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PROTECTIVE EFFECT OF SELENIUM AGAINST IONIZING RADIATION-INDUCED MALFORMATIONS IN MICE

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Abstract

A single dose of sodium selenite (0.5 mg Se/kg b.w.) was injected intraperitoneally into mice on day 9 of pregnancy, either 30 min or 2 h before 1.75 Gy whole body irradiation. Administration of selenite 2 h but not 30 min before irradiation resulted in a significant decrease in the number of malformed foetuses ($p<0.005$). The decrease in foetal 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. In addition, selenium pretreatment also protected against radiation-induced retardation of the sternum of the foetus.

Protective effect of pretreatment with various selenium compounds against radiation-induced damage has been shown in several investigations (1, 3). HOLLÓ & ZLATAROV (6) reported survival of all rats exposed to lethal radiation doses after the post-irradition treatment with selenate. Selenoaminoacids have shown a more powerful radioprotective effect than the analogous sulphuraminoacids and other sulphydryl radioprotectors, as tested in protein model system (10). No studies on radiation-induced malformations of foetuses seem to have been performed.

Since both inorganic and organic selenium compounds were shown to cross the placental barrier (2, 5), a possible protective effect of sodium selenite against radiation-induced embryonal and foetal toxicity was studied in the present investigation. The study was performed in mice subjected in an early stage of pregnancy to sublethal doses of roentgen irradiation and the rate of foetal survival, the foetus resorption, the incidence of foetal malformations as well as the skeletal development were monitored.

Material and Methods

Animals. Primiparous mice of C_3H strain, 3 to 5 months old, were kept on a 12 h light and 12 h dark cycle at a constant temperature of 23°C and relative humidity 50 to 55 per cent. The animals were given a standard laboratory diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum. The diet contained 0.1 to 0.2 mg Se/kg and 30 mg vitamin E/kg.

Chemicals. Sodium selenite ($Na_2SeO_3 \cdot 5\ H_2O$; analytical grade, E. Merck, Darmstadt, West Germany) was dissolved in distilled water and injected intraperitoneally (i.p.) in a dose of 0.5 mg Se/kg b.w. Control animals received i.p. injection of a corresponding volume of about 0.15 ml distilled H_2O.

Irradiation conditions. On day 9 of pregnancy the mice were exposed to whole body roentgen irradiation by a single dose of 1.75 Gy. 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 irradiation. The dose rate of exposure was 0.7 Gy/min as measured by lithium-fluoride thermoluminiscence dosimetry and checked by a simplex universal dosimeter.

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Table 1

Effect of selenite pretreatment (i.p.) on day 9 of gestation on the radiation-induced foetal mortality, resorption, and the body weight of surviving foetuses at day 18 of gestation in C_3H mice (numbers in parentheses represent per cent of total)

Experimental conditions	Litters	Total implants	Surviving foetuses	Dead foetuses	Resorptions	Body weight (g) of surviving foetuses (mean ± SD)
Controls	32	247	226 (91.5)	3 (1.2)	18 (7.3)	0.99±0.10
Se: 0.5 mg/kg bw irrad.: 0	22	109	102 (93.6)	0 (0)	7 (6.4)	1.00±0.14
Se: 0 Irrad.: 1.75 Gy	34	265	167 (63.0)	16 (6.0)	82 (30.9)	0.81±0.10
Se: 0.5 mg/kg bw 30 min before irrad.: 1.75 Gy	19	115	77 (67.0)	5 (4.3)	33 (28.7)	0.83±0.11
Se: 0.5 mg/kg bw 2 h before irrad.: 1.75 Gy	25	147	102 (69.4)	10 (6.8)	35 (23.8)	0.83±0.11

Table 2

Effect of selenite pretreatment (i.p.) on day 9 of gestation on the radiation-induced malformations in the foetus at day 18 of gestation in C_3H mice (numbers in parentheses represent per cent of total)

