FROM THE CENTRE OF RADIATION HYGIENE, INSTITUTE OF HYGIENE AND EPIDEMIOLOGY, 100 42 PRAGUE, CZECHOSLOVAKIA.

^{3}H-TDR LABELLING OF OSTEOPROGENITOR CELLS AFTER ^{226}RA INCORPORATION IN MICE

V. KOFRÁNEK, O. PAŘÍZEK, V. SVOBODA, D. BUBENÍKOVÁ and J. MACHEK

Based on standard autoradiographic methods, a technique using tritiated thymidine (^{3}H-TDR) to reveal deoxyribonucleic acid (DNA) synthesis in the nuclei of cells preparing to divide was developed several years ago (AMANO et coll. 1959) and has since been extensively used. The technique was also employed by KEMBER (1962) and TONNA & PAVELEC (1970) to investigate cell labelling indices in relation to postirradiation time, especially in osteogenic tissues of rats and mice exposed to external and internal irradiation. These authors found a considerable relationship between the number of cells taking up tritiated thymidine and the radiation dose.

In experiments with ^{226}Ra microdistribution in mouse femur and lumbar vertebra, using SST-autoradiography on organic foils, a different activity distribution in these analogous but topographically differently localized bone tissues was detected (KOFRÁNEK et coll. 1973). Assuming that the radiation dose to the populations of the proliferative cells at risk might be the critical factor in bone tumour induction (ICRP Publication No. 11, 1968), the interest was directed towards some quantitative changes occurring in the distinct compartments of dividing bone cells in relation to the dose from incorporated ^{226}Ra. The results are now reported.

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Material and Methods

Five-week-old SPF female mice of ICR strain, body weight approximately 23 g, were used in the experiment. The animals were injected intraperitoneally with $19.7 \pm 3.3\%$ (group BU) and $55.9 \pm 3.4\%$ μCi/kg body weight (group CU) of ^{226}Ra chloride in isotonic solution at pH 3 to 4 with calcium chloride carrier. At an interval of 2 hours to 28 days, both femurs and lumbar vertebrae were removed from 6 exsanguinated experimental mice and two control animals all given, 1 hour before killing, 1 μCi/g body weight of tritiated thymidine (^{3}H-TDR), specific activity 23.3 Ci/mM. One of the removed femurs and the 3rd lumbar vertebra from each mouse were examined with regard to the time course of ^{226}Ra activity distribution, by means of SST-autoradiography. This technique was described previously (KOFRÁNEK et coll. 1973).

The other femur and the 4th lumbar vertebra were electrolytically decalcified, histologically processed into 7 to 10 μm thick sections, and covered with Kodak AR-10 stripping film emulsion. After 1 month exposure the slides were photographically developed and histologically stained with Harris-hematoxylin and eosin. In some sections a modified coupling azo dye method for alkaline phosphatase was used for the topographic identification of the zones of cell compartments involved in new bone formation (PEARSE 1968).

As the main potentially hazardous sites of osteosarcoma induction first of all the endosteal surface of the trabecular bone was chosen and then the growth plates (proliferating zones) and furthermore the periosteal surfaces in distal epiphysis of the femur and in the lumbar vertebra (VAUGHAN 1970, LOUTIT & VAUGHAN 1971). In each of the above bone areas 200 to 500 osteoblast- and chondroblast-like cells, including the ^{3}H-TDR labelled cells were scored. Other cell compartments, i.e. osteocytes, osteoclasts, hypertrophic chondrocytes, vascular-endothelial cells, bone-marrow cells and cells found outside the strictly defined zones, were not taken into account (PRITCHARD 1968). In 8 to 10 sections, prepared at individual time intervals from femurs and lumbar vertebrae/per mouse, altogether more than 1.5×10^{6} cells were microscopically scored.

Because of the 1-hour interval between ^{3}H-TDR administration and the killing of the animals, the labelling was confined to cells under DNA synthesis (S phase) and to those which had proceeded to the postsynthetic phase (G_2) before mitosis. The labelling index values therefore indicate the proliferative activity of the cell compartments. The relative labelling index values were expressed in percentage of the labelling.

