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THE INFLUENCE OF SELENIUM, VITAMIN E, AND OESTROGEN ON THE DEVELOPMENT OF TUMOURS IN MICE EXPOSED TO ^{90}Sr

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The primary object of this experiment was to evaluate the potential role of the antioxidants, selenium and vitamin E, in the anti-tumour defence of mice internally irradiated with ^{90}Sr. Comparison in terms of neoplastic response was made between mice kept on a selenium and viatmin E deficient diet and mice given the same deficient diet but administered selenium and/or vitamin E in a controlled manner. The influence of simultaneous oestrogen treatment, known to promote radiogenic osteosarcoma induction, was also investigated. Non-irradiated mice were used as controls. Results are presented as crude and actuarial tumour incidence. No significant difference in tumour yield or actuarial tumour incidence was found when the differently treated mouse groups were compared, and accordingly no support was gained for the theory that the antioxidants selenium and vitamin E constitute a critical part of the complex defence system against neoplasms.

A tumour protective role of selenium and vitamin E has been claimed for a long time, based essentially on their interrelated antioxidative function, but has so far not been convincingly confirmed by in vivo experiments; in fact results have been rather conflicting (1–3). In the main the hypothesis originates from cell culture studies and the knowledge of the importance of antioxidising agents to meet oxidative stress situations in the living cell such as characteristically generated by irradiation and many chemical carcinogens. In order to illuminate the problem in the in vivo situation we used a well documented tumour induction model involving osteosarcomas, malignant lymphomas, inbred CBA mice and ^{90}Sr as the carcinogenic agent. This model offers reference data for a variety of tumour variables (4–7) also when influenced by biological modifiers like hormones (8, 9), and thus the opportunity to introduce or withdraw and evaluate the influence of exogenous or endogenous factors. Here we report on the influence of selenium, vitamin E, and oestrogen in terms of observed and histologically classified tumours as well as a calculation of the probability of developing tumours.

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Material and Methods

Animals and procedures. Female mice from our own CBA/S inbred strain (origin: CBA/Ca via MRC Harwell (/H) to Stockholm University (/SU) and FOA Sundbyberg (/S)) were used. The mice were divided into 9 groups and maintained in conventional animal rooms in Macrolon III cages, with 10 mice in each cage. Room temperature was 20–23 °C, relative humidity 50–60%, and the light/dark photoperiod 12/12 h. Feed and water was given ad libitum. Wood shavings were used as bedding (Beekay GLP bedding). From the age of 60 days and onwards all mice were kept on a specially prepared diet deficient in selenium and vitamin E. ^{90}Sr-injections were made at the age of 75 ± 3 days and weight 24 ± 4 g. Polyoestradiol phosphate was injected in selected mice at 30, 60, and 90 days after the exposure to ^{90}Sr. Selenium and/or vitamin E was continuously supplemented in selected groups by injection every second week with the first injection given at 105 days after the exposure to ^{90}Sr. After 14 months the same amounts were injected at 30-day intervals lasting for the rest of the

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life-span. The various combinations of treatments are depicted in Table 1. Permission for the investigation was obtained from the Swedish Ethical Committee for Animal Experimentation.

Diets. The mice were raised on a commercial diet (Brood Stock Feed for Rats and Mice-R3, Ewos AB, Södertälje), containing $\geqslant 0.14$ μg Se and 0.2 mg vitamin E per g feed. Throughout the experiment all mice were kept on a semi-synthetic diet (supplied by Ewos AB) deficient in Se (3–6 ng per g feed) and vitamin E (4–14 μg per g feed). This diet was prepared in six batches and stored at -18°C in sealed plastic bags filled with nitrogen gas. Each batch was used within less than 6 months and the animals were given fresh diet every week.

Biological control of selenium/vitamin E deficient diet. Juvenile CBA females fed this diet developed fatal dystrophic myocardial and muscular lesions typical of selenium/vitamin E deficiency, whereas no such effect was observed in adults given the same feed.

Vitamin E. α-Tocopherol acetate (E vitamin, 30 mg/ml, ACO AB, Solna) was diluted in sterile oleum arachidis and injected intramuscularly in doses of 0.5 mg/mouse.