Experimental conditions	No. foetuses inspected	No. of foetuses with one or more malformations	External malformations					Malformations seen after alizarin staining				
			Total	Short or kinked tail	Poly-, oligo- or syndactyly, finger hypertrophy	Umbilical hernia or omphalocele	Defects of external genitals	Total	Rib and vertebral malf.	14th rib	Anophthalmia or microphtalmia	Colobo-ma, deform. retina or iris
Controls	226	4 (1.8)	1	0	1	0	0	5	1	2	2	0
Se: 0.5 mg/kg bw Irrad.: 0	102	1 (1.0)	1	0	0	1	0	0	0	0	0	0
Se: 0 Irrad.: 1.75 Gy	167	90 (53.9)	30 (23.3)	13 (10.1)	7 (5.4)	7 (5.4)	3 (2.3)	99 (76.7)	53 (41.1)	6 (4.7)	2 (1.6)	38 (29.5)
Se: 0.5 mg/kg bw 30 min before irrad.: 1.75 Gy	77	44 (57.1)	13 (23.1)	6 (10.7)	1 (1.8)	6 (10.7)	0	43 (76.8)	23 (41.1)	6 (10.7)	1 (1.8)	13 (23.2)
Se: 0.5 mg/kg bw 2 h before irrad: 1.75 Gy	102	32* (31.4)	11 (26.2)	7 (16.7)	0	3 (7.1)	1 (2.4)	31 (73.8)	14 (33.3)	4 (9.5)	1 (2.4)	12 (28.6)

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

Experimental procedures. The female C_3H mice were mated overnight and examined for the presence of a vaginal plug next morning. The day on which the vaginal plug was found was denoted as day zero of pregnancy. The females were weighed daily from day 9 to day 18.

On day 9 of pregnancy the females were divided into 5 groups as follows: 1) control, 2) i.p. injection of selenite, 3) irradiation, 4) i.p. injection of selenite 30 min before irradiation, 5) i.p. injection of selenite 2 h before irradiation.

In a preliminary investigation the LD_{50} (i.p.) of sodium selenite for C_3H female mice was found to be 2.0 to 2.5 mg Se/kg b.w. Consequently, the experimental animals were given a single i.p. injection of 0.5 mg Se/kg b.w. Controls received a corre-

Table 3

Effect of selenite pretreatment (i.p.) on day 9 of gestation on the radiation-induced sternum retardation in the foetus on day 18 of gestation in C_3H-mice

Group No.	C_3H-mice, mothers treated with Se and irrad.	No. of foetuses inspected	Sternum retardation (% of inspected)	Sternum retardation and malformations (% of retarded)
1	Controls	226	1 (0.4)	1
2	Se: 0.5 mg/kg bw Irrad: 0	102	4 (3.9)	1 (25.0)
3	Se: 0 Irrad.: 1.75 Gy	167	93 (56.0)	59 (63.0)
4	Se: 0.5 mg/kg bw. 30 min before irrad.: 1.75 Gy	77	23 (29.9)	15 (65.2)
5	Se: 0.5 mg/kg bw 2 h before irrad.: 1.75 Gy	102	39 (38.2)	11 (28.2)

sponding volume of distilled water. After that the mice (groups 3, 4, 5) were submitted to a single dose of whole body irradiation (1.75 Gy).

On day 18 of gestation the animals were killed by cervical dislocation and dissected. The pregnant uterus was examined for the number of total implantations, resorptions, living and dead foetuses, normal and grossly malformed foetuses (13). The foetuses were individually weighed and examined after 3 to 5 days of fixation in ethanol. All foetuses were checked for external malformations (exencephaly, umbilical hernia, club foot, syndactyly, ectodactyly, adactyly) under dissection microscope. The foetuses were then cleared in potassium hydroxide (1% solution) and stained with alizarin red S for examination of skeletal malformations (4, 14). In addition, the retardation of the sternum was studied. The absence of one or several ossification centres, or an asymmetric and very irregular arrangement of these centres were considered as an indicator of the retardation; these signs were collectively named as sternum retardation.

Statistics. Differences between the results in individual groups were statistically tested using a chi-square test (11).