The differences among the observed relative frequencies were tested by χ^2 test using a method described by BLOM (1954), where the χ^2 test is constructed with the aid of a matrix of orthogonal standardized coefficients which convert the low relative frequencies transformed by a Poisson approximation to uncorrelated standardized normal values. The mathematical procedure was programmed for a 2116 C HEWLETT-PACKARD computer and the computations carried out accordingly.

Table

The time course of the experimental labelling index values together with average control values of osteoprogenitors in the mouse femur (distal epiphysis) and lumbar vetebra after administration of 19.7 μCi (BU group of mice) and 55.9 μCi (CU group of mice) of ^{226}Ra/kg body weight. c.v. = average value of the control labelling index

Time	Femur—distal epiphysis						Lumbar vertebra					
	Periosteum c.v. = 0.92		Growth plate c.v. = 3.21		Endosteum c.v. = 6.56		Periosteum c.v. = 0.44		Growth plate c.v. = 2.44		Endosteum c.v. = 2.27	
	BU	CU	BU	CU	BU	CU	BU	CU	BU	CU	BU	CU
2 h	0.50	0.00*	0.59*	0.60*	6.33	2.34*	0 04*	0.00*	0.05*	0.00*	6.14*	1.81
6 h	0.07*	0.03*	0.45*	0.05*	5.25*	0.38*	0.02*	0.01*	0.02*	0.01*	2.49	0.11*
24 h	0.10*	0.12*	0.36*	1.37	1.12*	0.09*	0.01*	0.02*	0.06*	0.00*	2.45	0.03*
3 d	0.62	0.53	3.32	2.42	0.60*	0.06*	0.02*	0.02*	0.22*	0.01*	3.34*	0.41*
7 d	0.38*	0.17*	1.45*	1.17*	3.11*	1.15*	0.04*	0.00*	0.22*	0.06*	2.01	0.68*
14 d	0.25*	0.73	1.52*	1.64*	5.94	4.21*	—	0.42	—	0.34*	—	1.71
28 d	0.05*	0.10*	1.15*	0.94*	1.37*	3.80	0.00*	0.13	0.89	0.00*	1.59	1.24

* $p < 0.05$

Results

In the control animals the highest labelling index was found in femur endosteum and its minimal values were observed in periosteum. In the distal epiphysis of the femur about one third higher values (basic) were found than in the vertebra.

The Table gives time courses of experimental labelling index values in distal epiphysis of the femur and in lumbar vertebra after administration of 19.7 and 55.9 μCi of ^{226}Ra/kg body weight, with statistical significance to the control values. It is evident that especially in the growth plates of both bones the number of labelled cells decreased markedly soon after ^{226}Ra injection; the decrease being related to the given dose of nuclide.

Fig. 1 gives the time course of experimental labelling index values both for the vertebral and femoral endosteum. The vertebral endosteum exhibits lowered values of a rather persisting character after the injection of a higher level of activity (CU), while after the administration of a lower activity (BU) the labelling index is raised above the control value throughout the first four time intervals. In the femoral endosteum the depression phase is protracted until the third day, being followed by a rise of the index near to the control value. The experimental values of the labelling index are again related to the amounts of ^{226}Ra administered.

The correlation of labelling index values with dose levels in the corresponding bone areas reveals some relationship between the values of the rising dose and decreasing values. In comparison to the Table, Fig. 2 demonstrates the time course of the accumulated dose and the dose rate in femoral and vertebral endosteum of mice after the injection of 19.7 and 55.9 μCi ^{226}Ra/kg body weight.

