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Effects of selenium inorganic and two new organic compounds supplementation on morphotic blood elements and antioxidant status in mice

Selenium is assimilated in the alimentary canal; its organic compounds show higher capacity of assimilation and the most favourable therapeutic effects are observed in combination with vitamins A, C and E (1). Selenium deficiency may lead to diseases in certain human populations. Combined E and Se deficiency resulted in significantly lower total (oxidised-reduced) mitochondrial coenzyme Q-9 (Co Q-9) concentration compared with control rats supplemented with dietary E and Se (5). Selenium supplementation dramatically reduced the incidence of Keshan disease (12), an osteoarthropathy characterized by necrosis of the cartilag. The involvement of Se in immune responses has been well documented. It appears to affect non-specific immune system, humoral immunity, cellular immunity and cytotoxicity (7), although cell mediated immunity is principally affected by Se deficiency. Some authors (6) report that low blood levels of selenium correlate with a higher prevalence and incidence of cancer. The metalloid element as a structural component of the active centre of glutatione peroxidase enzymes (SeGSH-Px) plays a major role in the intercellular antioxidant system (10). Selenium cumulation was determined in liver, kidney, heart, lung, adrenals, thyroid, spleen, pancreas, ovaries, brain, muscle and plasma, after selenium supplementation (15).

In our laboratory we produced two organic compounds, 4-(-o-tolyl-)-selenosemicarbazide of p-chlorobenzoic acid in the reaction of addition o-tolyl isoselenocyanate and p-chlorobenzoic acid hydrazide (11) and 3-(-p-chlorobenzoylamino-)-2-(-o-tolylimino-)-4-phenyl-4-selenazoline by condensing of 4-(-o-tolyl-)-selenosemicarbazide of p-chlorobenzoic acid with omega-bromoacetophenone. To determine the biological properties of these compounds the analysis of selenium cumulating in organs was performed, its effects on blood formation, phagocytes and antioxidative properties in mouse neutrophiles after the supplementation with two organic compounds were examined and compared with the effects induced by inorganic Na_2SeO_3.

MATERIAL AND METHODS

SYNTHESIS OF COMPOUNDS

1. 4-(o-tolilo)-selenosemicarbazide p-chlorobenzoic acid.

The compound was obtained by addition of 0.1mol p-chlorbenzoic acid hydrazide by warming 0.5h in 30cm^3 of methanol. The compound obtained in the form of sediment was filtered and four times crystallized from 90% ethanol. The obtained compound of t.t. 163-165 ^{0}C was identified by burning and percent determination of the content of carbon, hydrogen and nitrogen and by spectral measurements within the range of UV, IR and
HN-MR.

2. 3-(p-chlorbenzoilamino-)-2-(o-tolylimino-)-4-phenyl-4-selenazoline

The synthesis of the compound was obtained by condensing equal mol quantities of 4- (o-tolyl-)-selenosemicarbazide of p-chlorobenzoic acid and omega-bromoacetofenone, warming up the mixture to the temp. 250^{0}C in 40cm^3 methanol in a sequence of 3 h, next it was neutralized by sodium acetate water solution and four times crystallized with the use of 20 cm^3 95% ethanol each time. The obtained compound of tt 184-186^{0}C was identified as in the synthesis of selenosemicarbazide.

The syntheses of the compounds follow the reaction:

$o\text{-}CH_3\text{-}C_6H_4NC\ (Se) + H_2N\text{-}NH\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p \rightarrow$

$\rightarrow$ $o\text{-}CH_3\text{-}C_6H_4\text{-}NH\text{-}C(Se)\text{-}NH\text{-}NH\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p$

4-(o-tolyl-)-selenosemicarbazide p-chlorobenzoic acid

Calculated: 49.12%C, 3.84%H, 11.46%N

Obtained : 48.80%C, 3.68%H, 11.17%N

The spectrum UV λmax/ε [nm] of the band: 234/23205, 271/13200.The spectrum IR - the band of CO (amid I) group was determined at 1662 cm^{-1} HN-MR:DMSO-d6:10.67(s,1H,NH), 10.10 (s,1H,NH),7.12-7.99 (m,8H,ar),2.18 (s,3H,CH_3-ar).

$o\text{-}CH_3\text{-}C_6H_4NH\text{-}C(Se)\text{-}NH\text{-}NH\text{-}CO\text{-}C_6H_4Cl\text{-}p + Br\text{-}CO\text{-}CH_2\text{-}C_6H_5 \rightarrow$

$\rightarrow$ p-Cl-C_6H_4-CO-NH-N$=$C-C_6H_5

o-$CH_3C_6H_4$-N=C — CH

\ /

Se

3-(p-chlorobenzoilamino-)-2- (o-tolylimino-)-4-phenyl-4-selenazoline

Calculated: 59.17%C, 3.88%H, 9.00%

Obtained: 59.09% C,3.06%H, 8.90%N

UV λmax/ε [nm] of the band: 233/30445,the spectrum IR - the band of CO (amid I) group was determined at 1678cm^{-1}, H-NMR : $CDCl_3$ 11.656 (s,1H,NH), 7.405-6.988 (m.,13H,ar), 6.279 (s,1H,N-C=CH), 2.340 (s,3H,CH_3-ar).

