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INFLUENCE OF THE ADMINISTRATION OF SELENIUM COMPOUNDS ON TISSUE MAGNESIUM CONCENTRATION IN RATS

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Abstract

Magnesium and selenium belong to important bioelements. Magnesium is the second most abundant intracellular macroelement, which takes part in the metabolism of carbohydrates, nucleic acids, protein and lipids. Selenium is an essential microelement, whose deficit has been stated in many different pathological states. Much research on safe and effective selenium supplementation has been performed for the last fifty years but the results still remain unsatisfactory.

The aim of our study was to investigate the influence of inorganic sodium selenite Na_2SeO_3 and two selenoorganic compounds synthetized at our chair on magnesium concentration in tissues of adolescent male Wistar rats. Inorganic selenite was administered as a water solution, whereas organic compounds: 4-(o-tolilo)-selenosemikarbazyd of 2-chlorobenzoic acid of a chain structure (ORG-C) and 3-(o-chlorobenzoylamino)-2-(o-tolylimino)-4--methyl-4-selenazoline of a ring structure (ORG-R) were suspended in emulsion (oil, arabic gum and water). Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg Se g^{-1} b.w. once a day for a period of 10 days. The control group was treated with saline. The administration was performed with use of a stomach tube.

In comparison to the control group, selenium supplementation caused decrease in magnesium concentration in kidney and lung tissues, but did not cause any changes in the brain and heart muscle. In the liver and spleen it was only ring selenazoline that affected magnesium concentration, increasing it in the liver and decreasing in the spleen. In the femoral muscle it was only the selenosemicarbazide chain that exerted the significant effect causing a decrease in Mg concentration vs the control group.

Selenium supplementation influences the tissue magnesium concentrations depending on tissue and structure of the supplement. Irrespective of the administered compound, it lowered magnesium in kidneys and lungs but caused no changes in the brain and heart muscle. In the liver, spleen and femoral muscle, alterations in the magnesium concentration were dependent on the provided supplement.

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Key words: male rats, organoselenium compounds, supplementation, magnesium.

WPŁYW PODAWANIA ZWIĄZKÓW SELENU NA TKANKOWE STĘŻENIE MAGNEZU U SZCZURÓW

Abstrakt

Magnez i selen należą do biopierwiastków bardzo ważnych dla prawidłowego funkcjonowania organizmu. Magnez jest drugim co do ilości makropierwiastkiem wewnątrzkomórkowym, który odgrywa istotną rolę w metabolizmie węglowodanów, kwasów nukleinowych białek i lipidów. Selen jest niezbędnym mikroelementem, którego deficyt został stwierdzony w różnych stanach patologicznych. Przez ostatnie 50 lat prowadzono rozległe badania nad skuteczną i bezpieczną suplementacją tego pierwiastka, ale uzyskane wyniki nie są do końca satysfakcjonujące.

Celem pracy było zbadanie wpływu nieorganicznego selenianu(IV) sodu Na_2SeO_3 i dwóch organicznych związków selenu o różnej budowie na stężenie magnezu w tkankach młodych samców szczurów rasy Wistar. Nieorganiczny selenian(IV) sodu podawano w postaci wodnego roztworu, natomiast organiczne związki selenu: 4-(o-tolilo)-selenosemikarbazyd kwasu 2-chlorobenzesowego (ORG-C, budowa łańcuchowa) i 3-(2-chlorobenzoiloamino-)-2-(o-toliloimino-)-4-metylo-4-selenazolina (ORG-R, budowa pierścieniowa) w formie emulsji złożonej z oleju, gumy arabskiej i wody. Grupa kontrolna otrzymywała sól fizjologiczną. Związki podawano sondą dożołądkowo w dawce $5 \cdot 10^{-4}$ mg Se g^{-1} m.c. 1 raz dziennie przez okres 10 dni.

