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THE INFLUENCE OF SELENIUM COMPOUNDS OF DIFFERENT STRUCTURE ON MORPHOLOGY, BLOOD BIOCHEMISTRY AND PHAGOCYTIC CAPABILITY OF GRANULOCYTES IN RATS

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ABSTRACT

Background. Selenium belongs to important microelements. Numerous studies have revealed relationships between its deficiency and occurrence of diverse illnesses, but the question of the proper form and dose of Se-supplementation still remains unsolved.

Objective. In the present study the influence of different selenium compounds on blood morphology and biochemistry as well as on phagocytic capacity of granulocytes and NBT test in rats was investigated.

Material and methods. Adolescent male Wistar rats were divided into four groups (ten animals each): I – control, received saline; II – received sodium selenite Na_2SeO_3; III – received selenoorganic compound A of chain structure 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid; IV – received selenoorganic compound B of cyclic structure 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline. The administration was performed by stomach tube at a dose of $5 \cdot 10^{-4}$ mg Se g^{-1} b.w. once a day for 10 days.

Results. Selenium compounds treatment decreased haematocrit. Erythrocytes number was unchanged in all groups receiving Se vs. control, whereas leucocytes number was depressed in groups II and IV. Haemoglobin was significantly decreased in group III. White blood count was altered in groups II and III, where all parameters were markedly decreased except for lymphocytes in group III and remained unchanged in group IV. The outcomes regarding selenium effect on biochemistry parameters of blood showed that urea remained unchanged, glucose was statistically decreased in groups II and III, whereas cholesterol was significantly diminished in group II and increased in group III vs. control. Results concerning phagocytosis and NBT test displayed that % of positive cells were decreased in groups II and III, whereas remained unaltered in group IV vs. control.

Conclusions. As cyclic selenoorganic compound B did not cause many significant changes of the studied parameters it may be suggested that after further researches it could be taken into account as a possible selenium supplement.

Key words: *selenium, blood morphology, blood biochemistry, phagocytosis, rats.*

STRESZCZENIE

Wprowadzenie. Selen należy do mikropierwiastków o dużym znaczeniu dla organizmu. Badania naukowe wykazały istnienie zależności pomiędzy niedoborem selenu a występowaniem wielu poważnych schorzeń, jednakże kwestia doboru odpowiedniej formy i dawki stosowanej w suplementacji nadal pozostaje nierozwiązana.

Cel. W przeprowadzonym doświadczeniu badano wpływ podawania różnych związków selenu na morfologię krwi i biochemię krwi oraz zdolności fagocytarne granulocytów i test NBT u szczurów.

Materiał i metody. Młode szczury samce rasy *Wistar* podzielono na cztery grupy (po 10 zwierząt): I – kontrola, otrzymywała sól fizjologiczną; II – otrzymywała selenian(IV) sodu Na_2SeO_3; III – otrzymywała organiczny związek selenu A o budowie łańcuchowej 4-(o-tolilo-)-selenosemikarbazyd kwasu 2-chlorobenzoesowego; IV - otrzymywała organiczny związek selenu B o budowie cyklicznej 3-(2-chlorobenzoiloamino-)-2-(o-toliloimino-)-4-metylo-4-selenazolinę. Związki podawane były sondą dożołądkowo w dawce $5 \cdot 10^{-4}$ mg Se g^{-1} m.c. raz dziennie przez okres 10 dni.

Wyniki. Podawanie związków selenu obniżyło hematokryt. Liczba erytrocytów we wszystkich grupach pozostała niezmieniona w stosunku do kontroli a liczba leukocytów była zmniejszona w grupach II i IV. Poziom hemoglobiny został obniżony statystycznie w grupie III. Obraz białych elementów morfotycznych w porównaniu do kontroli uległ zmianie w grupach II i III gdzie wszystkie wskaźniki zostały istotnie statystycznie obniżone za wyjątkiem limfocytów w grupie III, natomiast w grupie IV nie zaobserwowano żadnych zmian. Analizując wpływ podawania selenu na parametry biochemiczne krwi stwierdzono, że stężenie mocznika pozostało niezmienione. Stężenie glukozy w przypadku grup II i III uległo istotnemu

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statystycznie obniżeniu. Stężenie cholesterolu w II grupie było istotnie obniżone a w III podwyższone w porównaniu z kontrolą. Badania fagocytozy i wyniki testu NBT wykazały, że % pozytywnych komórek uległ obniżeniu w grupach II i III w stosunku do grupy kontrolnej i pozostał niezmieniony w grupie IV.