Results

The dose of sodium selenite used (approximately 1/5 of LD_{50}) did not cause any death or apparent clinical signs of selenium intoxication in mothers during the whole period of experiment. The irradiation of pregnant mice with 1.75 Gy resulted in a significant ($p<0.005$) decrease of the number of surviving foetuses and their body weight, and a significant ($p<0.005$) increase of dead foetuses and resorptions (Table 1). This is in agreement with the previously described teratogenic effects of whole body irradiation in this strain of mice (13). Treatment with sodium selenite in non-irradiated animals did not influence these parameters as compared with controls. The pretreatment of animals with selenite either 30 min or 2 h before irradiation resulted in a slight increase of the number of surviving foetuses and in a decrease of resorptions. These effects were, however, statistically not significant. The number of dead foetuses and the body weight of surviving foetuses were not affected by the selenite pretreatment (Table 1).

The irradiation highly increased both the number of malformed foetuses (having one or more malformations) and the total number of malformations as compared with the non-irradiated controls (Table 2). No change in the number and type of malformations was observed when selenite was administered 30 min before irradiation. However, selenite treatment 2 h before irradiation significantly reduced (<0.005) the number of malformed foetuses.

A detailed analysis of the data in Table 2 indicates

that the over-all decrease of the number of malformations in the irradiated groups, which were pretreated with selenite, was not accompanied by any marked change in their qualitative pattern.

Sodium selenite given 30 min before irradiation significantly diminished ($p<0.005$) the frequency of sternum retardation (Table 3). No further reduction was observed in the group treated with selenite 2 h before irradiation. Out of the group exposed only to irradiation, 2/3 of foetuses had both sternum retardation and malformation. In the group treated with selenite 30 min before irradiation, the same frequency of retardation and malformation was observed. However, in the group pretreated with selenite 2 h before irradiation, only 1/3 of the number of foetuses had both sternum retardation and malformation.

Discussion

Selenium in various inorganic and organic forms was reported to prevent radiation damage both in in vivo and in vitro experiments (for survey, see 9). The protective effects of selenium have only been studied in adult animals and expressed in terms of decreased mortality.

In the present investigation the effects of selenite on foetal development were studied in pregnant mice subjected to selenite treatment followed by irradiation. Administration of sodium selenite did not reduce the frequency of resorptions or surviving foetuses but decreased the number of malformed foetuses provided selenium was given 2 h before irradiation. This agrees well with the relatively slow transplacental passage of selenium as described by BÉNARD (2). In his experiments the ^{75}Se-selenite did not appaer in the mouse foetus until 2 h after the intraperitoneal application of sodium selenite.

The different foetal malformations were reduced in the same proportion. This indicates that the selenite-mediated protection against a variety of radiation-induced foetal malformations operates by the same mechanism.

In contrast to the time-dependent selenite protection against the radiation-induced malformations, the effect on sternum retardation was already observed in the group given selenite 30 min before irradiation. This indicates the existence of at least two independent pathways for the selenite-mediated protection against radiation-induced disturbances in the development of the foetuses.

It is generally agreed that biologic effects of ionizing radiation in the cell result from a free radical damage to DNA. Furthermore, free radicals may exert a deleterious peroxidative effect also on cellular membranes, leading to impaired function or death of the cell. The mechanism of the radioprotective effect of selenium is at present unknown. In recent years, biochemical functions of selenium have been extensively studied (reviewed in 9). So far, only one selenium-containing compound with a specific physiologic function has been identified in animal tissue, the enzyme glutathione peroxidase (8). It is believed that this seleno-enzyme is involved together with vitamin E, superoxide dismutases and catalases in the protective mechanism against oxidative cellular damage caused by highly reactive oxygen species and by secondary peroxiradicals (12). Another possible interpretation is that selenium protection against radiation damage could be mediated by mechanisms analogous to those of sulphydryl-containing radioprotectors (7). These agents apparently react by trapping of free radicals, by protection of protein sulphydryl groups through mixed disulphide formation, and by hydrogen atom donation. However, the knowledge about metabolic conversion of selenite selenium into selenohydryl substances such as Se-aminoacids, and their incorporation into body proteins is far from complete.

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