W porównaniu z grupą kontrolną nieotrzymującą selenu, suplementacja związkami Se wpłynęła na statystyczny spadek stężenia magnezu w tkankach nerki i płuca, natomiast nie spowodowała żadnych zmian w tkance mózgu i mięśnia serca. W tkance wątroby i śledziony jedynie cykliczna selenazolina wpłynęła na stężenie magnezu – w wątrobie zaobserwowano wzrost, a w śledzionie spadek. W tkance mięśnia uda jedynie łańcuchowy selenosemikarbazyd wywarł istotny wpływ, powodując obniżenie stężenia Mg w stosunku do grupy kontrolnej.

Suplementacja selenu wpływa na tkankowe stężenie magnezu w sposób zależny od rodzaju tkanki i struktury zastosowanego suplementu. Niezależnie od budowy podawanego związku, zaobserwowano obniżenie stężenia magnezu w tkance nerki i płuca, natomiast nie zauważono żadnych zmian w mózgu i mięśniu serca. W tkankach wątroby, śledziony i mięśnia uda zmiany stężenia magnezu były zależne od rodzaju podawanego związku.

Słowa kluczowe: szczury samce, organiczne związki selenu, suplementacja, magnez.

INTRODUCTION

Magnesium is the second most abundant intracellular macroelement (Telci et al. 2002). It takes part in the metabolism of carbohydrates, nucleic acids, protein and lipids (Barbosa et al. 2010). Many disorders of functions in a human body can be connected with magnesium deficiency e.g.: disturbance of the cardiovascular system and homeostasis of other bioelements, muscle weakness as well as decreased parathyroid hormone secretion (Shoback 2008, Assadi 2010).

Selenium belongs to essential trace bioelements and its deficiency in an organism may result in numerous severe diseases, for example a low selenium level has been found in cases of alimentary tract illnesses (Skelton et al. 2006), dermatic and nephrological disorders (Ingen-Housz-Oro et al. 2004, Zachara et al. 2004) and AIDS development after HIV-infection (Rayman 2000). The question of selenium supplementation is rather complicated because of the narrow range between therapeutic and toxic doses (Hawkes et al. 2008). Moreover, its bioavailability depends on the structure of a used supplement (Burk et al. 2006). For the last fifty years extensive research on supplementation of selenium has been carried out (Combs 2005, Řezanka, Sigler 2008, Selamoglu Talas et al. 2009), including both inorganic (sodium selenite or selenate) (Ivancic, Weiss 2001, Uezono et al. 2006) and organic compounds (selenomethionine, selenocyanates, selenic acids of a chain structure as well as compounds of a cyclic form e.g. ebselen which has a benzisoselenazolone structure) (Xia et al. 2004, Burket et al. 2006, Cui et al. 2008). However, the problem of safe and efficient Se-supplementation still remains unsolved.

Two selenium organic compounds synthesized at our chair: 3-(2-chlorobenzoylamino)-2-(o-tolylimino)-4-methyl-selenazoline (Musik et al. 2009) possessing an ebselen-like, ring structure and 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid (Musik et al. 2002b) of a chain structure were studied in regard of the possibility of their application as a selenium supplement. Taking into account the importance of magnesium for proper functions of a human organism, we investigated the effect of the oral administration of the above compounds on the Mg tissue concentrations in rats.

MATERIALS AND METHODS

The experiment was carried out on four groups of adolescent male Wistar rats (ten animals each):

Group I – control treated with saline (n=10);
Group II – treated with water solution of sodium selenite (Na_2SeO_3), n=10;
Group III – treated with 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid – compound ORG-C (n=10);
Group IV – treated with 3-(2-chlorobenzoylamino)-2-(o-tolylimino)-4-methyl-4--selenazoline – compound ORG-R (n=10).

The weight of the animals at the beginning of the study was within the range of 110-150 g. Organic compounds given to groups III and IV were suspended in emulsion composed of oil, arabic gum and water in the following proportions 2:1:1.5. Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg Se g^{-1} b.w. once a day for a period of 10 days. The administration was performed with use of a stomach tube. The body weight of animals was measured every day before Se administration. Rats had free access to standard feed LSM and drinking water.

