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Comparison of selenium distribution in mice organs after the supplementation with inorganic and organic selenium compound selenosemicarbazide

Multidisciplinary studies have proved that selenium is a key component of a number of functional selenoproteins which are present in the whole body and necessary to stay healthy. Selenium is a basic element of glutathione peroxidase enzymes, which reduce hydrogen peroxide and organic hyperoxides generated by both physiological and pathological processes (9). Various cell types, organs and tissues were investigated with regard to selenoproteins contents and selenium antioxidant enzymes activity in lung (8), blood (4, 12), thyroid gland (1), reproductive cells and male gonads , liver, kidney, brain (2, 10), muscle, hair (4) serum and urine (15). Selenium dietary and metabolism selenium alternations or deficiency have some relationship to cancer incidence myocardiopathy in humans (Keshan disease), pancreatic degeneration (14) and Alzheimer disease (7). To test the protective qualities of selenium supplementation a number of trials was performed using inorganic selenium formulation alone, inorganic selenium compounds combined with vitamins and minerals and organic formulation (12) and mainly seleno-DL-methionine, propyl 2-2-diphenyl diselenite (2). It seems that synthetic organic compounds (11) and natural high selenium yeast (6) are the most effective and safest forms of selenium preventing selenium intoxication. Partially positive results encourage the search for the right therapeutic formulation of selenium. In our laboratory we have produced an organic compound, 4-(o-tolyl)-selenosemicarbazide of o-chlorobenzoic acid in the reaction of addition of o-tolilo isoselenocyanate and o-cholorobenzoic acid hydrazide. It is a relatively well absorbable selenium compound. The aim of our study was to determine the concentration and distribution of selenium in several organs and tissues in mice after

selenium supplementation with our newly synthesised organic formula in comparison to the effects of supplementation with inorganic compounds.

MATERIAL AND METHODS

THE SYNTHESIS OF 4-O-TOLYL-SELENOSEMICARBAZIDE OF O-CHLOROBENZOIC ACID

The compound was obtained in the reaction of addition of adequate semiproducts and the final product was subjected to chemical analysis. 0.2 M of o-tolyl isoselenocyanate and 0.2 M of o-chlorobenzoic acid hydrazide in 50 cm^3 of methanol was heated and kept at a boiling point for half an hour. The obtained compound was drained off and a solid product was crystallised three times in 50 cm^3 of 95% ethanol 4-o-tolyl-selenosemicarbazide and was then identified by combusting and determining the proportional contents of carbon, hydrogen and nitrogen. Calculated: 49.12% of carbon, 3.84% of hydrogen, 11.46% of nitrogen. Obtained in the process of combustion and in spectral measurements in the range of UV, IR, H-NMR. 48.91% of carbon, 3.72% of hydrogen, 11.27% of nitrogen.

The spectrum UV – λ_{max}[nm]/ε of the band: 280/13880. The spectrum IR – the band of CO (amid I) group was determined at 1672 cm^{-1}. H NMR: DMSO – d6: 10.521 (s,1H, NH), 10.141 (s, 1H, NH), 9.775 (s, 1H, NH), 7.165 - 7.939 (m, 8H, ar), 2.208 (s, 3H, CH_3-ar). The compound which is insoluble in water and has the molecular formula: o-CH_3-C_6H_4-NH-CSe-NH-NH-CO-C_6H_4-o-Cl, was left for biological experiments.