Both organic compounds insoluble in water were left till the time of biological tests.

DESCRIPTION OF THE EXPERIMENT

Selenosemicarbazide and selenazoline was suspended in the emulsion containing olive oil, arabic gum and water in the following proportions: 2:1:1.5. The compound suspended in this emulsion was given to SWISS mice in stomach tube at the dose of 10^{-3} mg Se per g^{-1} of body mass for 10 days. The control group was made of 10 female mice without selenium supplementation. The animals, aged 8 weeks, with comparable body mass, were kept in identical conditions. The body mass before the experiment ranged from 24 to 27g, after the experiment ranking from 25.2±1.3 g to 25.8±1.7 g. In the group supplemented with sodium selenite IV the body mass was 24.8±1.6 g, after the supplementation with selenosemicarbazide being 25.4 ±1.2g and selenazoline 24.9±1.7g.No statistically significant differences were observed. The animals

received a standard LSM fodder and water without limitations. Blood was collected from heparinised test tubes and the numbers of erythrocytes, leukocytes, hematocrit as well as haemoglobin concentration were determined. In a smear stained with May-Grunwald's method the percentage of white morphotic blood elements was determined which served as the basis for calculating the absolute number of cells in one mm^3 of blood. Phagocyte reaction with Bato-Latex (Difco, USA) was used to assess phagocyte capacity of neutrophils. Oxidoreductional potential of neutrophils was examined with the NBT test.

RESULTS

The results of blood parameters including erythrocytes, haematocrit, haemoglobin concentration and leukocytes were not changed (Tab.1).The examination of the leukocytes composition in mice following the administration of selenium compounds revealed complete absence of monocytes as well as statistically decreased lymphocytes in the group supplemented with Na_2SeO_3 and selenosemicarbazide compared to the controls, however, the number of neutophiles was increased in the group supplemented with both organic compounds (seleno-semicarbazide 980±134 and selenazoline 1040±965 in comparison to the control group (800±179).

Table 1. Morphological parameters of blood in SWISS mice after the supplementation sodium selenite IV, selenosemicarbazide and selenazoline to the control group

Mouse group	Haemoglobin (g%)	Heamatocrit (%)	Erythrocytes (10^6 /mm^3)	Leucocytes (10^3 /mm^3)
Controls without Se supplementation	11.978±1.230	46.100 ± 1.673	9.060 ± 0.285	2.980 ± 1.417
After sodium selenite IV supplementation	13.366 ± 0.411	45.200 ± 2.588	8.866 ± 0.519	2.120 ± 0.605
After selenosemicarbazide supplementation	13.178 ± 0.517	46.900 ± 0.961	9.188 ± 0.198	2.120 ± 0.455
After selenazoline supplementation	12.424 ± 1.442	46.200 ± 4.944	9.006 ± 0.953	2.260 ± 0.873

The highest bacillus content was observed after selenazoline administration (tab. 2), which was statistically significant. The examination of phagocyting function and intracellular processes of nitroblutetrazolium caused reduction the administration of selenium compounds (tab. 3).

Table 2. White blood count in SWISS mice after the supplementation with sodium selenite IV, selenosemicarbazide and selenazoline to the control group

Mouse group	Neutrophils number mm^{-3}	Bacillus number mm^{-3}	Monocytes number mm^{-3}	Lymphocytes number mm^{-3}
Controls without Se supplementation	800 ± 179	70 ± 52	8.00 ± 79	2516 ± 1228
After sodium selenite IV supplementation	300 ± 670	30 ± 33	0.000(*)	1519 ± 541 (*)
After selenosemicarbazide supplementation	980 ± 134 (**)	44 ± 13	0.000(*)	1565 ± 448 (*)

After selenazoline supplementation	1040 ± 965 (***)	198 ± 96 (***,***)	3.000 ± 6.70	1952 ± 867

Table 3. The comparison of granulocite phagocytic abilities and NBT in SWISS mice following the supplementation with sodium selenite IV, selenosemicarbazide and selenazoline against the control group

