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EFFECTS OF DIETARY SUPPLEMENTATION WITH SELENOMETHIONINE ON THE TERATOGENIC EFFECT OF IONIZING RADIATION IN MICE

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Abstract

Female C_3H mice were fed a standard pellet diet (containing 0.2 ppm Se and 30 ppm vit. E) or the same diet supplemented with 0.8 ppm (low dose) or 3.4 ppm (high dose) of selenomethionine for 10 weeks. After mating with males receiving the standard diet the mice were subjected, on the 9th day of pregnancy, to whole body roentgen irradiation of 1.75 Gy. On day 18 of gestation the frequency of resorptions, mortality and the incidence of fetal malformations were studied. Supplementation with Se-methionine resulted in a significant but dose-independent decrease ($p<0.005$) of the number of malformed fetuses from 62 per cent in the irradiated controls to 47 per cent in the low Se-group and high Se-group, respectively. In addition, the number of total malformations as well as fetal resorptions were significantly decreased in a dose-independent manner in the supplemented groups. The decrease in fetal malformations occurred proportionally for all the major malformations observed, i.e. short or kinked tail, rib and vertebral malformations, coloboma and deformation of retina and iris. Glutathione peroxidase activity in whole blood of Se-methionine fed mice was significantly increased. Thus, Se-rich diet may result in scavenaging of radiation-induced hydroperoxides.

Selenoamino acids and sodium selenite given parenterally have been shown to increase survival of laboratory animals exposed to lethal doses of roentgen irradiation (1, 3, 7). However, experiments with selenium (Se) supplementation in the diet have been unsuccessful in protecting rats exposed to chronic irradiation (8).

Single intraperitoneal administration of a high dose (0.5 mg/kg b.w.) of sodium selenite reduced also the incidence of radiation-induced fetal malformations in mice (4). The time interval between Se-administration and irradiation, necessary for Se-pretreatment to be effective, corresponded well to the reported time course of the uptake of Se into embryonal tissues (2, 6).

In the present investigation we have examined if supplementation of a laboratory diet with Se could also diminish radiation-induced teratogenicity in mice. Since exposure to Se occurs normally from organic Se-compounds in the food (10, 17), the diet in these experiments was supplemented with selenomethionine and fed to female mice for 10 weeks before mating and whole body irradiation. In order to relate a possible protective effect to the functional body Se, blood GSH-Px was measured in the groups of animals studied.

Materials and Methods

Animals and diets. Primiparous one-month-old C_3H mice were kept under standard housing conditions (23°C, 50–55% rel. humidity, 12 hours light/dark cycle). The animals received a standard (Ewos, Södertälje, Sweden) or Se-supplemented laboratory diet and tap water ad libitum. The standard diet contained 0.15 to 0.20 ppm Se and 30 ppm vit. E.

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Seleno-D,L-methionine (Sigma, St Louis, Mo, USA) was added to the standard diet before pelleting in amounts corresponding to 0.8 ppm (low dose) and 3.4 ppm (high dose) of Se. The final content of Se in the supplemented diets was checked using atomic absorption spectrometry.

Determination of glutathione peroxidase (GSH-Px) activity. GSH-Px activities in whole blood were measured by a slightly modified method of PAGLIA & VALENTINE (12), using hydrogen peroxidase as the substrate coupled with glutathione reductase. Blood samples of 50 μl were taken by orbital puncture with heparinized Microcaps. Hemolysates were prepared by adding 500 μl of 0.05 mol/l sodium phosphate buffer pH 7.0/0.5% (v/v) Triton X-100 to a 1:1.5 dilution of the blood in physiologic saline. Since the addition of an equal volume of Drabkin's reagent to the hemolysates was found to decrease the GSH-Px activity in a time-dependent manner, it was replaced by a solution of KCN and $NaNO_2$ (0.5 mg/ml of each) in distilled water. With this method of converting hemoglobin to cyanomethemoglobin the GSH-Px activity was stable for at least 7 hours. Final assay concentrations were 0.05 mol/l sodium phosphate buffer (pH 7.0), 4.5 mmol/l reduced glutathione, 3.4 mmol/l sodium azide, 4.5 mmol/l EDTA, 0.25 mmol/l NADPH, 0.3 U/ml glutathione reductase and 0.1375 mmol/l H_2O_2. The decrease in NADPH concentration at 25°C was measured by continuous registration of the absorbance at 340 nm. The assays were conducted with an AKES sample processor-spectrophotometer (Vitatron, Dieren, The Netherlands). One enzyme unit of activity was defined as 1 μl NADPH oxidized/min and the results expressed as U/g Hb. Hemoglobin was measured by the cyanomethemoglobin method (Merckotest Hemoglobin, E. Merck, Darmstadt, Germany).

