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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 17 of pregnancy, 2h before 1.5 and 3.0 Gy whole body gamma irradiation. Irradiation produced a growth impairment in the newborns. Weight improvement as a function of age of offspring from irradiated mice decreased proportionally to applied dose. Administration of selenite results also in a decrease of growth rate. Nevertheless, when irradiation and selenite treatments were combined, no additive effect can be observed, suggesting that selenium pretreatment would exert some protection against radiation-induced retardation of growth.

EFEITOS BIOLÓGICOS DA RADIAÇÃO DE ^{60}Co EM EMBRIÕES DE CAMUNDONGOS NA PRESENÇA DE SELENITO DE SÓDIO

RESUMO

Doses únicas de selenito de sódio (0,5 mg Se/kg peso corporal) foram injetadas por via intraperitoneal em camundongos fêmeas no 17º dia de prenhez, 2 horas antes de receberem doses de 1,5 e 3,0 Gy de radiação gama de ^{60}Co. A irradiação produziu um crescimento deficiente nos animais recém-nascidos. O incremento de peso da prole, em função da idade, decresceu proporcionalmente com as doses aplicadas. Só a administração de selênio também resultou num decréscimo da taxa de incremento de peso da progênie. Todavia quando o tratamento com selênio e irradiação foram combinados, um efeito aditivo não pode ser observado, sugerindo que o tratamento prévio exerceria alguma proteção contra o retardo de crescimento induzido pela radiação.

INTRODUCTION

Radiation effects are mediated in part by the generation of oxygen-derived free radicals and hydrogen peroxide.

Two detoxification systems defend the organism from their harmful effects: catalase and the glutathione cycle. The glutathione cycle metabolizes H_2O_2 by using reduced glutathione as a substrate for H_2O_2 in a reaction catalyzed by glutathione peroxidase (glutathione-hydrogen-peroxide oxireductase, EC 1.11.1.9) producing oxidized glutathione and 2 H_2O.

$$2\ GSH + H_2O_2 \longrightarrow GSSG + 2\ H_2O$$

GSSG can then be converted to GSH by glutathione reductase, which couples the reduction of GSSG to the oxidation of NADPH.

$$GSSG + NADPH + H^+ \longrightarrow 2\ GSH + NADP^+$$ (Sprier et al,1985)

NADPH then regenerated by the hexose monophosphate shunt. Any loss of ability of the organism to complete the glutathione cycle leaves it vulnerable to oxidative damage.

Membrane polyinsaturated fatty acids are important biological targets of these toxic molecules which cause lipide peroxidation. Radiation damage to DNA is also known to result in base hydroperoxide. Glutathione is known to inhibit lipid peroxidation both chemically and through its interaction with the selenium-dependent glutathione peroxidase (GSH-Px). Recent studies have suggested the presence of a membrane-bond GSH-dependent peroxidase system (Batist et al, 1986).

Since both inorganic and organic selenium compounds were known to cross the placental barrier (Akan et al, 1985 b) a possible protective effect of sodium selenite against radiation-induced faetal effects was studies to two different doses of ^{60}Co gamma irradiation and the rate of newborn as well as their dams weight improvement were monitored.

MATERIAL AND METHODS

Animals. Primiparous mice of Swiss strain, born at the Institute, 3 months old, were kept in a natural light cycle. The mice were given a standard laboratory diet (NUVILAB CR1., NOVITAL) and top water as libitum. The diet contained 0.05 mg/Se/kg and 30 mg vitamin E Units/kg. Twently pregnant females were randomized into six groups, two groups of two and four groups of four being housed in separate polypropylene cages with wood bedding.

Chemicals. Sodium selenite ($Na_2SeO_2 5H_2O$; analytical grade, Riedel de Haen H.H. D3016 Seelze 1) was dissolved in quartz bidistilled water and injected intraperitoneally (ip) in a single dose of 0.5 mg Se/kg. Control animals received ip injection of a corresponding volume of about 0.15 ml bidistilled H_2O.

Irradiation conditions. On day 17 of pregnancy the mice were exposed to whole body ^{60}Co gamma irradiation from a Gammacell 220, Atomic Energy of Canada Ltd., by single doses of 1.5 and 3.0 Gy at 5.5 Gy/min. The animals were placed in a cardboard cylindric cage and were allowed to move freely during irradiation.

