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The effect of a delayed chronic DTPA treatment on the Pu microdistribution in rat bone: preliminary results using hemi-blocks of femurs

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Abstract

We have developed a combined autoradiographic and confocal microscope analysis to visualize the decorporation of Pu in bone after delayed and chronic DTPA treatment using hemi-blocks of femurs. ^{238}Pu-citrate was administered to rats by intravenous injection at 21 weeks of age. Treatment by adding Zn-DTPA (1 mM) to the drinking water for one month was performed at 42 and at 85 days after contamination. Bone decorporation was estimated at 10–15% and at 5–7% at the end of the first and the second treatment, respectively. Because the block thickness was larger than the range of α particles in the tissue (25–30 μm), quantitative α track recording can be performed with a spatial resolution of less than 60 μm. The autoradiographs were compared with confocal images of the hemi-femur block surface so that the different bone compartments could be easily identified. With this method a clear preferential decorporation of bone surfaces was visualized compared to controls. © 1998 Elsevier Science S.A.

Keywords: Plutonium; Bone; Microdistribution; Autoradiograph; Chelating agents; DTPA

1. Introduction

After acute contamination with soluble Pu (IV), delayed and chronic DTPA treatment can remove significant amounts of the actinide from the skeleton [1,2]. This site is one of the main target organs for the induction of stochastic and nonstochastic effects. New chelating agents, such as 3,4,3-LIHOPO, appear much more efficient than DTPA for this delayed decorporation [3].

The main effect of actinide retention in bone is the α-dose delivered from the bone surfaces to the target cells, mainly osteoblasts and bone marrow cells. Obviously, the distribution ratio of α-emitters on bone surface and within the bone volume is a critical parameter for bone pathology induction. Autoradiographic studies have shown that this distribution varies depending on the actinide and the time post contamination [4–7]. Thus, it appears that the health benefits of chelating agent treatment could be more dependent on the qualitative alteration of the actinide distribution in bone than on the total amount of actinides removed from the skeleton.

The aim of this study was to compare health benefits in rats and quantitative decorporation after Pu contamination by adding Zn-DTPA to the drinking water. The actinide microdistribution in bone was characterized using a combination of solid track detector autoradiographs (CR39) and confocal images of hemi-femur block surfaces. This paper reports the quantitative decorporation of Pu in the skeleton after two chronic Zn-DTPA treatments for one month and the preliminary Pu microdistribution results.

2. Experimental details

All experiments on animals were performed by scientists authorized by the French Ministry of Agriculture to carry out these procedures. Sixty male Sprague Dawley rats were injected intravenously with 37 kBq kg^{-1} ^{238}Pu in 0.1 M citrate at the age of 21 weeks. Two groups of 30 animals were studied: contaminated controls and DTPA treated animals. Then, for this last group, Zn-DTPA was added to the drinking water for 30 days, beginning at 42 and at 85 days after contamination. During each treatment period, three rats of each group were maintained in metabolic cages to collect urine and faeces daily for Pu excretion measurement. Three control rats were sacrificed at 2, 42, 72, 85 and 115 days after contamination, whereas three DTPA-treated rats were killed at the end of each treatment. At this time, the other animals were kept until their death

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to estimate specific bone pathology incidence. Organ retention and excretion of Pu of the killed animals was measured either by X-ray spectrometry or liquid scintillation.

One femur from each animal was used for the histological study. It was fixed in 4% paraformaldehyde and embedded in an epoxy resin (Spurr). Hemi-blocks of femur were prepared using a low speed diamond wheel saw and the block surface was treated with a grinding and polishing machine. Autoradiographs of hemi-blocks were performed using the CR39 solid track method. After a suitable contact exposure period, the detector was etched in 12 M KOH at 85°C for 20 min. Images of the block stained with propidium iodide (PI 50 μg ml^{-1} in distilled water) were recorded using confocal microscopy (BIORAD MRC1024 Laser Scanning Confocal System). Mapping of the whole autoradiograph and block surface confocal images were obtained using a motorized stage and a software program we have developed.

3. Results

Fig. 1 shows the urinary excretion of plutonium during the first DTPA treatment. A 3- to 4-fold increase of this excretion was observed as compared to untreated controls but the decorporation appeared to decrease gradually throughout the treatment. During the second treatment, the DTPA efficacy decreased and, after 30 days, the cumulated urinary excretion was only two times larger than that of untreated controls. Measurements of the Pu decorporation in femurs show a 10–15% DTPA efficacy at the end of the first treatment, whereas a 5–7% efficacy was observed at the end of the second treatment.

Fig. 2 shows autoradiographs obtained before and after polishing a femur hemi-block and the confocal image of the polished surface. Resolution of the autoradiographs appeared to be increased by polishing and well defined images of the different bone compartments could be obtained by confocal microscopy even for a bad orientation of this femur, as shown in Fig. 2.

Fig. 3 compares the retention of plutonium in the femur at the end of the second DTPA treatment and in untreated control. A decrease of α track density was observed in the treated animal over most bone surface structures such as periosteum, endosteum, and trabecular bone. Similar differences between treated and controls were observed for each animal at the end of the second treatment, whereas it was not observed systematically at the end of the first DTPA treatment.