Selenium + vitamin E. Sodium selenite corresponding to Se^{4+} 0.6 mg/ml and α-tocopherol acetate 30 mg/ml (Tokosel vet., Pherrovet AB, Malmö) was diluted in sterile water and injected intraperitoneally in doses of 10 μg Se and 0.5 mg α-tocopherol acetate per mouse.

Oestrogen. Polyoestradiol phosphate (Estradurin, LEO AB, Malmö), with a long-term release of oestradiol-17β (approximately 30 days), was diluted in sterile water and injected subcutaneously in doses of 0.12 mg per mouse.

^{90}Sr treatment. Carrier-free ^{90}Sr-nitrate in 1N nitric acid (supplied by Amersham International, Amersham, England) was diluted in saline to an activity of 1 455 kBq per ml. The solution was in equilibrium concerning ^{90}Sr and ^{90}Y when administered to the mice by intraperitoneal injection. The activities injected were 0.00 or 9.25 kBq (0.25 μCi) ^{90}Sr per g body weight.

Pathology. The mice were allowed to live until they were moribund, and then euthanized with ether. Complete autopsies were performed, including both gross and microscopic evaluations. Dorso-ventral radiographs were used to detect early bone lesions. Tissues were fixed in Stieve's solution for 12 h, and cross sections for microscopy were prepared as described previously (10). Sections of the hard tissues were stained by van Gieson's method, and sections of the soft tissues with hematoxylin and eosin (HE). Special stains were used when required. The histologic typing of tumours was done in accordance with the system recommended by the European Late Effects Project Group (EULEP) Committee on Pathology Standardization (11).

Statistics. Neoplastic responses were expressed as the crude number and percentage of various tumour types found in each group of mice. Calculation of the probability of developing osteosarcoma, or malignant lymphoma involved appropriate correction for competing mortality, i.e., actuarial tumour incidence and actuarial time of tumour appearance (12, 13) was used along with log-rank testing. Kaplan-Meier survival curves (Figure) were used to illustrate the pattern of occurrence of osteosarcomas and/or malignant lymphomas according to treatment group.

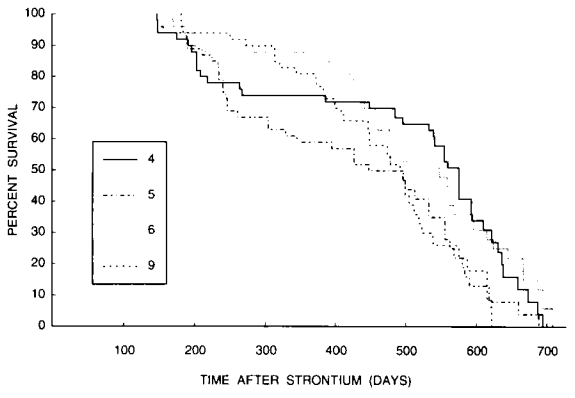

Figure. Kaplan-Meier plot of the development of malignant tumours (osteosarcomas and/or lymphomas) after treatment with ^{90}Sr (group 4), ^{90}Sr + vitamin E (group 5), ^{90}Sr + vitamin E/selenium (group 6), and ^{90}Sr + vitamin E/selenium + oestrogen (group 9).

Results

Crude tumour parameters. All neoplastic lesions diagnosed in each group are presented according to their histologic type, by number and percentage, in tabulated form (Table 1).

Actuarial tumour parameters. The probability of tumour development and time to tumour development have been calculated for each group and are presented in text and tabulated form (Table 2). The mean times for the actuarial appearance of tumours were shorter for mice with an additional exposure to oestrogen, in comparison with the figures obtained from animals only treated with ^{90}Sr. This was probably true also for mice given vitamin E in addition to the ^{90}Sr. The oestrogen may have a depressing effect on the actuarial incidence of malignant lymphomas. However, the hormone had no significant effect on the incidence rate of the lymphomas. The non-significantly higher actuarial incidences and lower mean induction times of the osteosarcomas in the mice treated with oestrogen as compared to those not treated with the hormone, suggest that oestrogen acts acceleratingly on the tumour formation. In order to examine this suggestion we have combined the two parameters (actuarial incidence and induction time) in a 'log-rank' test. The figures in Table 3 have all been obtained in comparison with the time-corresponding numbers of osteosarcomas in the mice treated with ^{90}Sr only.