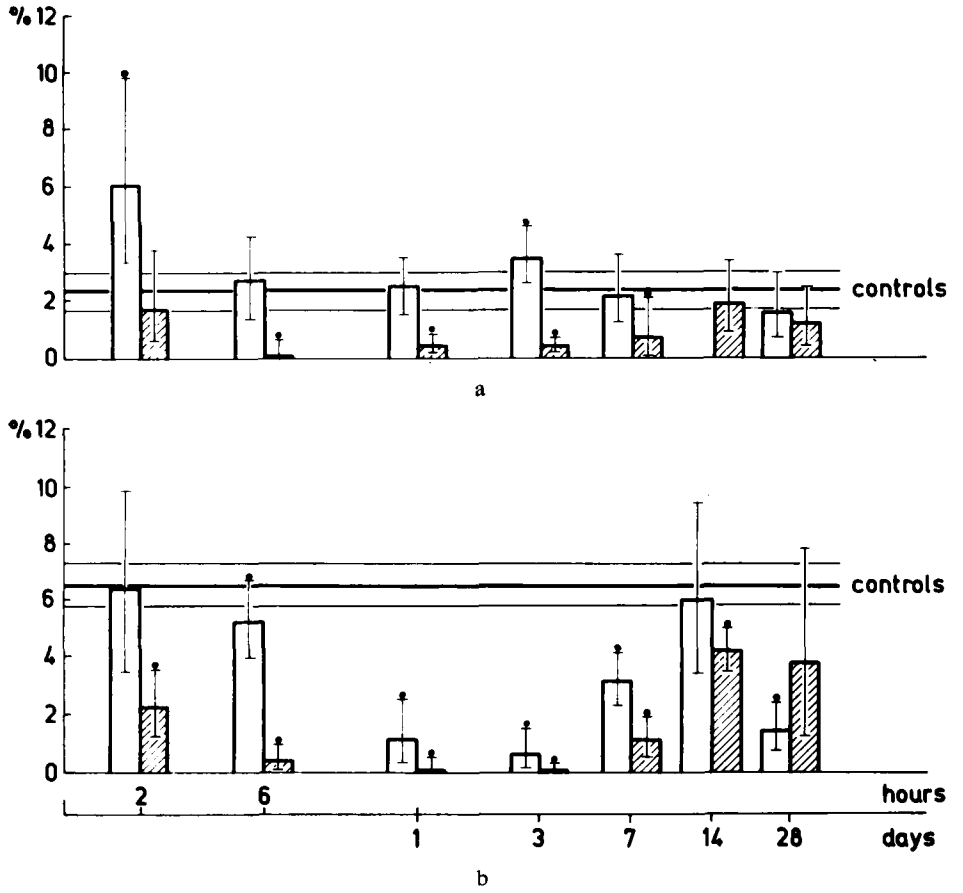

Fig. 1. The time course of the per cent ^{3}H-TDR labelling of osteoprogenitor cells in a) endosteum of lumbar vertebra and b) distal femoral epiphysis of mice after single injections of two ^{226}Ra levels (with 95 % confidence limits of the mean). BU (unfilled bars) = group of mice after injection of 19.7 μCi ^{226}Ra/kg body weight. CU (hatched bars) = group of mice after injection of 55.9 μCi ^{226}Ra/kg body weight. Pointed values = $p < 0.05$.

Discussion

The generally accepted idea that the cells at risk are only those which are dividing, was applied in the present experiment. By using ^{3}H-thymidine labelling, the proliferative potential of osteogenic cells was related to the amounts of ^{226}Ra injected and to the time course of the accumulated dose and dose rate in selected bone areas. It was ascertained that the changes of labelling index values in vertebral and femoral growth plates, as well as the changes of those in vertebral and femoral endosteum, are dose dependent.

The time courses of relative labelling index values in femoral endosteum differed from the changes of the values in vertebral endosteum (Fig. 1). The differences