DESCRIPTION OF THE EXPERIMENT

Selenosemicarbazide 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 10 eight-week-old, female SWISS mice in a stomach tube at the dose of 10^{-3}mg Se per g^{-1} of body mass every day for the period of 10 days. The reference group consisted of 10 female mice fed with sodium IV selenite (Na_2SeO_3) also 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 dose was set at the subtoxic level in order to determine the accumulation of selenium in the form of selenosemicarbazide compared with commonly investigated sodium selenite as well as to detect immunological effects of down-regulatory reaction. Selenium was given at different concentrations: 0.1 - 1.0 mg Se per kg^{-1} (15) and 0.5 - 8.0 mg Se per kg^{-1} of body mass (10). The animals were of similar age, comparable body mass and were kept in identical conditions. The body mass before

the experiment ranged from 24 to 27 g, after the experiment ranging from 25.2 ± 1.3 g to 25.8 ± 1.7 g. In the group supplemented with sodium selenite the body mass was 24.8 ± 1.6 g, after supplementation with selenosemicarbazide being 25.4 ± 1.2 g. No statistically significant differences were observed. The animals were receiving a standard LSM fodder and water without limitations. After 10 days of the experiment the animals were put to death and their organs were collected to determine the contents of selenium.

SELENIUM CONTENT IN ORGANS

The selenium organ content was studied in heart, brain, lungs, thigh and intercostal muscles. After collecting the organs they were weighted and frozen till the time of mineralisation. The mineralisation was carried out according to Shimoishi (5). 500 mg of the tissue in 10 cm^3 of concentrated nitric acid was placed in a Kjeldhal's flask and heated for about one hour at the temperature of 150° C in a silicone bath. It was then cooled, 3 cm^3 1M solution of urea was added and the solution was heated for 10 minutes. The solution was then cooled again and 10 cm^3 of distilled water and 1 cm^3 of 0.6% DANB (1,2-diamino-4-nitrobenzene – made by Fluka) solution was added. In order to obtain 5-nitropiazselenole the solution was left standing for two hours and then extracted with 5 cm^3 of toluene. In order to remove tissue-derived chromophoric substances the solution was shaken in 20 cm^3 of 1 M solution of sodium hydroxide and then toluene solutions were washed with 10 cm^3 of 7.5 M solution of hydrochloric acid. The selenium content was established spectrophotometrically (specord M-40, Carl Zeiss Jena) by determining the absorbance of toluene solution at the wavelength of 350 nm. The concentration was calculated from the standard curve plotted from DANB and standard solution of 100 μg Se/cm^3 in 0.1 M solution of hydrochloric acid. Se values were given as per gram wet weight.

RESULTS

Selenium concentrations in the control group as well as in the groups supplemented with inorganic selenium compounds and selenosemicarbazides were determined in several organs and tissues. In the control group of mice without any selenium supplementation the concentration varied in different tissues and organs. In our study the lowest selenium concentration was found in lungs (1.75 μg per 1 g of the tissue), higher concentrations being found in heart (8.75 μg/ 1g) and intercostal muscles (10.13 μg/1g). We compared the increase in selenium concentrations in the selected tissues after supplementation with inorganic selenium compounds with that with selenosemicarbazide. Despite supplementation we found no increase in selenium concentration in heart (9.08 μg/g

and 8.75 μg/g). Investigating selenium concentrations in brain we found an insignificant increase after supplementation with inorganic selenium compounds (32.00 μg/g) and a statistically significant increase ($p<0.05$) after the supplementation with selenosemicarbazide (38.4 μg/g). A multifold, statistically significant increase in selenium level after supplementation was found in lungs; it is worth noticing, however, that many times higher concentrations were observed after the supplementation with organic com-

Table 1. Selenium concentration in brain, heart and lung after selenium compounds supplementation

Mouse group	Heart		Brain		Lung	
	average organ mass ± SD(g)	average Se content μg/1g tissue ± SD	average organ mass ± SD(g)	average Se content μg/1g tissue ± SD	average organ mass ± SD(g)	average Se content μg/1g tissue ± SD
Controls without Se supplementation	0.18 ± 0.04	8.79 ± 0.07	0.40 ± 0.05	26.18 ± 7.20	0.20 ± 0.01	1.75 ± 0.72
After sodium IV selenite supplementation	0.17 ± 0.01	9.08 ± 2.05	0.45 ± 0.05	32.00 ± 2.70	0.19 ± 0,01	**6.79 ± 2.70***
After selenosemicarbazide supplementation	0.17 ± 0.01	8.75 ± 3.50	0.39 ± 0.04	**38.04 ± 10.20***	0.20 ± 0.02	**11.81 ± 1.80*** (**)

SD – standard deviation.

