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THE INFLUENCE OF AGE AND SEX ON SELENIUM DISTRIBUTION AND GLUTATHIONE PEROXIDASE ACTIVITY IN PLASMA AND ERYTHROCYTES OF SELENIUM-ADEQUATE AND SUPPLEMENTED RATS.

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The experiment was performed on Sprague-Dawley male rats weighing 203, 103 and 53 g, and female 99 g. Animals were fed for 2 weeks a diet containing 0,1 and 2,0 ppm of Se (Na_2SeO_3 added). It was observed that the daily Se intake per kg of BW is lowered with an increase in animals body weight. Se-supplementation caused a significant increase of Se content in plasma and red blood cells. The highest concentration of Se in plasma and in RBC was found in females. GSH-Px activity was higher in RBC of all male rats receiving a Se-supplemented diet, but not in females. In plasma these differences between Se-adequate and supplemented rats were significant in youngest male rats and in females. These results suggest that age and sex of rats affect the concentration of Se and GSH-Px activity in plasma and RBC of rats.

Key words: *age, sex, selenium, glutathione peroxidase*

INTRODUCTION

In 1957, Schwartz and Foltz (1) had shown, that Se deficiency in rats leads to an increased susceptibility to liver necrosis. The antioxidant property of Se was explained by the discovery of Rotruck et al. (2), that Se is an integral part of glutathione peroxidase (GSH-Px, EC 1.11.19.), which is responsible for hydroperoxides destruction. Thus, the pathological lesions that occured with Se deficiency are thought to be caused by peroxidative damage, because of depressed GSH-Px activity. Lipid peroxidation increases in liver microsomes

of rats as a function of age. This peroxidation is mediated predominantly by cytochrome P-450 enzyme activity (3). Also, the expression of superoxide dismutase and catalase which is important for protection of mammalian cells from oxidative damage decrease with age in the liver of rats (4). Selenium level in diet or nutrition might influence the aging process through free radical reactions and the antioxidant status of an organism plays a role in the aging process.

The minimal Se requirement for rats was estimated to be 0,1 μg/kg DM of diet (5). In rats, the effects of Se deficiency may lead to liver necrosis, nutritional myodegeneration, growth retardation, hair loss and cataracts. Burk et al. (6) observed that the appearance of biochemical manifestations of Se-deficiency are sex-related. Male rats were found to be more susceptible to Se deprivation, because of the higher requirement for this microelement.

However, in Se-deficient female lambs the body-weight response to Se supplementation was higher in comparison to male lambs (7). Since Behne and Hofer-Bosse (8) observed sex-related differences in liver Se content, the present study was performed to determine whether age and sex have any impact on Se distribution and GSH-Px activity in plasma and RBC of Se-adequate and supplemented rats.

MATERIAL AND METHODS

Sprague-Dawley rats (Harland Industries, IN, USA) were housed individually in stainless steel cages in a room with controlled temperature, humidity and lighting. All rats were offered free access to food and deionized water. The basal diet contained: 37% sucrose, 31,5% corn starch, 15% casein, 10% corn oil, 2% cellulose, 3,5% mineral mixture * and 1% vitamin mixture **. Analysis of the basal diet revealed selenium in the amount of 0,01 ppm. This diet was supplemented with Na_2SeO_3 to a final concentration of 0,1 and 2,0 ppm. The weight of the rats and food intake was monitored periodically. Animals received experimental diets for 2 weeks. After this period rats were treated with i.p. injection of pentobarbital (Nembutal, 70 mg/kg BW). While unconscious, rats were sacrificed by cervical displacement and blood was collected by heart puncture. Heparin was used as an anticoagulant. Red blood cells were separated using centrifugation (10 min, 2500 × g, +4°C). After removal of plasma, RBC were washed 2 times with ice cold 1,15% KCl and then hemolized in cold deionized water (1 : 9 vol). Determinations of selenium concentration were performed using gas chromatography with electron cap-

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* Mineral mixture — AIN-76

** Vitamin mixture — Teklad 40060

ture, according to the method of McCarthy et al. (9). GSH-Px activity was estimated by the method of Beutler (10). Hydrogen peroxide and tertiary-buthyl hydroperoxide were used as substrates for GSH-Px and peroxidase (Px) activity estimation, respectively. One unit of enzyme activity is equivalent to one μmol of NADPH oxidized per minute at 25°C. Hemoglobin concentration in RBC was measured using cyanomethemoglobin method.

For statistical evaluation, ANOVA was used. Means with $p<0;05$ were considered statistically significant.

RESULTS

Table 1. The weight and daily Se intake of rats given Se-adequate (0,1 ppm) and Se-supplemented (2,0 ppm) diet.