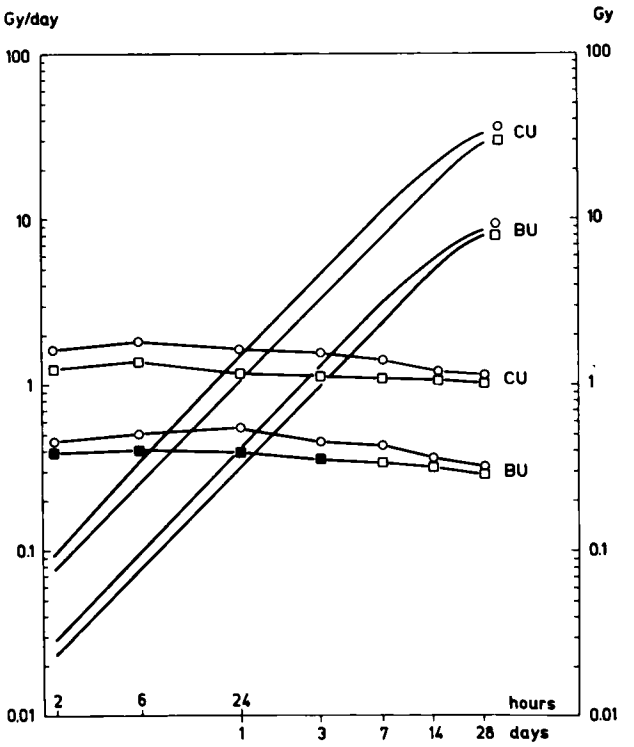

Fig. 2. The time course of the accumulated dose in Gy (oblique curves) and dose rate in Gy/day (horizontal curves) in endosteum of lumbar vertebra (□) and distal femoral epiphysis (○) after injection of 19.7 and 55.9 μCi/ kg of ^{226}Ra in mice. (Labelling indices below the control values = empty symbols and over the control values = full symbols.)

between the responses of both compartments of dividing cells might probably be either due to differences in the distribution of radiation dose, or due to a changed time table for cell ageing, which is apparently not synchronized for all the skeletal cell compartments associated with a given structure, or because of both. The metaphyseal endosteal osteoblasts seem to differ morphologically and biochemically from other osteogenic cells (TONNA 1965).

The general character of the dose-response curve after injection of bone-seeking nuclides is the increased incidence of bone tumours with increasing amount of isotope administered. After a maximum is reached the further increase in dose is followed by a decline in the incidence. This has been confirmed experimentally in mice after a single injection of nuclide by many authors, e.g. FINKEL & BISKIS (1959) with ^{226}Ra, ^{90}Sr and ^{45}Ca, HUG et coll. (1969) with ^{224}Ra or NILSSON (1970) with ^{90}Sr.

With regard to the experimental results (Table, Fig. 2) it is suggested that bone areas exhibiting relatively low dose rates after ^{226}Ra incorporation preserve some normal or raised levels of proliferative activity of osteoprogenitors. If the values of dose rate and accumulated dose are higher, the proliferation is, on the contrary, inhibited.

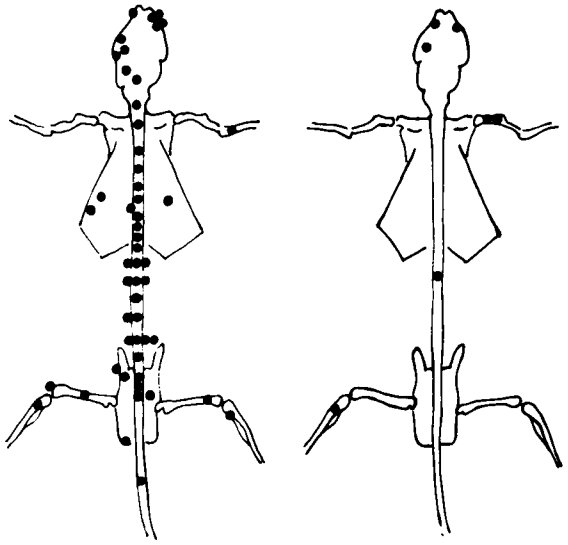

Fig. 3. The topographical localization of osteosarcomas after ^{226}Ra administrations in mice. The left mouse represents a 52 per cent incidence of osteosarcomas in a group of 100 mice with 24.6 μCi/kg and the right mouse represents only a 7 per cent incidence of osteosarcomas in a group of 100 mice with 70.5 μCi/kg of injected ^{226}Ra (KOFRÁNEK et coll., 1976).

Assuming that the neoplastic effect depends on the dose distribution and on the kinetic behaviour of target cells, the differences in the proliferative capacity of osteoprogenitor cell compartments might be related to the potential malignant transformation of dividing bone cell precursors. The decrease of labelling index values after higher doses might be compatible with rising dose of radiation, above a maximum follows a decline in its incidence. At least the experimental results (KOFRÁNEK et coll. 1976) with osteosarcoma production after ^{226}Ra administration (Fig. 3) in comparison with approximately similar nuclide levels administered in the present experiment with ^{3}H-TDR, are fully in agreement with findings by FINKEL & BISKIS (1959) in mice receiving injections of 28.8 and 50 μCi ^{226}Ra/kg body weight, respectively. In all these cases the osteosarcoma production after administration of more than 50 μCi ^{226}Ra/kg body weight, as well as proliferative activity of osteoprogenitors, was reduced.