Mouse group	Neutrophils number mm^{-3}	Phagocytosis % of positive cells	NBT test % of positive cells
Controls without Se supplementation	800 ± 179	61.20 ± 7.430	7.000 ± 1.000
After sodium selenite IV supplementation	300 ± 670	25.600 ± 20.500 (*)	4.400 ± 1.673 (*)
After selenosemicarbazide supplementation	980 ± 134 (**)	36.00 ± 13.416 (*)	2.800 ± 1.095 (*)
After selenazoline supplementation	1040 ± 965 (***)	48.00 ± 26.496 (***)	3.600 ± 2.190 (*)

SD – standard deviation

* Statistical significance in comparison with the control group $p.<0.05$

** Statistical significance after selenosemicarbazide supplementation in comparison with sodium selenite supplementation

*** Statistical significance after selenazoline supplementation in comparison with sodium selenite supplementation

**** Statistical significance after selenosemicarbazide supplementation in comparison with selenazoline supplementation

DISCUSSION

Selenium was shown to function as an antioxidant that may enhance immunity during microbial infection. The aim of our research was to find adequate form of selenium compound offering the highest selenium intake and to examine its influence upon haemopoesis and immunity. Thus we were looking for the most effective forms of selenium compounds. We compared two forms of organic selenium with inorganic Na_2SeO_3. The results showed that inorganic compounds, which do not dissolve in water, dissolve well in the emulsion whose composition was presented in the "Material and methods" section. Coxackie virus was isolated from the blood of patients with Keshan disease and is thought likely to be a cofactor in the development of cardiomyopathy (3,4). It seems probable, therefore, that human selenium deficiency similarly affects the viral genome resulting in the development of the heart pathology. Our studies of haemopoesis supplementation with selenium found no changes. Selenium deficiency also depresses the effectiveness of immune cells, especially immunoglobulin production and antiviral resistance. Selenium supplementation boots cellular immunity by T cells function and (indirectly) B cells function activation and by protecting the immune cells against antioxidative stress-induced damage (13). Our study examined the effects of selenium supplementation upon phagocytosis and NBT test (14). Selenium influence upon the immune functions is dose-dependent. It acts both as a stimulant (9) and immunosuppressant. The dose used in our investigations, 10^{-3} mg/1g resulted in decreased phagocyting activity. Theoretically, the way it was used could have resulted in increased neutrophils reactivity (8). Reduction properties inside neutrophils are important for intracellular metabolism and killing

the bacteria phagocyted. Selenium compound supplementation and its intake by the enzymes resulted in decreased NBT test due to selenium oxido-reductive properties. Complete NBT reduction to dipharmazan needs two electrons. Reduction can have a four-stage course. At the first stage a relatively stable NBT free radical forms; it can dismutate to NBT^{+2} and monopharmazan or it can react with monopharmazan reductor. Dicatione form NBT^{+2} is yellow, however, the colour reduction, thus loss of potential and tear of tetrazolic rings, leads to changed spectral properties (the appearance of intense blue colour) (2). Our investigations found that selenium compounds supplementation and intake by the enzymes resulted in decreased NBT test.

CONCLUSIONS

After analyzing the experiments with two newly organic compounds of selen, synthesized in our laboratory, it was found that they reveal different satisfactory degree of combining into the studied tissues and organs and an ability to regulate investigated immunological parameters.

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SUMMARY

Two organic compounds, 4-(o-tolilo-)-selenosemicarbazide of p-chlorobenzoic acid and 3-(p-chlorobenzoylamino-)-2-(o-tolylimino-)-4-phenyl-4-selenazoline were compared to the effects of the supplementation with inorganic Na_2SeO_3. Studies were carried out in four groups consisting of 10 female mice each of SWISS strain. Three of them were supplemented with different selenium formula at the dose of 10^{-3} mg Se per g over the period of 10 day. The blood samples were collected to heparinized test tubes; the red blood and white blood count, hematocrit and haemoglobin concentration were studied. The influence of selenium compounds on phagocytosis and NBT test was determined.

Wpływ suplementacji selenu nieorganicznego i dwóch nowych organicznych związków na morfologię krwi i właściwości antyoksydacyjne u myszy

Dwa organiczne związki 4-(o-tolilo-)-selenosemikarbazyd kwasu p-chlorobenzoesowego i 3-(p-chlorobenzoilo-amino-)-2-(o-toliloimino-)-4-fenylo-4-selenazolina były porównywane z nie-organicznym Na_2SeO_3. Badania wykonano w czterech grupach po 10 samic myszy szczepu SWISS. Trzy grupy suplementowano różnymi związkami selenu w dawce 10^{-3} mg Se na g masy ciała w ciągu 10 dni, czwartą grupę stanowiła kontrola bez suplementacji. Zwierzęta karmiono paszą standardową LSM i pojono wodą *ad libitum*. Krew pobierano do probówek heparynizowanych i oznaczano białe ciałka, hematokryt i hemoglobinę. Określano wpływ związków selenu na zdolności fagocytarne i potencjał oksydoredukcyjny neutrofili.