Table 1

Crude tumour incidences (percentage) in female CBA mice fed a selenium and vitamin E deficient diet, and treated with ^{90}Sr, vitamin E, selenium, and oestrogen as described in materials and methods.

Group	1	2	3	4	5	6	7	8	9
Treatments	Control	– E – –	E/Se	^{90}Sr – –	^{90}Sr E –	^{90}Sr – E/Se –	^{90}Sr – oestrog.	^{90}Sr E oestrog.	^{90}Sr E/Se oestrog.
No. of mice at start of experiment	30	30	30	51	50	50	50	52	51
No. of mice examined*	29	28	29	51	50	50	44	52	50
Survival time, days**	700 ± 21	725 ± 12	734 ± 17	491 ± 25	425 ± 23	505 ± 23	436 ± 16	447 ± 13	453 ± 16
Malignant lymphoma	1(3)†	2(7)	8(28)	17(33)	20(40)	15(30)	9(21)	8(15)	8(16)
Histiocytic sarcoma	2		2	–	–	1		–	
Mastcell tumour	–	–	1			1§			
Bronchiolo-alveolar adenoma	3(10)	–		4(8)	4(8)	4(8)	4(9)	3(6)	5(10)
Bronchiolo-alveolar carcinoma	–			1(2)	2(4)			1(2)	1(2)
Hepatocellular adenoma	6(21)	9(32)	10(34)	2(4)	4(8)	4(8)	–	1(2)	–
Hepatocellular carcinoma	–	2(7)	3(10)	1(2)	–			–	
Mal. haemangioendothelioma	–	–	1	2		–			–
Mammary glands									
adenocarcinoma	–	–				2	1	1	–
sarcoma	2			1					
Ovaries									
adenoma				2					
granulosa cell tumour				2		3	–		
carcinoma	–		1					–	
Uterus									
adenoma	–			–					1
adenocarcinoma	–			–		–	–		4
leiomyoma		–		1		1			1
Harderian gl. tumour		–				2	–		
Pituitary adenoma					1			1	
Integument									
keratoacantoma		–				–		–	2
sebaceous carcinoma	–	–		–	–			1	
undiff. carcinoma	–				1				
Sustentaculum									
fibroma		–				1			
fibrosarcoma		–	1	1			1		–
Ameloblastoma	–							1	
Osteosarcoma	1(3)	–		25(49)	19(38)	29(58)	15(34)	27(52)	30(60)
Total No. of osteosarcomas	1			37	32	52	17	48	51
Osteosarcomas per mouse***	1.00	–		1.48	1.68	1.79	1.13	1.78	1.70

* Animals found dead and autolyzed were not microscopically examined.
** Mean ± standard error of the mean.
*** Mean number of osteosarcomas in osteosarcoma bearing mice.
† Number of animals with tumour (percentage).
§ Generalized.

As shown by the data in Table 3 oestrogen exerts an obvious accelerating effect on ^{90}Sr-induced bone tumours, whereas selenium and vitamin E do not affect the tumourigenesis. A corresponding test performed on malignant lymphomas was negative. However, the increase in the actuarial incidence of lymphomas was very steep during the first 100 days after the appearance of the first animal demonstrating this malignancy after combined treatment with ^{90}Sr, selenium and vitamin E. After this initial period of time only very few cases were observed. The actuarial incidence of lymphomas in animals treated with oestrogen exhibited a much slower increase with time, but this increase remained the same during the entire period of examination. The increase in actuarial incidences of malignant lymphomas per day during these initial 100 days are shown in Table 4. The stars indicate a significantly slower increase rate of malignant lymphomas in oestrogen-treated animals as compared to those only given ^{90}Sr, or ^{90}Sr in

Table 2

Actuarial incidence (A) and mean time of actuarial appearance (T) of osteosarcomas and malignant lymphomas