Group	Sex	Se in diet ppm	Weight of rats (g)		Daiy Se intake μg/kg Bw
			Initial	Final	
1	M	0.1	200.4±9.0	241.3±28.3	5.6± 1.7
		2.0	206.5±7.3	268.6±14.2	110.1±10.1
2	M	0.1	103.9±8.0	268.6±14.2	110.1±10.1
		2.0	102.3±6.4	171.2±13.7	156.9±24.3
3	M	0.1	52.5±4.2	108.6±17.8	10.4± 1.7
		2.0	53.6±4.0	106.6± 9.3	186.4±31.0
4	F	0.1	98.4±5.6	142.5±13.9	7.5± 1.2
		2.0	100.5±7.3	145.9± 5.9	153.8±18.0

Means±SD for 7 rats.

Table 1 presents data regarding the weight and Se daily intake in rats. The oldest male rats receiving a Se-supplemented diet (2,0 ppm) have significantly higher body gains in comparison to rats fed a Se-adequate diet (0,1 ppm Se). Daily intake of Se depends on its content in the diet and is inversely proportional to increases in body weight. The results of Px and GSH-Px activity in plasma and RBC are shown in table 2. Se-supplementation caused an increase in Px activity in RBC of all male rats and in plasma youngest male rats. Differences of GSH-Px activity between Se-supplemented and Se-adequate rats were seen in the plasma of youngest male animals and in females. This difference was significant in RBC for all groups of male animals. Se-supplementation caused an increase of this microelement in plasma as well as in the RBC of investigated groups of rats (Table 3). The highest concentration of Se in plasma and RBC was found in Se-supplementated females rates.

Table 2 The influence of 2 weeks Se-supplementation on peroxidase and GSH-Px activity in plasma and red blood cells (RBC) of rats

Group	Se in diet ppm	Peroxidase activity		GSG-Px activity	
		Plasma U/ml	RBC U/mg Hb	Plasma U/ml	RBC U/mg Hb
1	0.1	1.94 ± 0.23^{a}	0.44 ± 0.06^{bc}	1.54 ± 0.18	0.29 ± 0.04^{c}
	2.0	2.11 ± 0.27^{a}	0.56 ± 0.10^{a}	1.72 ± 0.25^{a}	0.49 ± 0.09^{a}
2	0.1	1.73 ± 0.25^{ab}	0.41 ± 0.06^{c}	1.20 ± 0.24^{cdd}	0.29 ± 0.8^{c}
	2.0	1.79 ± 0.24^{b}	0.51 ± 0.07^{ab}	1.39 ± 0.31^{bc}	0.38 ± 0.8^{b}
3	0.1	1.01 ± 0.12^{d}	0.40 ± 0.10^{c}	0.80 ± 0.15^{e}	0.30 ± 0.06^{c}
	2.0	$1.480.31^{c}$	0.56 ± 0.08^{a}	1.18 ± 0.21^{d}	0.45 ± 0.10^{a}
4	0.1	1.66 ± 0.34^{bc}	0.42 ± 0.05^{c}	0.97 ± 0.16^{e}	0.35 ± 0.05^{b}
	2.0	1.86 ± 0.17^{ab}	0.44 ± 0.05^{bc}	1.44 ± 0.24^{b}	0.35 ± 0.06^{b}

Means $\pm$SD for 7 rats. Means with unlike supperscript letters differ significantly at $p = 0.05$.

Table 3. The influence of 2 weeks Se-supplementation on Se concentration in plasma and red blood cells (RBC) of rats.

Group	Se in diet ppm	Selenium concentration	
		Plasma ng/ml	RBC ng/mg Hb
1	0.1	151.9 ± 7.7^{c}	1.45 ± 0.10^{d}
	2.0	213.9 ± 15.3^{b}	2.13 ± 0.28^{c}
2	0.1	156.9 ± 10.3^{c}	1.60 ± 0.22^{d}
	2.0	222.1 ± 22.4^{b}	2.28 ± 0.35^{c}
3	0.1	157.7 ± 8.9^{c}	1.68 ± 0.27^{d}
	2.0	214.1 ± 18.7^{b}	2.69 ± 0.26^{b}
4	0.1	154.0 ± 7.4^{c}	1.57 ± 0.24^{d}
	2.0	271.4 ± 27.0^{a}	3.06 ± 0.34^{a}

Means $\pm$SD for 7 rats. Means with unlike superscript letters differ significantly at $p = 0.05$.