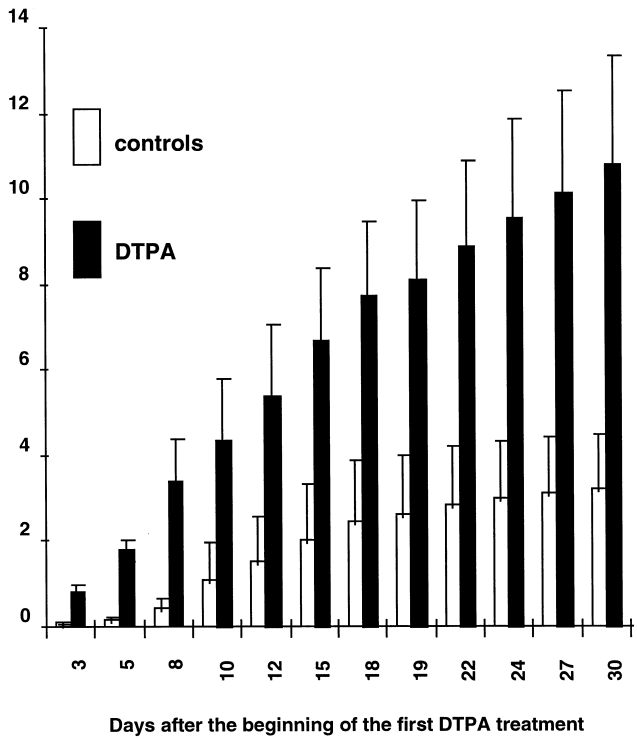


Fig. 1. Evolution of the cumulated urinary excretion of Pu in DTPA treated and control rats as a function of time following the beginning of the DTPA treatment. Mean values+standard deviation (n=3).

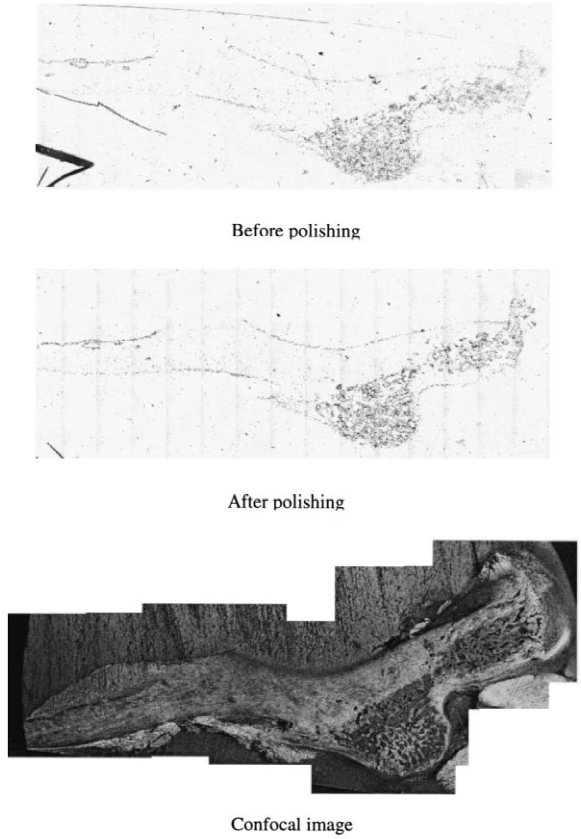
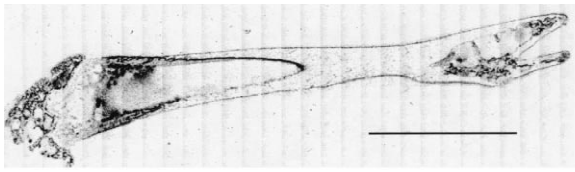
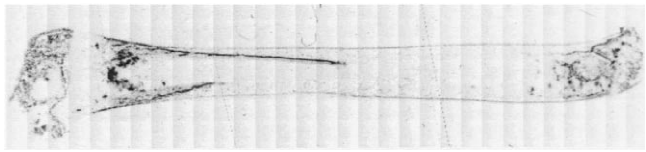


Fig. 2. Comparative autoradiographs before and after polishing and the confocal image of the polished block surface. Femur of a rat killed 2 days after Pu contamination after a 15 day exposure period.



untreated femur



DTPA treated femur

Fig. 3. Comparative autoradiographs of DTPA and control femur at the end of the second DTPA treatment 115 days after contamination after a 15 day exposure period. Bar: 1 cm.

4. Discussion

The Pu decorporation parameters we have measured were very similar to those reported by adding Zn-DTPA to the drinking water. At the end of the second treatment, the global bone decorporation was low, about 20%. Our preliminary experiments have shown that quantitative autohistoradiographs were difficult to obtain on standard thin sections. This was due to individual variation of the tissue section thickness. Thus, because of the short range of α particles in biological tissue (<30 μm), quantitative results might be obtained on tissue sections having a thickness more than this α range. To our knowledge such autoradiographic approach has not been reported for bone.

Polishing of the block seemed to increase the autoradiographic resolution due to the actual plane surface of the block obtained by this process. In such experimental conditions, we have estimated an autoradiographic spatial resolution of 0 to 60 μm assuming a 25 μm range of α particles in the tissue block and that only α particles reaching the CR39 detector with an angle less than 55° could be recorded as previously reported for high LET particles [8].

In conclusion, this study shows that, even for a Pu bone decorporation as low as 20% the autoradiographic method developed systematically visualized differences between DTPA-treated rats and untreated controls. The main advantage of this method is that it is not very time consuming and that potentially it provides quantitative autoradiographs even on large bone blocks. Further studies are in progress to determine the density of α tracks recorded per unit of solid detector area. This will be performed by image analysis combining the autoradiograph and confocal images to define the different bone compartment areas.

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