Group treatment	All types of osteosarcomas	Osteoblastic osteosarcomas	Fibroblastic osteosarcomas	Malignant lymphomas
4	A; 0.576 ± 0.090	0.521 ± 0.092	0.032§	0.349 ± 0.073
^{90}Sr	T; 568 ± 17	567 ± 19	534	319 ± 48
5	A; 0.508 ± 0.103	0.440 ± 0.109	0.123 ± 0.079	0.432 ± 0.078
^{90}Sr + vit-E	T; 510 ± 18*	526 ± 17	537 ± 21	282 ± 30
6	A; 0.664 ± 0.083	0.577 ± 0.093	0.221 ± 0.091	0.322 ± 0.080
^{90}Sr + vit-E/Se	T; 529 ± 18	537 ± 21	549 ± 33	393 ± 46
7	A; 0.413 ± 0.107	0.306 ± 0.102	0§	0.227 ± 0.068
^{90}Sr + oestrogen	T; 484 ± 18**	494 ± 20	–	284 ± 39
8	A; 0.672 ± 0.084	0.530 ± 0.098	0.245 ± 0.111	0.159 ± 0.51*
^{90}Sr + vit-E + oestrogen	T; 467 ± 12***	574 ± 14	499 ± 42	292 ± 33
9	A; 0.655 ± 0.079	0.560 ± 0.090	0.180 ± 0.086	0.150 ± 0.052*
^{90}Sr + vit-E/Se + oestrogen	T; 452 ± 15***	463 ± 15***	482 ± 30	300 ± 35

A = actuarial incidence based on tumour appearance up to the level of 10 surviving mice.
T = mean time (days) between ^{90}Sr-injection and actuarial appearance of tumours.
The stars denote significant deviations from the corresponding values obtained from animals only treated with ^{90}Sr (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).
§ Value is below reference value.

Table 3

Osteosarcoma susceptibility (Z) calculated by log-rank testing of actuarial incidences and induction times

Group	Treatment	Z	p
5	^{90}Sr + vit-E	1.25	–
6	^{90}Sr + vit-E/Se	0.27	
7	^{90}Sr + oestrogen	4.14	< 0.001
8	^{90}Sr + vit-E + oestrogen	4.52	< 0.001
9	^{90}Sr + vit-E/Se + oestrogen	3.47	< 0.001

combination with selenium or vitamin E (***$p < 0.001$; *$p < 0.05$ respectively). Hence, oestrogen seems to have a retarding effect on ^{90}Sr induced malignant lymphomas in the initial phase of their formation.

Discussion

The main observations in this study are: a) a decreased crude incidence of malignant lymphomas (ML) in oestrogen-treated groups compared with relevant controls (Table 1); b) a decreased incidence rate of ML during a certain period of 100 days in oestrogen groups (Table 4); c) an enhancement of the appearance, but no significant effect on the crude or actuarial incidence of osteosarcomas in oestrogen groups (Tables 1–3), and finally and particularly important d) no significant effects of vitamin E or vitamin E/Se on ^{90}Sr induced osteosarcomas.

Radiation is well known to cause oxidative stress by radiolysis in exposed cells and tissues. The generation of free oxygen radicals in living cells represents one of the most serious threats to cellular molecules and thus to the biochemical and physiological cell functions (14). Evolu-

Table 4

Increase in actuarial incidences of malignant lymphomas (ML) per day during the initial 100 days after appearance of the first ML

Group	Treatment	Day of first appearance of ML	The increase in actuarial inc./day
4	^{90}Sr	148	$(2.47 \pm 0.42)10^{-3}$
5	^{90}Sr + vit-E	148	$(2.26 \pm 0.31)10^{-3}$
6	^{90}Sr + vit-E/Se	170	$(2.13 \pm 0.78)10^{-3}$
7	^{90}Sr + oestrogen	171	$(1.51 \pm 0.12)10^{-3}$*
8	^{90}Sr + vit-E + oestrogen	178	$(0.60 \pm 0.05)10^{-3}$***
9	^{90}Sr + vit-E/Se + oestrogen	182	$(0.50 \pm 0.04)10^{-3}$***

tion has met this problem with an efficient enzymatic and non-enzymatic antioxidant defence system. Selenium and vitamin E have long been known to play an important role in this system and have therefore been suggested for use as a tumour protective treatment. The results of the present study do not support this idea. The data instead show that the tumour response, and more clearly the probability of tumour development, following treatment with a tumourigenic amount of ^{90}Sr, was of similar magnitude in animals deprived of selenium and/or vitamin E as in those continuously injected with supranutritional amounts of the same substances (Figure). This observation has a special value in the fact that the experimental model concerns induction and development of two histogenetically diverse tumour types in a mouse strain not predisposed to develop these tumours.

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