ELSEVIER

Journal of Alloys and Compounds 271–273 (1998) 100–102

Journal of ALLOYS AND COMPOUNDS

Extraction of Pu from rat bone by formic acid/guanidine treatment after intravenous ^{238}Pu-citrate administration: influence of time after contamination and of a chronic DTPA treatment

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Abstract

A formic acid/guanidine treatment was used to characterize the kinetics of Pu extraction from bone powder at different times after Pu contamination, and after DTPA treatment. Male Sprague-Dawley were contaminated by intravenous injection of 37 kBq ^{238}Pu(IV)-citrate either at 3 or 6 months of age. DTPA treatment was performed between days 42 and 72 post-contamination by adding Zn-DTPA to the drinking water. The animals were killed 2 or 85 days after contamination. Significant differences in the extraction kinetics of Pu were observed depending on age, time post-contamination and DTPA treatment. This method allows us to estimate the Pu internalization in the bone volume with increasing time post-contamination and the preferential Pu decorporation of bone surfaces by the DTPA treatment. © 1998 Elsevier Science S.A.

Keywords: Plutonium; Bone; Biological ligands; Chelating agents

1. Introduction

After specific mineral and organic extraction of bone components, early studies have shown that most of the Pu retained in bone was bound to the organic matrix [1]. In vitro experiments suggested that bone chondroitin sulfate-proteins and sialoproteins could be the main organic ligands [2,3]. We have recently developed a formic acid/guanidine sequential extraction method to estimate the Pu retention in the mineral and organic matrix of bone. Such extraction prevents the chelation of Pu that might occur during the usual EDTA demineralization procedure [4]. This new procedure was applied to 3-month-old rats, 48 h after intravenous injection of ^{238}Pu(IV)-citrate when most of the Pu was retained on bone surfaces. During the formic acid extraction, addition of citrate was needed to inhibit the deposit of the extracted Pu on the organic residue. In these experiments, the actual distribution of Pu in the mineral and in the organic bone component was difficult to establish but less than 20% of Pu appeared bound to the organic matrix [4].

The aim of this study was to characterize the effect of time post-contamination and the influence of a chronic and delayed DTPA treatment on the kinetics of Pu extraction during formic acid/guanidine treatments.

2. Experimental details

Three groups of anesthetized male Sprague-Dawley rats were administered with 37 kBq of ^{238}Pu(IV)-citrate by intravenous injection either at 3 months (groups A and B) or at 6 months of age (group C). Animals of group B were treated by adding Zn-DTPA to the drinking water (1 mM) from days 42 to 72 post-contamination. Rats from groups A and B were killed 85 days after contamination, whereas rats from group C were killed 2 days after contamination. The femora were removed and kept frozen at −80°C until the extraction experiments. Formic acid/guanidine extraction were performed as previously described on 1 g of powdered femur obtained by milling into small particles of 150–250 μm in size using a freezer-mill [4]. The powder was de-fatted in acetone and rinsed in distilled water just before extraction. Two extractions with 0.3 M formic acid, pH 3, containing 0–0.3 M citrate were performed for 48 h (20 ml per g of bone powder), and then one extraction was performed in 4 M guanidine, pH 7.4, for 24 h (20 ml per g of bone powder). Aliquots of the extracts were centrifuged

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0925-8388/98/$19.00
PII S0925-8388(98)00033-4

Table 1
Percentage of Pu extracted from femurs of adult rats 48 h after contamination with ^{238}Pu-citrate in formic acid/guanidine media with different citrate concentrations in the formic acid

Extraction media:time of extraction	Extracted Pu as % of initial bone powder activity (group C)			
	Without citrate	Citrate, 0.01 M	Citrate, 0.1 M	Citrate, 0.3 M
F1:1 h	26.3±3.7	47.7±2.7	65.7±5.6	72.0±2.9
F1:24 h	4.3±3.6	60.4±4.3	67.7±8.9	73.0±3.9
F1:48 h	2.8±1.6	56.4±6.7	66.4±10.4	70.7±2.8
F2:48 h	14.1±4.8	14.1±2.9	20.3±6.9	20.9±2.2
G1:24 h	59.7±1.2	25.5±1.5	9.5±0.9	4.8±0.5

Mean value±standard deviation (n=3). F1, first formic acid extract; F2, second formic acid extract; G1, first guanidine extract.

and their Pu contents were measured by liquid scintillation counting.

Some animals from groups A and B were kept in metabolism cages during the time corresponding to the DTPA treatment. The experimental procedures used to measure Pu retention and excretion are described in detail by Le Naour et al. [5].

3. Results

About 10% of the total Pu content was extracted during the de-fatting step, and at the end of the formic acid/guanidine extraction only a small residue remained which contained negligible amounts of Pu. The Pu recovered in each extraction medium was expressed as percent of the total Pu recovered during the formic acid/guanidine treatments. Tables 1 and 2 show the amount of Pu extracted in formic acid with different citrate concentrations and in guanidine from femurs obtained 2 or 85 days after contamination, respectively.

Without citrate, i.e. animal groups A and C, a significant decrease of the extracted Pu was observed between 1 and 24 h after the beginning of the first formic acid treatment. The second formic acid treatment extracted about 10 times more Pu than the first, and 60 to 70% of the total Pu was extracted by guanidine.