Irradiation conditions. The mice (9th day of gestation) were exposed to whole body roentgen irradiation in a single dose of 1.75 Gy at a dose rate 0.7 Gy/min as measured by lithium-fluoride thermoluminiscence dosimetry and checked by a simplex universal dosimeter. Physical parameters of irradiation were as follows: 250 kV, 15 mA, 0.5 mm Cu added filtration. The animals were placed in a plastic cage (distance from the target 70 cm) and were allowed to move freely during the irradiation.

Experimental design. A total of 330 female mice were fed 1) standard diet, 2) low Se-supplemented diet or 3) high Se-supplemented diet. The body weights and feed consumption were checked twice a week. Blood samples for GSH-Px analysis were taken by orbital puncture before the start of the experiment and after 10 weeks feeding the diets. After 10 weeks the females were mated overnight and the presence of a vaginal plug the next morning was denoted as day 0 of pregnancy. On the 9th day of gestation half of the number of mice in each dietary group were irradiated. The whole body irradiation dose of 1.75 Gy was chosen to induce approximately 50 per cent fetal mortality and malformation incidence as established in preliminary investigations with this mouse strain. The remaining animals served as controls for possible toxic effects of Se.

On the 18th day of gestation the mice were killed by cervical dislocation. The pregnant uterus was inspected for number of total implantations, resorptions and number of living and dead fetuses. After 3–5 days' fixation in 70 per cent ethanol all fetuses were checked for external malformations under a dissecting microscope. Alizarin red-S staining was used for examination of skeletal and eye malformations (5, 18).

Statistical methods. Differences between the results in individual groups were statistically tested using a chi-square test (16) or where appropriate with Student's t-test.

Results

The weight gains of the female mice fed Se-supplemented diets were well within the range of control values during the whole period of investigation (not shown), suggesting an absence of toxic effects of dietary Se at the concentrations used. While the control group showed a slight (12%) increase in blood GSH-Px activity, a significant elevation ($p<0.005$) was seen in the supplemented low-Se (+69%) and high-Se (+88%) groups at the end of the feeding period. However, the difference between the latter two groups was not statistically significant (Table 1).

Irradiation of pregnant mice on day 9 of gestation resulted in a marked decrease of the number of surviving fetuses due to resorption and fetal death (Table 2). Supplementation of the diet with selenomethionine reduced the resorption incidence from 53 per cent in the irradiated control group to 36 and 37 per cent, respectively, in the low- and high-Se groups. The number of surviving fetuses increased to 59 and 55 per cent in low- and high-Se groups of

Table 1

Glutathione peroxidase (GSH-Px) activity in whole blood of female C_3H mice fed for 10 weeks standard and Se-methionine supplemented diets. The values represent means ± SD

Treatment groups	Diet		Blood GSH-Px (U/g Hb) Feeding (day)		Change (%)
	Se-methionine suppl. (ppm)	Total Se (ppm)	0	75	
Control (n=15)	–	0.2	148.5±10.2	166.4±23.9	+12.1
Low Se (n=14)	0.8	1.0	147.4±17.0	249.5±24.2	+69.3*
High Se (n=15)	3.4	3.6	140.9±11.2	265.2±22.9	+88.2*,**

* Significantly different (t-test) from controls (p<0.005).
** Not significantly different (p<0.1) from low-Se.

Table 2

Effect of dietary Se-methionine supplementation on radiation-induced fetal mortality and resorptions on the 18th day of gestation in C_3H mice. Per cent in parentheses

Experimental groups	No. of litters	No. of total implants	No. of surviving foetuses Per cent total implants	No. of dead foetuses after day 15 Per cent total implants	No. of resorptions Per cent total implants
Control. No irrad.	24	184	172 (93.6)	1 (0.5)	11 (6)
Control. Irrad.: 1.75 Gy	20	180	71 (39.5)	13 (7.2)	96 (53.3)
Low Se (+0.8 ppm). No irrad.	24	142	129 (90.8)	1 (0.7)	12 (8.5)
Low Se (+0.8 ppm). Irrad.: 1.75 Gy	32	200	118 (59.0)*	9 (4.5)	73 (36.5)*
High Se (+3.4 ppm). No irrad.	21	139	134 (96.4)	0 (0)	5 (3.6)
High Se (+3.4 ppm). Irrad.: 1.75 Gy	28	182	100 (55.0)*	14 (7.7)	68 (37.3)*

* p<0.005 (significantly different from irradiated control group).

mice, respectively, as compared with the irradiated non-supplemented controls which showed 39 per cent living fetuses. The differences between the irradiated controls and the Se-treatment groups as regards both the number of resorptions and fetal survival rates were statistically significant ($p<0.005$).