Wnioski. Ponieważ organiczny związek cykliczny B nie spowodował istotnych statystycznie zmian wielu badanych parametrów można byłoby sugerować, że przeprowadzenie dalszych badań pozwoli rozważyć jego zastosowanie jako suplementu selenu.

Słowa kluczowe: *selen, morfologia krwi, biochemia krwi, fagocytoza, szczury.*

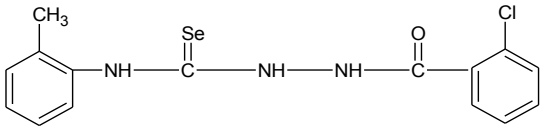
INTRODUCTION

Selenium belongs to essential bioelements. During the last year numerous researches concerning its metabolism have been undertaken resulting in evidence of relationships between selenium level in organism and the state of health. Selenium deficit has been found to show connections with higher incidence of cancer, hypertension hepatonecrosis and *Friedreich's* ataxia [6, 17, 19]. Relationships between selenium level in organism and immune system and thyroid gland functions [2, 3, 4] as well as alleviating influence of selenocompounds in cases of chemiotherapy side effects have also been reported [7]. Selenium deficit can cause development disturbances [16] and has been found to occur in patients with numerous illnesses [8, 20, 21]. However, the excess of this element may also bring about severe impairment of functions of organism [9].

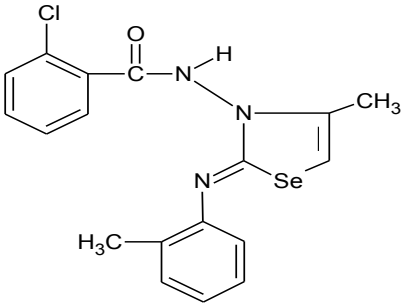
The question on the proper dose and form of selenium supplementation is a difficult and complex problem, all the more so because selenium level in organism can depend on diet [5]. As selenoorganic compounds have been found to be less toxic than inorganic ones, the growing concern in new, efficient selenoorganic supplements has been observed [18]. Our previous studies have also revealed that selenium administered in organic form is better assimilated than inorganic sodium selenite [13]. The aim of the present study was to evaluate the influence of two selenoorganic compounds of different structure, synthesized in our chair, on morphological and biochemical parameters in blood as well as on phagocytic capacity of granulocytes and NBT test in rats. The observed results were also compared with those obtained when inorganic selenite was applied.

MATERIAL AND METHODS

Two selenoorganic compounds were synthesized in our chair: compound A (chain structure) 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid [13] and compound B (ring structure) 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline [15].



compound A



compound B

The experiment was performed on adolescent male Wistar rats (110 - 150 g body mass). After an acclimatization period of three days the animals were randomly divided into four groups (ten animals each): group I (control)- treated with saline; group II (Na_2SeO_3) - treated with sodium selenite; group III (Se-chain) - treated with compound A; group IV (Se-ring) - treated with compound B. Sodium selenite was given in form of water solution. As organic compounds given to groups III and IV were insoluble in water they 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^{-1} of b.w. once a day for a period of 10 days. The administered dose and period of experiment were chosen taking into account previous studies, both ours and performed by other authors [1, 4, 10, 11, 12]. Body mass of each animal was measured every day before Se-administration and the appropriate amount of selenium compound was calculated. Rats had free access to standard feed LSM and drinking water. The study was performed according to statutory bioethical standards and approved by the Local Ethical Commission of Medical University of Lublin, acceptance no. 65/AM/2004.

After the end of the experiment animals were sacrificed under pentothal narcosis and samples of blood

were collected to heparinized test tubes. Morphological parameters (red blood cells, white blood cells, haematocrit and haemoglobin as well as white blood count) were determined using CELL-DYN 1700 system. Biological parameters (urea, glucose and cholesterol) were determined using KONELAB 60 PRIME analyzer with the help of commercial reagents and diagnostic kits. The neutrophil oxidation-reduction potential was examined using NBT test (Nitro Blue Tetrazolium test), in which the positive cells were those whose oxidated form of a yellow water-soluble dye was converted into a dark-blue water-insoluble diformazan upon reduction. In the smears stained with May-Grunwald method the percentage of white morphotic elements of blood was determined which was used to calculate the absolute cell number in mm^3 of blood. To evaluate the neutrophil phagocitic capacity, the phagocitic reaction Bacto-Latex (Difco, USA) was used. In both tests 100 cells were calculated. In the phagocitic test the cells with at least 3 latex granules were considered the positive ones. In the NBT test the positive cells were those in which big formazan granules were observed. The number of positive cells in 100 analyzed cells was determined by the test indicators.

