Osteosarcoma induction in mice by the alpha-emitting nuclides, plutonium-239, americium-241 and uranium-233

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Introduction

Following either occupational or environmental exposure of individuals to alpha-emitting isotopes of plutonium (Pu), americium (Am) or uranium (U), their entry into the bloodstream will result in long-term retention in the skeleton and a consequent risk of osteosarcoma and leukaemia. The International Commission on Radiological Protection (ICRP) has recently adopted models for the behaviour of radionuclides in the skeleton which take account of the physiological processes of bone remodelling and growth (1,2). The models treat Pu and Am similarly, allowing for burial of surface deposits during mineral deposition and transfer to the marrow and release to the circulation during bone resorption (1). Thus, the models predict that ^{239}Pu and ^{241}Am are similarly effective in inducing osteosarcoma and leukaemia. Uranium behaves more like calcium and the alkaline earth elements and the model assumes more rapid transfer from surfaces to bone volume (2). Because a greater proportion of alpha energy from U isotopes is deposited harmlessly in bone mineral, they are predicted to be less effective in causing malignancy. No changes have been made to the assumptions that the sensitive cells for osteosarcoma induction are uniformly distributed on endosteal bone surfaces and those for leukaemia induction are uniformly distributed through red bone marrow. Furthermore, radionuclides are assumed to initially deposit uniformly on endosteal bone surfaces.

A number of finding are at variance with model predictions. Experimental studies using rats (3) and mice (4) have shown that ^{239}Pu is about three times as effective as ^{241}Am in inducing osteosarcoma per unit average skeletal dose. Autoradiographic studies of mouse (5) and rat (6) bone have shown differences in the initial distribution of ^{239}Pu, ^{241}Am and ^{233}U as well as their subsequent behaviour. The induction of osteosarcoma but absence or very low incidence of leukaemias in humans given radium isotopes suggest that alpha irradiation from bone surfaces may be ineffective in causing leukaemia, possibly due to heterogeneity in the distribution of sensitive cells in the marrow (7).

This paper presents results from a study of the comparative toxicity of ^{239}Pu, ^{241}Am and ^{233}U in mice, comparing tumour incidence and the distribution of alpha dose within the skeleton. Completed results for osteosarcoma induction are reported; leukaemia diagnoses are not yet complete.

Materials and Methods

Male CBA/H mice were obtained from the Medical Research Council (MRC) Radiobiology Unit, Chilton, Didcot, Oxon, OX11 0RD. This strain a very low spontaneous incidence of osteosarcoma and leukaemia. Groups of 5 mice were housed together. Food (type RMI Expanded, Special Diet Services, Witham, Essex) and water were freely available at all times. Animal care and handling conformed to the UK Animals (Scientific Procedures) Act 1986.

Groups of 12 week old mice (50-100) were given intraperitoneal injections of ^{239}Pu, ^{241}Am or ^{233}U as the citrate complexes. Each nuclide was administered to three groups of animals at levels of activity estimated to give average bone doses of about 0.2-0.3 Gy, 0.5-1.0 Gy and 1.3-1.6 Gy, calculated to 500 days after administration; a further control group (100) were given inactive solution. To avoid chemical toxicity to the kidneys from ^{233}U, each nuclide was administered as 9 injections over a 3 week period. All groups were treated similarly. The mice were followed for their life-span and either died or were killed when moribund or a visible tumour was present. Osteosarcomas were identified by x-ray examination of the skeleton at death and the tumours confirmed by histological examination. Other organs and tissues were taken for histological examination as appropriate.

Results

Table 1 summarises tumour diagnoses for each group of animals, counting all observations including cases where individual animals were shown to have more than one tumour or tumour type. High incidences of liver and lung tumours were observed for all experimental groups and the control group, as expected with this mouse strain (8,9). A variety of other soft tissue tumours were identified with low incidences (see footnotes to Table 1). Small numbers of blood cell tumours were also observed; final diagnoses are in progress.

Table 1. Number of tumours by type

Group	^{239}Pu			^{241}Am			^{233}U			Control
Average skeletal dose to 500 days (Gy)	0.2	0.5	1.3	0.3	0.9	1.6	0.3	0.9	1.3	0
No of animals	100	60	50	100	75	50	100	60	50	100
Osteosarcoma	2	5	14	0	3	10	2	1	2	1
Other skeletal tumours[a]	0	1	5	3	4	0	3	0	0	6
Leukaemias / lymphomas[b]	5	6	1	6	0	0	2	1	1	2
Other haemopoietic disorders[c]	2	1	1	3	4	1	4	2	3	5
Liver tumours[d]	94	76	48	86	69	43	83	66	42	80
Lung tumours[e]	40	23	15	36	24	10	40	19	16	37
Other soft tissue tumours[f]	10	12	10	8	11	8	19	18	30	19

[a] skeletal haemangioma, haemangiosarcoma, osteoma, odontoma, sarcoma, mastocytoma ; [b] myeloid leukaemia, lymphatic leukaemia, malignant lymphoma; [c] histiocytic sarcoma, haemangiosarcoma, haemangioma; [d] hepatocellular adenoma and carcinoma; [e] bronchiolo-alveolar adenoma and carcinoma; [f] islet adenoma and carcinoma, tubular adenoma and carcinoma, adrenal cortical adenoma, adrenal phaeochromocytoma, neuroendocrine carcinoma, meningeal sarcoma, malignant paraganglioma, malignant schwannoma, rhabdomyosarcoma, sarcoma, leiomyoma, trichoepithelioma and squamous cell carcinoma (skin)