The effect of dietary Se on the incidence of radiation-induced fetal malformations is shown in Table 3. As was the case with fetotoxicity, irradiation strongly increased both the number of malformed fetuses (having one or more malformations) and the total number of malformations as compared with the non-irradiated controls. Groups of mice on the selenium-supplemented diets showed a significant but dose-independent decrease ($p<0.005$) in the number of malformed fetuses down to 47 per cent in both groups as compared with 62 per cent in the irradiated non-supplemented group. The number of total malformations was also significantly reduced in a dose-independent manner. The observed decrease in the supplemented groups occurred proportionally, i.e. with unchanged relative proportions between the major malformations observed such as short or kinked tail, rib and vertebral malformations, coloboma and deformation of iris (Table 3). Retardation of the sternum amounting to about 60 per cent in irradiated control animals was unaffected by Se-rich diet (not shown).

Discussion

The results of the present investigation show that 10 weeks' feeding with 0.8 ppm or 3.4 ppm selenomethionine supplemented in the diet reduced radiation-induced fetotoxicity and the number of fetal malformations in mice exposed to whole body roentgen irradiation. The Se-supplemented diets were given after weaning at an age of one month. In a

Table 3

Effect of dietary Se-methionine supplementation on fetal malformation incidence after whole body irradiation of C_3H mice on the 9th day of gestation

Experimental conditions	No. of fetuses inspected	No. of malformed fetuses	Total No. of malformations	External malformations					Malformations seen after alizarin staining				
				Total	Short or kinked tail	Poly-, oligo- or syndactyly, finger hypertrophy	Umbilical hernia or omphalocele	Cleft palate	Total	Rib and vertebral malformations	14th rib	Anophthalmia or microphthalmia	Coloboma, deform. retina or iris
Control No irrad.	172	2 (1.2)	2	0	0	0	0	0	2	1	0	1	0
Control Irrad.: 1.75 Gy	71	44 (62.0)	55	12	7	3	2	0	43	30	1	4	8
Low Se (+0.8 ppm) No irrad.	129	2 (1.6)	2	1	0	0	1	0	1	0	0	1	0
Low Se (+0.8 ppm) Irrad.: 1.75 Gy	118	56 (47.4)*	70	13	2	5	6	0	57	35	7	2	13
High Se (+3.4 ppm) No irrad.	134	3 (2.2)	4	4	1	0	3	0	0	0	0	0	0
High Se (+3.4 ppm) Irrad.: 1.75 Gy	100	47 (47.0)*	60	9	3	3	2	1	51	36	3	5	7

* $p<0.005$ (significantly different from irradiated control group).

previous investigation, a decrease in radiation-induced malformations in mice has been observed after a single parenteral pretreatment with sodium selenite (4). The observed decrease occurred proportionally for all the major malformations detected. A similar reduction of malformations with no marked changes in their qualitative pattern was also seen in the present investigation.

The mechanism of the radioprotective effect of dietary Se is at present unclear. It is generally agreed that biologic effects of ionizing radiation in the cell result mainly from free radicals produced by radiolysis of the cell water giving rise to damage to DNA. Furthermore, free radicals may have a deleterious effect on cellular membranes through peroxidation of membrane lipids, thus causing disturbances in cell functions (14). Se, as an essential component of GSH-Px (13) together with vit. E, superoxidase dismutase and catalase prevents the accumulation of reactive oxygen intermediates and may thus also be involved in the inactivation of irradiation-induced secondary radicals (15).

The significantly increased GSH-Px blood concentration of the mice fed with Se-supplemented diets in the present study would support the latter proposition. The absence of a dose-response relationship for the protective effects of dietary Se on teratogenicity induced by ionizing radiation might be related to the small differences in blood GSH-Px activities observed between the low- and high-Se groups. In other studies there was, however, no obvious relationship between generally assumed radiosensitivity of normal tissues and various neoplastic cell lines and the cellular content of GSH-Px (11).

Another explanation could be that protection by Se against radiation damage is mediated by mechanisms analogous to those of sulphydryl-containing radioprotectors (9). These agents apparently react by trapping free radicals, by protection of protein sulphydryl groups through mixed disulphide formation, and by hydrogen atom donation. This type of radioprotective action of Se may, however, be more true for single high-dose injection of Se in pregnant mice two hours before irradiation where no increase in the GSH-Px blood concentration was found (unpublished).

Finally, it should be noted that the lowest dietary concentrations of Se which in the present investigation reduced the radiation-induced malformations in mice is only about five times higher than the recommended dietary Se levels for this and other animal species (17). This would make studies of a possible preventive action of Se against ionizing radiation interesting even within the range of dietary intake of Se in man.

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