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. Values were considered significant with $p < 0.05$. Contrast analysis was also performed to evaluate significance of differences between connected groups. The differences between group I and connected groups II + III + IV (selenium supplementation) as well as between group II (inorganic selenium) and connected groups III + IV (organic selenium) were estimated. Values were considered significant with $p < 0.05$.

RESULTS

Comparing the results obtained for groups receiving selenium compounds with those observed in control group provided with saline we found that haematocrit was decreased in groups II and III. The number of leucocytes was unchanged in group III and diminished in groups II and IV. Haemoglobin was decreased in group III. Erythrocytes number was not altered in all supplemented groups. The presented outcomes were collected in Table 1.

White blood count was changed in groups II and III, where all parameters were markedly decreased except lymphocytes in group III. In group IV (cyclic, organic selenocompound) no changes vs. control were observed (Table 2).

The results of contrast analysis concerning morphology were shown in Table 3. Selenium supplementation regardless of its form decreased haematocrit and number of some white blood cells (neutrophils, bacillus and monocytes). Organic selenium supplements in turn enhanced number of leucocytes as well as lymphocytes and monocytes in comparison with widely applied inorganic supplement Na_2SeO_3 (Table 3).

Selenium treatment did not influence urea concentration in plasma vs. control. Glucose was decreased in groups II and III, whereas cholesterol was diminished in group II and enhanced in group III vs. control. In group IV biochemical parameters remained unchanged. The results concerning phagocytosis and NBT test showed that % of positive cell were decreased in groups II and III. Cyclic organic compound (group IV) did not cause significant changes vs. control (Table 4).

The results of contrast analysis concerning biochemistry, phagocytosis and NBT test showed that selenium significantly decreased phagocytic capability as well as % of positive cells in NBT test and glucose concentration. Organic selenium treatment caused increase in cholesterol concentration (Table 5).

Table 1. Blood morphological parameters

GROUP	HAEMOGLOBIN (g %) $\overline{X} \pm SD$	HAEMATOCRIT (g %) $\overline{X} \pm SD$	ERYTHROCYTES (10^6 mm^{-3}) $\overline{X} \pm SD$	LEUCOCYTES (10^3 mm^{-3}) $\overline{X} \pm SD$
I	14.40 ± 2.17	49.09 ± 4.88	9.02 ± 2.22	3.46 ± 0.92
II	14.16 ± 2.23	37.54 ± 2.34 ** (H)	8.75 ± 1.28	2.21 ± 0.67 * (H)
III	10.72 ± 2.10 *, X (H)	34.00 ± 4.93 *** (H)	7.61 ± 2.27	4.32 ± 0.89 C (H)
IV	14.84 ± 1.22	41.52 ± 3.67 X (H)	8.75 ± 1.42	2.07 ± 0.52 *, Z (H)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ 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
H *Tukey's* test HSD

Table 2. White blood amount

GROUP	NEUTROPHILS number mm $^{-3}$ $\overline{X}$ ± SD	BACILLUS number mm^{-3} $\overline{X}$ ± SD	MONOCYTES number mm $^{-3}$ $\overline{X}$ ± SD	LIMPHOCYTES number mm $^{-3}$ $\overline{X}$ ± SD
I	1098 ± 228	187 ± 55	166 ± 32	1912 ± 197
II	777 ± 158 * (H)	69 ± 22 *** (H)	54 ± 18 *** (H)	867 ± 113 ** (H)
III	734 ± 119,20 ** (H)	86 ± 21 *** (H)	68 ± 19 *** (H)	1889 ± 3699 ^{B} (H)
IV	955 ± 155	227 ± 82	125 ± 35	1746 ± 764 ^{A} (H)

* 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
^{H}*Tukey's* test HSD

Table 3. Contrast analysis between group I (Control) and connected groups II + III + IV (selenium supplementation) as well as between group II (inorganic selenium) and connected groups III + IV (organic selenium) of blood morphology.