After the experiment, the animals were sacrificed under pentothal narcosis and the tissues of their kidneys, liver, brain, spleen, femoral muscle, heart muscle and lungs were collected. Ten per cent (w/v) tissue homogenates were prepared in 0.1 mol dm^{-3} Tris – HCl buffer, pH = 7.4. Supernatants were obtained by centrifugation at 5000 x g for 30 min. The prepared material was stored at -18°C.

Magnesium concentration in the supernatants was measured by the reaction with xylidyl blue (diagnostic set Liquick Cor-MG 60), using the colorimetric method. The wavelength was 520 nm. The assays were carried out with the help of a SPECORD M40 (Zeiss Jena) spectrophotometer.

Comparisons between the control and tested groups as well as between the selenium supplemented groups were made using c-Cochran-Cox test. The values were considered significant at $p < 0.05$.

The study was performed according to the statutory bioethical standards and approved by the Local Ethics Commission of the Medical University of Lublin, approval 65/AM/2004.

RESULTS AND DISCUSSION

The results obtained in the present experiment provided evidence to support interaction between magnesium and selenium. This effect was mainly observed in kidney and lung tissues. In comparison with the control group (without Se), selenium supplementation, regardless of its form, caused a decrease in the magnesium tissue concentration. In contrast, none of the Se supplement caused any changes in the brain and heart muscle. In the liver and spleen, it was only ring selenazoline that affected the magnesium concentration, casuing its increase in the liver and a decrease in the spleen. In the femoral muscle it was only chain selenosemicarbazide that exerted a significant effect vs the control group, causing a decrease in the Mg concentration.

Our comparison between the Se-treated groups showed that in some tissues the influence of selenium on Mg tissue concentration was dependent on its form. In the liver, ring selenazoline (group IV) increased Mg in comparison with the other groups receiving selenocompounds, significantly vs group III (given chain selenosemicarbazide). In the spleen, organic compounds diminished the Mg concentration in comparison with inorganic selenite and in group IV this effect was significant vs group III. In the femoral muscle, a distinct difference between organic compounds was displayed, namely chain selenosemicarbazide decreased Mg whereas ring selenazoline enhanced the level of this element, an effect which was evident vs group II (sodium selenite) and group III (selenosemicarbazide).

All the results of determinations are presented in Table 1.

Table 1

Magnesium tissue concentration in rats receiving different selenium supplements

Group	Magnesium tissue concentration (mmol kg^{-1} of wet tissue)						
	kidney $\overline{x}$ ± SD	liver $\overline{x}$ ± SD	brain $\overline{x}$ ± SD	spleen $\overline{x}$ ± SD	femoral muscle $\overline{x}$ ± SD	heart muscle $\overline{x}$ ± SD	lung $\overline{x}$ ± SD
Group I Control n=10	9.61 ± 0.37	5.30 ± 1.59	4.20 ± 0.44	9.75 ± 1.73	9.53 ± 1.39	5.85 ± 1.46	.71 ± 1.13
Group II Treated with Na_2SeO_3 n=10	5.15 ± 0.90 ***	5.79 ± 1.12	3.11 ± 0.26	9.47 ± 2.18	8.05 ± 1.08	5.89 ± 0.55	3.76 ± 0.98 ***
Group III Treated with ORG-C n=10	4.73 ± 1.45 **	5.74 ± 0.46	4.10 ± 0.43	7.62 ± 1.52	6.73 ± 0.72 **	6.00 ± 0.70	3.34 ± 0.35 ***
Group IV Treated with ORG-R n=10	5.09 ± 0.50 ***	7.08 ± 0.39 $^{*, Y}$	4.50 ± 0.58	5.04 ± 1.27 $^{**, \#}$	11.21 ± 1.62 $^{\#, Z}$	6.65 ± 0.50	2.79 ± 0.50 ***

Values are mean ± standard deviation
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$ vs. group I
$^{\#}$ $p < 0.01$ vs. group II
^{Y} $p < 0.01$; ^{Z} $p < 0.001$ vs. group III
n – number of animals in the group