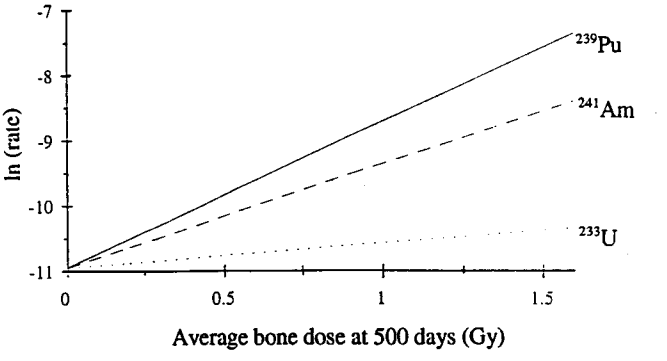

Figure 1. Incidence of osteosarcoma following administration of ^{239}Pu, ^{241}Am or ^{233}U

Osteosarcoma was seen in 2%, 8% and 18% of mice in the 0.2 Gy, 0.5 Gy and 1.3 Gy ^{239}Pu groups (5, 15 and 25 kBq kg^{-1}), respectively. The corresponding incidences of osteosarcoma in ^{241}Am groups were 0, 4% and 18%, respectively, and incidence in each ^{233}U group was about 2%. Only the highest dose groups had animals with more than one osteosarcoma, with one animal having two tumours in each nuclide group and 2 animals having three tumours in the ^{239}Pu group. A difference was apparent in the location of osteosarcomas in the highest dose ^{239}Pu and ^{241}Am groups in that about 70% in the ^{239}Pu group were in the appendicular skeleton, mainly femur, while a similar proportion in the ^{241}Am group were in the axial skeleton, mainly vertebrae. Osteosarcoma incidence as a function of dose was assessed using Poisson regression (Figure 1). Significance was tested by comparing changes in deviance with a χ^2 distribution. Dose was found to have a significant effect on incidence (change in deviance = 20.34 on 1

$p<<0.001$). However, the effect differed significantly between nuclides (change in deviance = 16.21 on 2 d.f.; $p<0.001$). There was evidence for a difference in regression slopes for ^{239}Pu and ^{241}Am (change in deviance = 3.86 on 1 d.f.; $p=0.05$) and a single slope for ^{239}Pu and ^{241}Am differed significantly from a slope for ^{233}U (change in deviance = 12.35 on 1 d.f.; $p<0.001$). For ^{233}U, the slope was consistent with no effect of dose. A further analysis will take account of ages at death and competing causes.

Discussion

The observed incidences of ^{239}Pu induced osteosarcoma in male CBA/H mice are consistent with reported incidences in females of the same strain of 7.5% and 30% after administration of 6 kBq kg^{-1} and 19 kBq kg^{-1} of ^{239}Pu, respectively (8), and the observation that females were more sensitive than males to osteosarcoma induction by a factor of 3 - 4 for ^{239}Pu and 4 - 6 for ^{226}Ra (9). It is considered that the difference may be due to greater turnover of bone in females. The observed difference in osteosarcoma induction by ^{239}Pu and ^{241}Am was smaller than reported previously. Bensted et al (4) reported that 17 out of 22 male rats given 108 kBq kg^{-1} ^{239}Pu developed osteosarcoma compared with 4 out of 19 given 92 kBq kg^{-1} ^{241}Am. Using small groups of animals (10 - 20), Taylor et al (3) showed a significantly greater incidence of osteosarcoma in male and female C57Bl/Do black mice given 105 kBq kg^{-1} and 32 kBq kg^{-1} of ^{239}Pu than in comparable groups given 102 kBq kg^{-1} and 30-34 kBq kg^{-1} of ^{241}Am. Similar studies using C57BL/Do albino mice showed greater osteosarcoma incidence with ^{239}Pu than ^{241}Am in female mice but no difference in male mice.

Autoradiographic studies have shown clear differences in the initial distribution of ^{239}Pu and ^{241}Am on bone surfaces in rodents (5,6). While ^{239}Pu is concentrated mainly on endosteal surfaces of trabecular and cortical bone, ^{241}Am is more widely distributed on bone surfaces including periosteal surfaces and internal cortical bone surfaces. Previous reports of greater sensitivity to osteosarcoma induction by ^{239}Pu than ^{241}Am have been attributed to greater doses from ^{239}Pu to trabecular surfaces (10). It is possible that differences in distribution and osteosarcoma incidence were accentuated by increased bone turnover caused by the deposited nuclides which is apparent, according to Loutit et al (9), at administered activities of 20 kBq kg^{-1} and greater.

Uranium has been shown to deposit initially on bone surfaces, including endosteal and periosteal surfaces, concentrating on growing surfaces (5,6). The low toxicity of ^{233}U compared with ^{239}Pu and ^{241}Am is consistent with more rapid burial of surface deposits, reflecting the chemical similarity of the uranyl ion and those of the alkaline earth elements, including calcium.

In conclusion, it would appear that the observed osteosarcoma incidences, although showing small differences between ^{239}Pu and ^{241}Am, are broadly consistent with ICRP model estimates of equivalent toxicity of ^{239}Pu and ^{241}Am and lower toxicity of ^{233}U.

Acknowledgements

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