	Haematocrit	Leucocytes	Neutrophils	Bacillus	Monocytes	Lymphocytes
I vs. II + III + IV	*** ↓		** ↓	*** ↓	*** ↓	
II vs. III + IV		* ↑			*** ↑	***↑

*p < 0.05; **p < 0.01; *** p < 0.001
↑ - increase; ↓ - decrease

Table 4. Blood biochemistry as well as phagocytic capability of granulocytes and NBT test.

GROUP	Urea mg/dl $\overline{X}$ ± SD	Glucose mg/dl $\overline{X}$ ± SD	Cholesterol mg/dl $\overline{X}$ ± SD	Phagocytosis % of positive cells $\overline{X}$ ± SD	NBT test % of positive cells $\overline{X}$ ± SD
I	34.45 ± 5.28	194 ± 19	91 ± 7	58.90±11.09	8.77±1.86
II	36.77 ± 11.71	138 ± 6 * (H)	68 ± 4 * (H)	33.40±8.31 *** (H)	4.60±1.27 ** (H)
III	37.73 ± 2.10	94 ± 6 *** (H)	153 ± 6 *** (H)	34.68±5.73 *** (H)	3.47±1.09 *** (H)
IV	32.90 ± 8.42	185 ± 28 ^{A,Z} (H)	81 ± 12 ^{C,Z} (H)	47.32±6.38 ^{A} (H)	8.60±2.59 ^{B,Z} (H)

* 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
^{H} *Tukey's* test HSD

DISCUSSION

Selenium supplementation remains an important question as its deficiency is observed in pathological states and supplementation may exert beneficial influence in many cases. One of the last studies displayed preventive action of selenium against mercury toxicity. Co-administration of selenium inhibited decrease in erythrocytes, leucocytes, haemoglobin and haematocrit observed in fish exposed to mercury [1].

Our researches on influence of selenoorganic compounds' treatment on blood morphology, both present and previously undertaken on mice, showed that changes of structure of the administered compound modified their effect in ambiguous way. Organic selenocompounds of the similar structure as those applied in

Table 5. Contrast analysis between group I (Control) and connected groups II + III + IV (selenium supplementation) as well as between group II (inorganic selenium) and connected groups III + IV (organic selenium) of blood biochemistry and phagocytic capability of granulocytes and NBT test.

	Phagocytosis	NBT	Glucose	Cholesterol
I vs. II + III + IV	*** ↓	** ↓	*** ↓	
II vs. III + IV				*** ↑

**p < 0.01; *** p < 0.001
↑ - increase; ↓ - decrease

this study did not change haemoglobin and haematocrit, whereas neutrophils were increased and lymphocytes diminished [14]. In the present experiment chain compound A decreased haemoglobin and haematocrit. Cyclic compound B only diminished leucocytes. In the present study inorganic selenite decreased haematocrit and leucocytes. These observations are consistent with those obtained in experiment undertaken on calves treated with 6 mg Se (Na_2SeO_3) + 300 U vitamin E per 45 kg b.w. The authors reported that in three-week-old animals hemoglobin and white blood cells were decreased. In four-week-old ones haematocrit was diminished [11]. *Katamoto* et al. also reported that in animals exposed to heat haematocrit was decreased after 8 days of selenium + vitamin E treatment [10].

In the case of blood biochemistry the present experiment revealed that only cyclic organic compound B did not cause any significant changes. The decrease in glucose and cholesterol observed in group II (selenite) was consistent with the outcomes reported by *El-Demerdash*. The author found that rats given 0.2 g Se/kg b.w. (as selenite) displayed decrease in cholesterol, glucose and urea both in healthy animals and in those additionally exposed to $AlCl_3$ [4].

Our previous studies concerned the effect of selenocompouns on NBT test as well as phagocytic capacity of granulocytes showed that chain and cyclic compounds of similar structures as compounds used in the present experiment as well as selenite caused decrease in these parameters [12, 14]. Regarding the compounds studied in the present experiment selenite and chain organic compound also caused decrease, whereas the little change in cyclic compound structure resulted in entirely different effect - no alterations were found. The question of relationships between selenium and phagocytosis has not been practically studied yet. In the only available article *Katamoto* et al. reported increase in NBT reduction by neutrophils in goats exposed to heat and provided with selenium + vitamin E for 8 days [10].

CONCLUSIONS

1. Selenorganic compound B 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline of cyclic structure disturbed morphology, biochemical parameters and phagocytic capacity of granulocytes in rats to the least degree, compared with chain organic 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid and inorganic sodium selenite.
2. The obtained outcomes let suggest that further studies would be reasonable to consider application of compound B as a selenium supplement.

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