In our previous experiment, we studied the effect of similar selenoorganic compounds on magnesium concentration in mice. The obtained results were the same in the brain, where no changes were observed. In the kidney and liver, inorganic selenite exerted the same effect causing significant decrease in the kidney and no alterations in the liver. A slight modification of the structure of an organic chain supplement (4-(o-tolyl)-selenosemicarbazide of 4-chlorobenzoic acid instead of 4-(o-tolyl)-selenosemicarbazide of 2-chlorobenzoic acid) resulted in distinct differences in its influence – in the kidney magnesium did not change significantly, whereas in the liver a decrease was observed. When the ring-structured supplement was applied, a more appreciable difference in the structure (3-(4-chlorobenzoylamino)-2--(o-tolylimino)-4-phenyl-4-selenazoline instead of 3-(2-chlorobenzoylamino)-2--(o-tolylimino)-4-methyl-4-selenazoline) altered its influence in the kidney, where no changes were noticed, and in the liver, where significant Mg depletion was determined. In the brain, the above modification of the supplement's structure did not change the effect of Se-supplementation (Musik et al. 2002a).

Relationships between Mg and Se have already been reported (Osada et al. 2002, Erdal et al. 2008). Both elements play an important role in the diabetes therapy (Matej-butrym, Schabowski 2008) and were reported to be effective agents for mercury toxicity (Shukla et al. 2007). Decrease in the serum magnesium and selenium was found in diabetic patients (Kamal et al. 2009). In epileptic patients treated with valproic acid, increased serum selenium was observed but the magnesium concentration in serum was unaltered (Hamed et al. 2004).

Investigating changes in the magnesium level in tissue of animals subjected to selenium supplementation seems to be advisable because such determinations are possible only using an animal model. In horses administered a selenium-containing diet, no alterations of the plasma Mg were observed, irrespective of both Se-source (selenite or Se-yeast) and Se-dose (Calamari et al. 2010). In rats receiving sodium selenite, enhanced fractional reabsorption of magnesium was displayed (Sakly et al. 2003). Inorganic selenium caused some increase in the Mg^{2+} accumulation in K562 cells (Jun--ying, Cun-Shuan 2009).

The effect of selenium can depend on other substances administered to experimental animals. In our study, Se did not alter Mg in the heart muscle of healthy rats. In rabbits administered daunorubicin, a significant decrease in the myocardial magnesium was accompanied by a slight increase in selenium (Šimunek et al. 2005). Rats treated with dietary cadmium showed depressed Se and Mg in the liver (Noël et al. 2004), although we did not observe any effect of the selenium treatment (sodium selenite or chain selenosemicarbazide groups) or an increase in the liver magnesium (ring selenazoline). Other factors can also influence the effect of Se-administration. In horses given selenium with vitamin E after exercise, a significant increase in serum Mg was observed, whereas before exercise no changes were

obtained (YUR et al. 2008). Sodium selenite caused only a slight enhancement of magnesium in lenses of rats exposed to cigarette smoke (DILSIZ et al. 1999). Relationships between magnesium and selenium in an organism are also dependent on time. In rats fed a magnesium-deficient diet the kidney selenium was increased after 7 days, whereas in the heart such an effect was not observed until day 70 (JIMENEZ et al. 1997). Similarly, in the present study, short-term administration of selenium compounds did not change the heart muscle magnesium.

CONCLUSIONS

1. Selenium supplementation influenced tissue magnesium concentrations depending on tissue and structure of the supplement.

2. Selenium supplementation, irrespective of an administered compound, caused magnesium depression in the kidney and lungs and no changes in the brain and heart muscle.

3. In the liver, spleen and femoral muscle, alterations of magnesium concentrations were was dependent on the provided supplement.

REFERENCES

ASSADI F. 2010. *Hypomagnesemia: an evidence-based approach to clinical cases.* Iran. J. Kidney Dis., 4: 13-19.

