	74·76 ± 7·61*	76·17 ± 7·61*
Aspartate aminotransferase (U·l^{-1})	38·66 ± 6·92	39·76 ± 7·19	31·53 ± 5·74	44·68 ± 9·22
Lactate dehydrogenase (U·l^{-1})	213·38 ± 17·73	213·38 ± 17·73	192·74 ± 18·93	202·74 ± 22·99
C-reactive protein	Nonreactive	Nonreactive	Nonreactive	Nonreactive

Statistically significant differences were determined using ANOVA followed by Tukey test: *$P < 0.05$, from control, $n = 10$/group.

Table 2. Effect of acute treatment with different doses of the organoselenium 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on red blood cells of rats

	Control	125 μg·kg^{-1}	250 μg·kg^{-1}	500 μg·kg^{-1}
Red blood cell (10^6·mm^{-3})	10·15 ± 1·24	8·70 ± 2·28	10·23 ± 1·92	9·61 ± 2·48
Hemoglobin (g·dl^{-1})	20·32 ± 1·05	21·00 ± 2·02	15·10 ± 1·02*	14·87 ± 1·05*
Hematocrit (%)	56·60 ± 1·45	54·57 ± 2·30	64·40 ± 4·08	55·05 ± 6·48

Statistically significant differences were determined using ANOVA followed by Tukey test: *$P < 0.01$, from control; $n = 10$/group.

Table 3. Effect of acute treatment with different doses of the organoselenium 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on white blood cells of rats

	Control	125 μg·kg^{-1}	250 μg·kg^{-1}	500 μg·kg^{-1}
White blood cell (10^3·mm^{-3})	10·08 ± 1·33	8·6 ± 1·45	18·09 ± 1·87*	18·06 ± 1·86*
Neutrophils (%)	19·16 ± 1·44	31·66 ± 2·30*	21·66 ± 3·16	10·66 ± 2·23*
Lymphocytes (%)	79·33 ± 2·10	71·50 ± 2·66	76·00 ± 3·35	91·33 ± 2·23 *
Monocytes (%)	0·66 ± 0·49	0·16 ± 0·10	0·66 ± 0·49	0
Eosinophils (%)	0·16 ± 0·10	0	0·16 ± 0·10	0
Basophils (%)	0	0	0	0

Statistically significant differences were determined using ANOVA followed by Tukey test: *$P < 0.05$, from control; $n = 10$/group.

discussed.[20] Organic compounds of Se have distinct activities, sometimes therapeutic and sometimes toxic.[16,21] The paradoxical effect of these compounds depends on the variety of chemical structure, doses, route and regimen of administration and animal species involved in the studies.[17] Considering that toxicological studies involving exposure to these compounds are scarce, the aim of our study was to evaluate the effects of acute exposure to a new organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one on biochemical and hematological parameters in male rats.

Here, we observed that the organoselenium 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one reduced cholesterol levels in all treated groups. According to our present results, i*n vitro* studies showed that tellurium, another chalcogen compound, inhibits cholesterol biosynthesis by interaction with the sulfhydryl groups of the enzyme squalene monooxygenase, a microsomal enzyme that catalyses the second step in the pathway for cholesterol biosynthesis. [22] *In vivo* studies also showed that repeated administration of diphenyl ditelluride (0.6–0.9 μmol·kg^{-1}) decreased blood cholesterol levels in rats.[23] Thus, we can suggest that our organoselenium compound also decreased cholesterol levels by interactions with the sulfhydryl groups of the enzyme squalene monooxygenase.

Additionally, we showed that the acute administration of the organoselenium induced mild hepatocellular damage evidenced by an increase in ALT activity in animals that received 250 and 500 μg·kg^{-1} of the compound, without any alterations in AST activity. Results from other studies are controversial. Straliotto *et al.*,[24] showed that acute intraperitoneal administration of diphenyl diselenide did not modify these plasma liver enzymes in rabbits, whereas Meotti *et al.*,[25] showed that subchronic subcutaneously administration of diphenyl diselenide increases both ALT and AST plasma enzymes in rats. It appears that the regime of administration contribute to the hepatotoxicity of these organochalcogens compounds in different animal species. At this point, it is important to emphasize that there are only few studies showing the effects of acute administration of other organochalcogens in rats; thus, we do not discard that chronic or subchronic administration of this organoselenium would increase both ALT and AST activities.

The compound used in the present study was not able to change glucose, triglycerides, creatinine, urea, LDH and CRP levels in the animals. This is in line with previous studies using other organochalcogens where it was observed that urea, creatinine, LDH and CRP levels also were not modified.[24–26] On the other hand, subcutaneous exposure of rats to diphenyl ditelluride was capable of decreasing triglyceride concentration and increasing CRP levels.[23]

Interestingly, we also showed that the acute administration of 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one promoted hematological disorders, as seen by changes on both red and white blood cells of male rats. Although hematocrit and RBC cells were not affected, hemoglobin decreased after organoselenium administration at doses of 250 and 500 $\mu g \cdot kg^{-1}$. *In vitro* studies showed that different organochalcogens caused toxic effects on human erythrocytes, including hemolysis.[27] Although the specific molecular targets that mediate organochalcogen toxicity are not known, these compounds can interact directly with low molecular thiols, oxidizing them to disulfides.[28] In fact, reduced cysteinyl residues from proteins also can react with these compounds, which may cause, in the case of the enzymes, the loss of their catalytic activity.[16,22] The impairment of the activity of sulfhydryl enzymes, such as δ-aminolevulinate-dehydratase (δ-ALA-D), may impair heme biosynthesis and possibly cause an unbalance of cellular heme-dependent metabolic pathways, decreasing the production of hemoglobin.[17,29,30] Because Se have a high affinity for SH groups on proteins and other endogenous biomolecules,[6] we may infer that this organoselenium compound decreased hemoglobin in our treated rats by interference on this route.

Furthermore, we found that WBC increased after acute organoselenium treatment at doses 250 and 500 $\mu g \cdot kg^{-1}$. The lowest dose of 125 $\mu g \cdot kg^{-1}$ of this compound increased neutrophil count, whereas the highest dose of 500 $\mu g \cdot kg^{-1}$ increased lymphocyte counts. Johnson *et al*.,[31] also showed a dose-dependent increase in proliferation of lymphocytes and other proinflammatory cytokines following 2 weeks of oral exposure to Se administered in the drinking water of rats. It is well known that Se is involved in the production of antibodies and modulation of the immune response.[32] However, it also is a toxic trace element on immune system.[31] Thus, we could assume that the increase in WBC by the organochalcogen tested here indicates an over-activation of the defense mechanisms of rats, through immunological mechanisms. This immune activation by this organochalcogen may be related to the inhibition of the enzyme adenosine deaminase (ADA), an enzyme involved in purine metabolism.[8] It is suggested that inhibition of ADA activity causes an increase in the concentration of adenosine, which acts as a sensor of tissue injury that culminates with stimulation of the immune system. [33]

In conclusion, we showed here that an acute exposure to the new organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one caused a decrease in serum cholesterol levels, liver toxicity and hematological disorders in male rats. However, further studies are required to determine the mechanisms that underline the effects caused by this compound.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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