DISCUSSION

Determination of GSH-Px activity as well as Se concentration in indicator materials (RBC, plasma) are used to characterize Se-status in rats. Since Se deprivation leads to a faster decrease of GSH-Px activity than Se levels in rat liver, it would appear that in low Se-status animals the maintenance of other Se-proteins has priority over GSH-Px. In such rats the pool size of these

proteins is larger than GSH-Px (11). Over 60% of Se in rat plasma is asociated with selenoprotein P (MW 57000), and this protein incorporates Se very rapidly (12, 13). Plasma transport of selenite is mediated by proteins with a MW of 68000. Sani et al. (14) indentified this protein as serum albumin. Plasma GSH-Px antigenically distinct from erythrocyte enzyme and its source may be the liver (15). It was steated that transport of Se in cells is accomplished by Se-binding proteins with a MW of 17000. Also, other selenoproteins from rat plasma and liver were identified (11, 16).

In Se-adequate rats Behne and Wolters (17) calculated that 82, 63 and only 9% of tissue selenium originated from GSH-Px in red blood cells, liver and plasma, respectively. However in Se-supplemented rats the activity of GSH-Px was found to be about 30% higher in RBC as well as in plasma compared to Se-adequate animals. It suggest that even in Se-adequate rats the activity of GSH-Px in plasma respond to changes in dietary Se intake.

The activity of this Se-dependent peroxidase in plasma was age related. The lowest GSH-Px activity was stated in the youngest animals. This observation is in agreement with results obtained by Rao et al. (4). It is interesting, that in all groups of male rats this increase was significant also in RBC. This is due to de novo synthesis of bone marrow erythroid precursors of RBC (18). The group of enzymes called glutathione-S-transferases (GST), whose activity is not dependent on Se, act also as peroxidases. Some isozymes of GST during Se and vitamin E deficiency have higher activity and are able to partly compensate the lose of GSH-Px activity. The efficiency of this compensation is limited. Harris and Stone (19) indicated that increased lipid peroxidation, occuring in advanced deficiency, inhibits microsomal GST of rats. Compared to Se-depleted rats in Se-adequate and Se-supplemented animals the participation of GST activity in total peroxidase decreases. We found that in plasma of Se-adequate rats 71% and in RBC 74% of the total Px activity originates from GSH-Px whereas in Se-supplemented rats 79% and 81%, respectively (Table 2).

Daily Se food intake of younger rats was found to be significantly higher than in older rates consuming the same diet (Table 1). Because younger animals possess faster protein turnover, this caused a significantly higher level of Se in RBC of Se-supplemented rats (Table 3). Se supplementation of the diet with sodium selenite (2,0 ppm of Se) for two weeks leads to significant increase of this microelement concentration in plasma as well as in RBC. Sunde (20) have shown that absorbed selenite is reductively catabolized to selenide via a series of glutathione-dependent reactions in erythrocytes and in the cytosol of other cells. Selenide becomes then bound to proteins or undergoes methylation and excerption. The exact nature of the Se binding into tissues and proteins, and its incorporation into selenoamino acids remains to be determined.

Selenium level in the diet might influence the metabolism of xenobiotics through free radical reactions and antioxidant status of an organism, through of the changes in glutathione peroxidase activity. Another way of changing xenobiotic metabolism is through the modification of cytochrome P-450 isozyme activity. An important property of many hepatic isozymes of this cytochrome is that they are inducible e.g. by barbiturates. It was found that phenobarbital induction of cytochrome P-450 in Se-deficient rats is limited (21). Wrighton and Elswick (22) have shown that this increase of P-450 in Se-deficient rats is about 50% of that observed in the control animals.

The impaired induction of cytochrome P-450 isozyme is the result of increased degradation of this cytochrome and/or decrease in translation of their mRNA coding (23). It appears that each of the dietary constituants, including Se, affect a rather wide range of P-450 isozymes. Same of these isozymes are sex specific. A male specific form of cytochrom P-450 has been shown to possess higher steroid and xenobiotic metabolizing activities (24, 25). The study of Fujita et al. (26) provided the evidence that differences in the amount of major male specific P-450 isozymes are responsible for age- and sex-associated differences in some of the drug metabolizing activities. It is possible, that sex and age related differences in cytochrome P-450 isozyme activity have an influence on Se concentration and GSH-Px activity in plasma and RBC. We steated in this study higher level of Se in RBC and plasma of female rats. This is in agreement with observation of Behne and Hofer-Bosse (8) who found higher liver Se concentration in female rats fed Se-adequate diet compared to male animals. It may be related to sex-differences in hormonal status. It is known, that steroid hormones affeckt Se retention and distribution (27).

These findings show some dificulties in Se-status characterization based on the Se concentration and GSH-Px activity in indicator tissues. In addition to many other factors, age and sex also seem to affect Se concentration and GSH-Px activity in plasma and RBC of rats.

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