BARBOSA F.T., BARBOSA L.T., JUCÁ M.J., CUNHA R.M. 2010. *Applications of magnesium sulfate in obstetrics and anesthesia.* Rev. Bras. Anestesiol., 60: 104-110.

BURK R.F., NORSWORTHY B.K., HILL K.E., MOTLEY A.K., BYRNE D.W. 2006. *Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial.* Cancer Epidemiol. Biomarkers Prev., 15 (4): 804-810.

CALAMARI L., ABENI F., BERTIN G. 2010. *Metabolic and hematological profiles in mature horses supplemented with different selenium sources and doses.* J. Anim. Sci., 88: 650--659.

COMBS G.F.Jr. 2005. *Current evidence and research needs to support a health claim for selenium and cancer prevention.* J. Nutr., 135: 343-347.

CUI X.R., TAKAHASHI K., SHIMAMURA T., KOYANAGI J., KOMADA F., SAITO S. 2008. *Preparation of 1,8--Di-O-alkylaloe-emodins and 15-Amino-, 15-Tthiocyano-, and 15-Selenocyanochrysophanol derivatives from Aloe-Emodin and studying their cytotoxic effects.* Chem. Pharm. Bull., 56: 497-503.

DILSIZ N., OLCUCU A., CAY M., NAZIROGLU M., ÇOBANOĐLU D. 1999. *Protective effects of selenium, vitamin C and vitamin E against oxidative stress of cigarette smoke in rat.* Cell Biochem. Funct., 17: 1-7.

ERDAL M., SAHIN M., HASIMI A., UCKAYA G., KUTLU M., SAGLAM K. 2008. *Trace elements levels in hashimoto thyroiditis patients with subclinical hypothyroidism.* Biol. Trace Elem. Res., 123: 1-7.

HAMED S.A., ABDELLAH M.M., EL-MELEGY N. 2004. *Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients.* J. Pharmacol. Sci., 96: 465-473.

HAWKES W.C., RICHTER B.D., ALKAN Z., SOUZA E.C., DERRICOTE M., MACKEY B.E., BONNEL E.L. 2008. *Response of selenium status indicators to supplementation of healthy North American men with high-selenium yeast.* Biol. Trace Elem. Res., 122: 107-121.

INGEN-HOUSZ-ORO S., BLANCHET-BARDON C., VRILLAT M., DUBERTRET L. 2004. *Vitamin and trace metal levels in recessive dystrophic epidermolysis bullosa.* J. Eur. Acad. Dermatol. Venereol., 18: 649-653.

IVANCIC J.Jr., WEISS W.P. 2001. *Effect of dietary sulfur and selenium concentrations on selenium balance of lactating holstein cows.* J. Dairy Sci., 84: 225-232.

JIMENEZ A., PLANELLS E., ARANDA P., LLOPIS J., SANCHEZ-VINAS M. 1997. *Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats.* J. Am. Coll. Nutr., 16: 175-180.

JUN-YING Y., CUN-SHUAN X. 2009. *Antitumor effects of a selenium hetoropoly complex in K562 cells.* Pharmacol. Rep., 61: 288-295.

KAMAL M., SALEM M., KHOLOUSI N., ASHMAWY K. 2009. *Evaluation of trace elements and malondialdehyde levels in type II diabetes mellitus.* Diabetes Metab. Syndr., 3: 214-218.

MATEJ-BUTRYM A., SCHABOWSKI J. 2008. *Znaczenie składników mineralnych w cukrzycy. [The significance of minerals in diabetes].* Fam. Med. Prim. Care Rev., 10: 212-217. (in Polish)

MUSIK I., PASTERNAK K., KIEŁCZYKOWSKA M. 2002a. *Wpływ organicznych związków selenu na stężenie magnezu w wybranych tkankach oraz wskaźniki krwi myszy. [The effect of organic selenium compounds on the magnesium concentration in the chosen tissues and blood indicators in mice].* Biul. Magnezol., 7: 316-323. (in Polish)

MUSIK I., KOZIOŁ-MONTEWKA M., TOŚ-LUTY S., DONICA H., PASTERNAK K., WAWRZYCKI S. 2002b. *Comparison of selenium distribution in mice organs after the supplementation with inorganic and organic selenium compound selenosemicarbazide.* Ann. UMCS, Med., 57: 15-21.