Experimental procedures. On day 17 of pregnancy the females were divided into 6 groups as follows:
1) Control, 2) ip injection of selenite, 3) Gamma irradiation with 1.5Gy; 4) ip injection of selenite 2h before 1.5 Gy gamma irradiation, 5) Gamma irradiation with 3.0 Gy, 6) ip injection of selenite 2h before 3.0 Gy irradiation.

Controls received a corresponding volume of bidistilled water. The weight of the offspring from each pregnant mouse as well as from the pregnant females herselves were recorded three times a week during at least 25 days from birth.

The litter of animals were nursed by their dams during the observation period.

Statistics. Means were compared by two factor analysis of variance using the Bonferroni test (Neter & Wassermon, 1974) or where appropriate with student's t-test.

RESULTS AND DISCUSSION

The effects of radiation on the rate of body weight improvement of newborns irradiated previously on 17 day of gestation were studied. The rate of weight gain as a function of age declined as the gamma radiation dose increased (Figure 1, Table I). Whereas the slope of the control had a value of 0.65 those of the mice irradiated with doses of 1.5 and 3 Gy were reduced to 0.32 and 0.25 respectively. Those unirradiated controls injected with 0.5 mg Se/kg b.w. had, however, a significantly lower weight gain (0.27) than the control group given water alone (0.65). This is in agreement with data from others (Franke & Potterm 1935) who described the growth retardation in young rats as a consequence of orally administered food-stuff containing selenium.

The dose of sodium selenite used, approximately 1/5 of DL_{50} (Cekan et al., 1985) did not cause any death or apparent clinical signs of selenium intoxication in mothers during the period of experiment. The irradiation of previously injected sodium selenite pregnant mice produced slight changes

in the body weight gain curves with slope values of 0.42 and 0,26 for doses of 1.5 and 3.0 Gy respectively, when compared with the control (0.27). Treatment with sodium selenite in irradiated mice influenced these parameters : while the slope of selenium control was disminished when compared with water control (0.27 against 0.65), the slope of the curve from newborns coming from dams treated with Se + 3.0 Gy presented no difference with those 3.0 Gy irradiated alone (Table I). So, it seems like selenium exerced some kind of radioprotection on growth impairment produced by ^{60}Co radiation or enhanced the recovery from radiation injury.

The number of offsprings were not significantly affected by selenium pretreatment as it is shown in Table II, in spite of a decrement in the alive offspring is shown as a consequence of irradiation. At the same time , there was no significant difference in radiation response between the selenium-treated pregnant animals and control pregnant animals when body weight after irradiation were analyzed (Table III).

Selenoamino acids and sodium selenite given parenterally have been shown to increase survival of laboratory animals exposed to lethal doses of roentgen irradiation (Badiello et al., 1975).

Dietary supplementation with selenomethionine resulted in a significant decrease of the number of malformed foetuses from single dose irradiated mice (Cekan et al., 1985 a). However, esperiments with selenium supplementation in the diet have been insucessful in protecting rats exposed to chronic irradiation (Hurt et al., 1971).

A single dose of sodium selenite (0.5 mg Se/kg) injected intraperitoneally into mice on day 9 of pregnancy 2h but not 30 min before whole body roentgen irradiation by a single dose of 1.75 Gy, results in a significant decrease in the number of malformed foetuses (Cekan et al., 1985 b).

Johnson (1977) studied the whole body retention following an intravenous injection of ^{75}Se-methionine in humans. Two hundred days after injection, the ^{75}Se body burden was reduced to 10% the initial value. So, the kinetics of elimination of Se appeared as quite slow, that would permit an ip administration to exert their action for a while.

Speier et al. (1985) working with clonal leukemia cultured cells , shown that glutatione peroxidase activity depended on a the medium Se concentration up to 2.6×10^{-8}M (sodium selecite, 5 mg/ml), above which a plateau occurred. They also observed no Se dependence or cell growth inhibition in serum free medium. However, it remains unknown whether the effect of Se on cell growth they observed represents a need for GSH peroxidase or for another unidentified Se-dependent activity.