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

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

pounds of selenium (Table 1). Measurements of selenium concentrations in thigh and intercostal muscles were also performed. We found an insignificant increase in selenium concentration in thigh muscles after supplementation. In intercostal muscles we found an insignificant increase in selenium concentration after supplementation with inorganic compounds and a statistically significant increase after supplementation with selenosemicarbazide (28.84 μg/g). We also found a significant increase in the mass of intercostal muscles (Table 2).

Table 2. Selenium concentration in thigh and intercostal muscle after selenium compounds supplementation

Mouse groupe	Thigh		Intercostal muscle	
	average organ mass ± SD(g)	average Se content μg/1g tissue ± SD	average organ mass ± SD(g)	average Se content μg/1g tissue ± SD
Controls without Se supplementation	0.51 ± 0.04	23.35 ± 6.20	0.20 ± 0.04	10.13 ± 1.50
After sodium IV selenite supplementation	0.45 ± 0.05	31.98 ± 9.00	**0.28 ± 0.02***	14.21 ± 7.00
After selenosemicarbazide supplementation	0.49 ± 0.04	28.40 ± 11.30	**0.27 ± 0.03 ***	**28.84 ± 12.10*** (**)

SD – standard deviation.

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

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

DISCUSSION

Positive results of selenium supplementation in numerous diseases, sometimes only in selenium deficiency or as prevention, encourage the search for investigation of the right form of selenium and an appropriate treatment regimen. In our preliminary investigations we compared the assimilation and distribution binding of selenium in different organs after the commonly used supplementation with inorganic compounds and our newly synthesised organic compound. The results of selenium binding varied both for different organs and for different selenium formulations. The selenium concentration in heart was quite surprising; despite supplementation selenium levels were highly stable in both groups. This result is especially interesting considering that some cardiological disorders are associated with selenium metabolism and its deficiency (14). Comparing selenium concentrations in different tissues in mice before supplementation, the selenium concentration in lungs was strikingly low compared with that in brain, thigh muscles and intercostal muscles (1.75 μg/g, 26.18 μg/g, 23.35 μg/g and 10.13 μg/g, respectively).

The selenium concentration in the lung tissue is strictly connected with the presence of glutathione peroxidase which scavenges hydrogen peroxide damaging lipid and phospholipid hydroperoxides generated *in vivo* by free radicals and other oxygen-derived substances. Chronic deficiency of selenium in a diet results in a gradual decrease in glutathione peroxidase levels and altered response to environmental stress (8). In regard to the selenium presence in lungs it is also important to notice that relatively harmless viruses

can become virulent by passing through a selenium deficient host (3). Selenium supplementation seems to be especially effective considering a multiple increase in its concentration (from 1.75 μg/g to 6.79 μg/g) after the administration of inorganic selenium compounds and even greater increase (to 11.81 μg/g) after the administration of selenosemicarbazide. The lung tissue seems to be especially sensitive to the selenium deficiency and although no positive effects have been achieved after selenium supplementation for cancer prevention in patients with a skin cancer (6) patients treated with selenium showed a significant reduction in mortality and incidences of a lung cancer (13). While administering selenium in two comparable forms: organic and inorganic it has been shown that the ability of the brain tissue to bind selenium compounds is greater in case of selenosemicarbazide (38.04 μg/g) in comparison with 32.00 μg/g for sodium selenate. Studies on selenium contents in thigh and intercostal muscles have shown not only diverse selenium binding abilities but also an increase in the muscle mass after supplementation with inorganic and organic compounds of selenium. Significant increase in selenium contents in the muscle tissue was found only in animals supplemented with selenosemicarbazide. In thigh muscles, however, a moderate increase in selenium contents was observed after its supplementation. Establishment of selenosemicarbazide as formula of selenium supplementation needs additional investigations of enzymatic activities. To sum up, we can say that a selenosemicarbazide compound synthesised in our laboratory is more easily assimilated than an inorganic selenium formulation. Our studies are only preliminary observations that should encourage further investigations.