MUSIK I., KIEŁCZYKOWSKA M., HORDYJEWSKA A., PASTERNAK K. 2009. *Influence of different forms of selenium supplementation on superoxide dismutase activity and total antioxidant status in rats.* Ann. UMCS, Pharm, 22: 95-101.

NOËL L., GUÉRIN T., KOLF-CLAUW M. 2004. *Subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health.* Food Chem. Toxicol., 42: 1203--1210.

OSADA H., WATANABE Y., NISHIMURA Y., YUKAWA M., SEKI K., SEKIYA S. 2002. *Profile of trace element concentrations in the feto-placental unit in relation to fetal growth.* Acta Obstet. Gynecol. Scand., 81: 931-937.

RAYMAN M.P. 2000. *The importance of selenium to human health.* Lancet, 356: 233- 241.

ŘEZANKA T., SIGLER K. 2008. *Biologically active compounds of semi-metals.* Phytochemistry, 69: 585-606.

SAKLY R., CHAOUCH A., EL HANI A., NAJJAR M.-F. 2003. *Effects of intraperitoneally administered vitamin E and selenium on calcium oxalate renal stone formation: experimental study in rat.* Ann. Urol. (Paris), 37: 47–50.

SELAMOGLU TALAS Z., YILMAZ I., OZDEMIR I., ATES B., GOK Y., CETINKAYA B. 2009. *Role of synthesized organoselenium compounds on protection of rat erythrocytes from DMBA-induced oxidative stress.* Biol. Trace Elem. Res., 128: 167-175.

SHOBACK D. 2008. *Clinical practice. Hypoparathyroidism.* N. Engl. J. Med., 359: 391-403.

SHUKLA S., SINGH V., JOSHI D. 2007. *Modulation of toxic effects of organic mercury by different antioxidants.* Toxicol. Int., 14: 67-71.

ŠIMŮNEK T., ŠTĚRBA M., HOLEČKOVÁ M., KAPLANOVÁ J., KLIMTOVÁ I., ADAMCOVÁ M., GERŠL V., HRDINA R. 2005. *Myocardial content of selected elements in experimental anthracycline-induced cardiomyopathy in rabbits.* BioMetals, 18: 163-169.

Skelton J.A., Havens P.L., Werlin S.L. 2006. *Nutrient deficiencies in tube-fed children*. Clin. Pediatr. (Phila), 45: 37-41.

Telci L., Esen F., Akcora D., Erden T., Canbolat A.T., Akpir K. 2002. *Evaluation of effects of magnesium sulphate in reducing intraoperative anaesthetic requirements.* Br. J. Anaesth., 89: 594-598.

Uezono Y., Toyohira Y., Yanagihara N., Wada A., Taniyama K. 2006. *Inhibition by selenium compounds of catecholamine secretion due to inhibition of Ca^{2+} influx in cultured bovine adrenal chromaffin cells*. J. Pharmacol. Sci., 101: 223–229.

Xia R., Ganther H.E., Egge A., Abramson J.J. 2004. *Selenium compounds modulate the calcium release channel/ryanodine receptor of rabbit skeletal muscle by oxidizing functional thiols*. Biochem. Pharmacol., 67: 2071–2079.

Yur F., Dede S., Deger Y., Kilicalp D. 2008. *Effects of vitamin E and selenium on serum trace and major elements in horses.* Biol. Trace Elem. Res., 125: 223-228.

Zachara B.A., Włodarczyk Z., Masztalerz M., Adamowicz A., Gromadzinska J., Wasowicz W. 2004. *Selenium concentration and glutathione peroxidase activities in blood of patients before and after allogenic kidney transplantation*. Biol. Trace Elem. Res., 97: 1-13.