GSH peroxidase is a selenium enzyme containing selenocystein at its catalytic site (Pryor, 1982). Lack of selenium leads to a deficiency in GSH peroxidase activity and consequently, decreased ability to oxidize glutathione and adequately catabolize H_2O_2. In studies on humans receiving total parenteral nutrition, a decrease of GSH peroxidase activity was observed during selenium deficiency and restoration of activity by Se replenishement (Pryor, 1982). Selenium intake (5.0 ppm) induces growth retardation, lack of growth hormone response to growth hormone releasing hormone (GHRM) in rats as established by Thorlacius-Ussing et al (1988). The same group shown that growth hormone after stimulation with GHRH and plasma somatomedin C were both drastically reduced in animals treated with 15 ppm sodium selenite (5.0 ppm selenium) in the drinking water (Thorlacius-ussing et al., 1987).

So far, the only selenium-containing compound with a specific physiological function identified in animal tissue is the enzyme glutathione peroxidase which 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 (Cekan et al., 1985 b). Some possible interpretation of protection against radiation damage could arrive by the study of mechanisms analogous to those of sulphydryl-containing radioprotectors. 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.

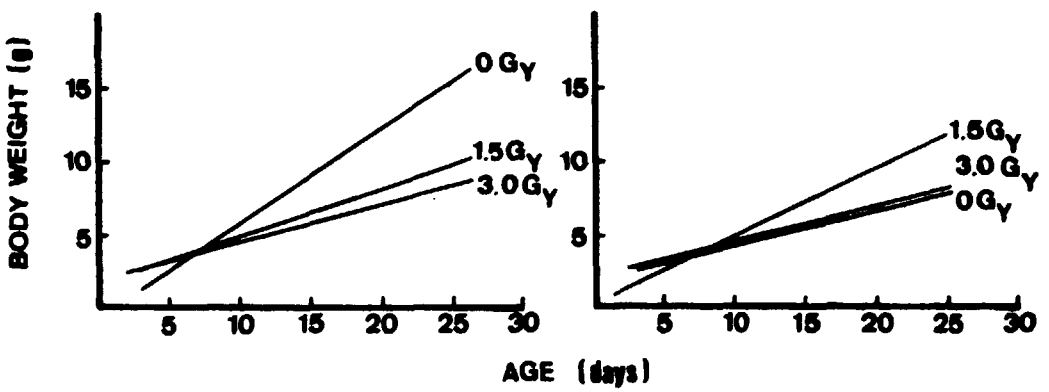

Fig. 1. Body weight gain curves as a function of age of mice ^{60}Co-irradiated in 17 day of gestation. a) H_2O injection b) 0.5 mg/kg b.w. sodium selenite injection.

Table I Slopes of body weight gain curves from birth to 25 day old of newborns coming from irradiated and selenium treated 17-day pregnant mice.

Treatment	Slope	Standard deviation	T for H0: parameter=0	Prob>|T|	R-Square
H_2O	0.65451075	0.02542714	25.741	0.0001	0.9246
Se	0.26559841	0.01398048	18.998	0.0001	0.7337
1.5 Gy	0.32575006	0.01126342	28.921	0.0001	0.8189
Se + 1.5 Gy	0.41566271	0.01354389	30.687	0.0001	0.8945
3.0 Gy	0.24627161	0.01243582	19.803	0.0001	0.7687
Se + 3.0 Gy	0.25675419	0.01080826	23.755	0.0001	0.8199

Table II Relationship of alive and total offsprings after treatment with ^{60}Co irradiation and sodium selenite of 17-day pregnant mice.

Treatment	Offspring (n = dam number) alive/total
H_2O	14/14 (n = 2)
1.5 Gy	33/33 (n = 4)
3.0 Gy	14/25 (n = 4)
Se	19/19 (n = 2)
Se + 1.5 Gy	26/34 (n = 4)
Se + 3.0 Gy	27/31 (n = 4)

Table III Increment in body weight from 3th to 15th day after ^{60}Co irradiation of Se treated and control animals.

Treatment	Average body weight increment
none	117.4 ± 24.5
1.5 Gy	117.3 ± 15.6
3.0 Gy	109.1 ± 2.0
Se (0.5 mg/kg b.w.)	108.5 ± 1.0
Se + 1.5 Gy	110.3 ± 1.2
Se + 3.0 Gy	98.4 ± 10.7

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