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SUMMARY

Studies on selenium organ content and its function in living organisms just like studies on other elements provide interesting results although their interpretation is not always clear. The aim of our study was to determine the concentration and distribution of selenium in several organs and tissues in mice after supplementation with our newly synthetized organic compound of selenium selenosemicarbazide (4-o-tolyl-selenosemicarbazide of o-chlorobenzoic acid) as compared to the effects of the supplementation with inorganic compounds. SWISS mice were fed with both types of compounds at the dose of 10^{-3}g Se per kg for the period of 10 days. The concentrations of selenium in brains of mice treated with selenocarbazide and sodium selenite were higher than in controls (38.04 μg g^{-1} and 32.00 μg g^{-1} vs. 26.18 μg g^{-1}). There was a statistically significant increase in the selenium contents in lungs after supplementation with selenosemicarbazide and sodium selenite (11.81 μg g^{-1} and 6.79 μg g^{-1} vs. 1.75 μg g^{-1} in controls). We found a statistically insignificant increase in selenium contents in intercostal muscles after supplementation with inorganic selenium compounds and a statistically

significant increase after the supplementation with selenosemicarbazide (10.13 $\mu g\ g^{-1}$; 14.21 $\mu g\ g^{-1}$ and 28.84 $\mu g\ g^{-1}$, respectively). Our investigations lead to a conclusion that 4-o-tolyl-seleno-semicarbazide of o-chlorobenzoic acid, an organic selenium compound may be more easily absorbed than inorganic sodium IV selenite.

Porównanie zawartości selenu w narządach myszy po suplementacji nieorganicznym i organicznym związkiem selenu selenosemikarbazydem

Badania zawartości oraz funkcji selenu w organizmach żywych, podobnie jak innych pierwiastków, dostarczają interesujących wyników, jakkolwiek interpretacje nie zawsze są jednoznaczne. Celem naszej pracy było oznaczenie stężenia i dystrybucji selenu w poszczególnych narządach i tkankach myszy po suplementacji 4-(o-tolilo)- selenosemikarbazydu kwasu o-chlorobenzoesowego w porównaniu z nieorganicznym selenianem IV sodu. Badany związek oraz nieorganiczny selenian IV sodu podawano myszom SWISS w dawce 10^{-3} g Se kg^{-1} masy przez 10 dni. Stwierdzono podwyższoną zawartość selenu w mózgu 31,98 mg g^{-1} w grupie suplementowanej selenianem IV sodu, 38,04 mg g^{-1} w grupie suplementowanej selenosemikarbazydem v s. z grupą kontrolną 26,18 mg g^{-1}. Kikakrotny i istotny statystycznie wzrost zawartości selenu stwierdzono w tkance płucnej 11.81 mg g^{-1} po suplementacji selenosemikarbazydem, 6.79 mg g^{-1} selenianem IV sodu v s. 1.75 mg g^{-1}. Również w mięśniach międzyżebrowych stwierdzono nieistotny wzrost selenu na gram masy tkanki po suplementacji selenem nieorganicznym i istotny statystycznie wzrost stężenia po suplementacji selenosemikarbazydem 10,13 mg g^{-1} w grupie pierwszej i odpowiednio 14,21 mg g^{-1}, v s. 28,84 mg g^{-1}.Nasze badania pozwalają na stwierdzenie, że związki organiczne selenu mogą być łatwiej przyswajalną formą Se w porównaniu z nieorganicznym selenianem IV sodu.