It has already been found by FINKEL et coll. (1964) that the initial dose rate value immediately after the injection of ^{226}Ra is an important factor influencing the production of osteosarcoma in the mouse. MÜLLER & LUZ (1975) in their ^{224}Ra and ^{227}Th experiments demonstrated that the incidence of osteosarcoma is closely related to the dose rate. The mean skeletal daily doses above 0.35 Gy/day rendered a lower incidence of bone tumours. The present experimental results seem to confirm these suggestions (Figs 2, 3).

Another interesting question is the localization of osteosarcomas in ^{226}Ra experiments with mice. The bone tumours induced by alpha emitting bone-seekers are predominantly situated in the axial skeleton and then in the long bones. On the left mice in Fig. 3, 43 per cent of primary osteosarcomas are located in axial skeleton,

6 per cent in long bones and 3 per cent in remaining sites. Especially in lumbar vertebrae, about 35 per cent of bone tumours of the whole axial skeleton including head are located.

A well known non-uniform distribution of nuclide is present in both these types of trabecular bone, which is in agreement with the experimental results showing different doses in endosteum of lumbar vertebra and femur after administration of either dose of ^{226}Ra (Fig. 2). This also might contribute to the higher osteosarcoma appearance especially in the lumbar vertebrae of mice.

Nevertheless, the relation between the compartment of osteoprogenitor cells at risk and induction of osteosarcomas after ^{226}Ra incorporation is a rather complicated problem when estimating radiation carcinogenesis. A series of another factors is involved including bone remodelling rate, duration of mitotic cycle, cellular environment, life span of cells, initial oncogenic dose etc., which all are influencing and affecting it. For further considerations about the induction of bone malignancy by alpha radiation the recent attempts to elaborate theoretical models may be of value among which the three-stage alpha particle model by MARSHALL & GROER (1975) seems to be very instrumental.

Acknowledgments

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SUMMARY

The time course of ^{3}H-TDR labelling index of osteoprogenitor cells and the doses in endosteum of lumbar vertebra and distal femoral epiphysis were autoradiographically determined in young female mice after single injections of 19.7 and 55.9 μCi/kg body weight of ^{226}Ra. The selected bone areas were examined in animals killed 2 hours to 28 days after the injection of nuclide. It was ascertained that changes in the relative labelling index are depending on the absorbed doses of alpha radiation. The possible relevance of these experimental findings for the explanation of the osteosarcoma induction and localization is discussed.

ZUSAMMENFASSUNG

Der Zeitverlauf des ^{3}H-Thymidin Markierungsindex der Progenitoren der Knochenzellen und die Dosen zum Endost des Rückenwirbels und der distalen Femurepiphyse wurden autoradiographisch bei jungen weiblichen Mäusen nach einzelnen Injektionen von 19,7 und 55,9 μCi/kg ^{226}Ra untersucht. Ausgewählte Knochenabschnitte wurden 2 Stunden bis 28 Tage nach der Injektion des Nukleids bei den Tieren untersucht. Es wurde festgestellt, dass die Veränderungen im relativen Markierungsindex von der absorbierten Dosis der Alphabestrahlung abhing. Die mögliche Relevanz dieser experimentellen Befunde für die Erklärung der Induktion von Osteosarkomen und deren Lokalisation wird diskutiert.

RÉSUMÉ

Les auteurs ont étudié par auto-radiographie sur de jeunes souris femelles, après des injections uniques de 19,7 et 55,9 μCi/kg de ^{226}Ra, l'évolution en fonction du temps de l'index marqueur de ^{3}H-TDR de cellules ostéoprogénitrices et ils ont étudié les doses dans l'endostéum de la vertèbre lombaire et dans l'épiphyse fémorale distale. Les régions osseuses sélectionnées ont été examinées sur des animaux tués de 2 heures à 28 jours après l'injection du radionuclide. Il a été établi que les modifications de l'index relatif de marquage dépendent des doses absorbées de radiation alpha. Les auteurs étudient l'intérêt possible de ces résultats expérimentaux pour expliquer l'induction d'ostéosarcome et sa localisation.

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