The addition of citrate inhibited the decrease of extracted Pu previously observed between 1 and 24 h after the beginning of the formic acid treatment. For a given citrate concentration, after the first hour in the formic acid medium, the amount of Pu extracted was always lower in group A than in group C. In the range of the citrate concentrations used, no significant alteration of the amount of solubilized Pu can be observed between 24 and 48 h. At the end of the first formic acid treatment the amount of Pu extracted gradually increased as the citrate concentration increased. In contrast, for each citrate concentration studied, the amount of Pu extracted during the second formic acid treatment was similar in both groups. The remaining Pu extracted by guanidine gradually decreased as the citrate concentration increased and, for a given citrate concentration, about twice as much Pu was solubilized in group A than in group C.

After DTPA treatment, the formic acid extractions were performed with 0.1 M citrate only. During the first incubation, 33.7% (SD=2.1, n=3), 41.5% (SD=5.4, n=3) and 41.0% (SD=8.8, n=3) of the total Pu was extracted after 1, 24 and 48 h, respectively; 32.8% (SD=3.7, n=3) of the total Pu was extracted during the second formic acid treatment, and 24.6% (SD=2.6, n=3) was solubilized by guanidine. Thus, for each time studied, significant differences in Pu extracted from the femora of groups A and B were observed during the formic acid treatments only (Student's test, $P<0.01$).

4. Discussion

The results obtained 48 h after contamination of 6-month-old rats can be compared with those previously reported in 3-month-old rats after a similar post-contamination delay [4]. During the first formic acid treatment, without citrate, the decrease of the solubilized Pu observed

Table 2
Percentage of Pu extracted from femurs of adult rats 85 days after contamination with ^{238}Pu-citrate in formic acid/guanidine media with different citrate concentrations in the formic acid

Extraction media:time of extraction	Extracted Pu as % of initial bone powder activity (group A)			
	Without citrate	Citrate, 0.01 M	Citrate, 0.1 M	Citrate, 0.3 M
F1:1 h	8.3±3.0	34.3±1.7	52.0±6.8	63.6±4.6
F1:24 h	0.5±0.4	48.2±15.3	61.7±6.2	75.4±3.9
F1:48 h	1.4±0.6	45.1±23.6	63.7±7.5	74.1±1.6
F2:48 h	22.0±4.8	12.1±3.9	11.6±1.1	14.5±1.9
G1:24 h	69.7±3.7	42.7±19.7	24.6±8.5	11.5±1.5

Mean value±standard deviation (n=3). F1, first formic acid extract; F2, second formic acid extract; G1, first guanidine extract.

at 1 and 24 h after the beginning of the treatment of 6-month-old rats show it redeposited on the bone residue. This was not observed for 3-month-old rats. These results suggest that the solubilized Pu has a greater affinity for the bone residue of the oldest rats than for those of 3-month-old rats. This could be explained either by an increased amount of extracted minerals related to aging, which prevent stabilization of the low-molecular weight Pu complexes, or by a greater affinity of solubilized Pu for the biological matrix of the residue. At the end of the guanidine extraction 77.3% (SD=0.7) and 59.7% (SD=1.2) of the total Pu content was solubilized on 3- and 6-months-old rats, respectively. These results suggested that, at early times after contamination, the retention ratio of Pu in the mineral and the organic matrix of bone increased depending on the age of the animals.

The kinetics of Pu extraction in the first formic acid medium seemed to be delayed when the time post-contamination increased from 2 to 85 days. This could show the transfer of Pu in the bone volume with time post-contamination. At the end of the guanidine treatment about twice as much Pu was solubilized from bone 85 days after contamination than from bone 2 days after contamination. Thus, a different deposition ratio of Pu in the mineral and organic matrix could be expected depending on the time post-contamination.

During the DTPA treatment, the urinary excretion of Pu was increased about four times when compared to untreated controls. Thus, the chronic chelating agent treatment decreased the skeleton retention by about 15% [5]. This decorporation was similar to that reported previously when Zn-DTPA was added to the drinking water [6]. After DTPA treatment, the kinetics of Pu extraction in the formic acid medium was slower than that observed without treatment. This suggests that, after the decorporation treatment, more Pu was present in the bone volume than without treatment. However, the fraction of Pu extracted from the organic matrix by the guanidine treatment was not altered by the DTPA treatment.

Most of these results are in accordance with those previously reported after studying the localization of Pu in bone by autoradiographic methods [7].

Further studies are in progress to improve the kinetics of our Pu extraction for a better quantitative analysis of actinide diffusion within the bone volume. For that purpose, other actinides, such as ^{233}U, ^{237}Np, and ^{241}Am, will be studied. This could also provide new information on the relative deposition of actinides in the mineral and organic bone matrix depending on the radionuclide considered.

Acknowledgements

This work was partly funded by COGEMA (Programme d'Intérêt Commun, D10 CEA/COGEMA).

References

[1] I.A. Tseveleva, Biokhimiya 25 (1960) 636.
[2] A.R. Chipperfield, D.M. Taylor, Eur. J. Biochem. 17 (1970) 581.
[3] D.M. Taylor, Health Phys. 22 (1972) 575.
[4] B. Ramounet, P. Fritsch, Int. J. Radiat. Biol. 73(1) (1998) 83.
[5] H. Le Naour, B. Ramounet, L. Le Foll, P. Fritsch, Submitted to J. Alloys Compounds, August 1997, (in this issue).
[6] G.N. Stradling, Radiat. Prot. Dosim. 53 (1994) 297.
[7] M. Ellender, J.W. Haines, J.D. Harrison, Hum. Exp. Tox. 14 (1995) 38.