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A STUDY OF IRRADIATED BONE: PART III. ^{99m}Tc PYROPHOSPHATE AUTORADIOGRAPHIC CHANGES¹

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ABSTRACT

The macroautoradiographic and microautoradiographic localization of ^{99m}Tc pyrophosphate ($^{99m}\text{TcPPi}$) was studied in X-irradiated bone of rabbits up to one year post-irradiation. In cortical bone, $^{99m}\text{TcPPi}$ was concentrated on bone surfaces near vasculature. Both forming and resorbing bone surfaces were comparably labeled at 2 hours post-injection. Uptake on the surface of sites of haversian bone remodeling was observed to be at least part of the increased $^{99m}\text{TcPPi}$ observed in irradiated bone in camera images (7). In irradiated trabecular bone 12 months following irradiation a patchy decrease in $^{99m}\text{TcPPi}$ uptake was correlated with localized decreases in vasculature.

INTRODUCTION.

Since their introduction a number of studies have been published on the autoradiographic localization of ^{99m}Tc labeled imaging agents in bone (1-5). The objective of this article is to report the changes in ^{99m}Tc pyrophosphate ($^{99m}\text{TcPPi}$) localization in bone as observed autoradiographically following localized irradiation of bone. This study is a part of a larger investigation of post-irradiation effects in bone (6,7) designed to evaluate histological sites of, and pathophysiological mechanisms responsible for, the alterations taking place in the camera images of irradiated bone. The study was also performed to further document the sites of $^{99m}\text{TcPPi}$ localization in tissues and to advance the understanding of the mechanisms responsible for the accumulation of this agent.

MATERIALS AND METHODS

The left hind limbs of adult male New Zealand albino rabbits were sham-irradiated (controls), or

X-irradiated with either a single dose of 1756 rad or a fractionated dose of 4650 rad over a period of three weeks. The procedure of irradiation has been described previously (6,7).

Macroautoradiography. Macroautoradiographs were made from the tibial shaft and the femoral head from both hind legs of 3 rabbits from each dose regimen at 1, 3, 6 and 12 months post-irradiation. The animals were injected intravenously with 2 mCi/kg body weight of $^{99m}\text{TcPPi}$ * 2 hours prior to sacrifice. Bone specimens were placed in 95% ethanol for fixation, and dehydration was carried out using acetone under vacuum. Monitoring of fixing and dehydrating solutions showed a negligible loss of activity. The specimens were then embedded in Ward's Bioplastic (8) to preserve the soft tissues (marrow) and to prevent loss of detail by penetration of the high energy conversion electrons and low energy X-rays of $^{99m}\text{TcPPi}$ through air gaps along bone surfaces (9). Sections of bone

* Pyrophosphate obtained from Phosphotec kits supplied by E. R. Squibb and Sons, Inc.

and plastic 2 mm thick were cut with a Gillings - Bronwell thin-sectioning machine (10), cleaned with 95% ethanol and air dried. The sections were attached to labeled 3" x 5" cards to maintain their identity, covered with plastic wrap to prevent chemography (11), and placed in contact with Kodak No-Screen or RP Royal X-Omatic Medical X-Ray Film in Kodak X-Ray Exposure Holders for 12 hours. The films were developed by hand or X-Omat, respectively. The total time from animal sacrifice to placing the sections in contact with the X-ray film averaged 8 hours.

In two additional rabbits, at 1 day after irradiation with the single dose, the gross distribution characteristics of $^{99m}\text{TcPPi}$ were assayed with macroautoradiography at five minutes after injection. After death, the hind legs of the rabbits were disarticulated and frozen in liquid nitrogen, and transverse sections 2 mm thick were cut in the thin-sectioning machine. Autoradiographs were obtained by placing the specimens in contact with the X-ray film for 12 hours in a freezer.

Microautoradiography. In two control rabbits, and in one of the single dose irradiated rabbits sacrificed at each of the times listed above, a more detailed analysis of the autoradiographic localization of $^{99m}\text{TcPPi}$ was made. In these animals smaller specimens of cortical bone, with the soft tissues removed, were embedded in Ward's Bioplastic. Sections of embedded bone 200 microns thick were cut in the thin-sectioning machine, attached to glass microscope slides with Eastman 910 adhesive, and ground to 50 microns with dry sandpaper (12). The slides were cleaned in 95% ethanol, dipped in Kodak NTB-2 emulsion (11), stored in a refrigerator for 12 hours, and developed (11).

RESULTS AND DISCUSSION

Microautoradiographs. In the section of bone from the rabbits used in the microautoradiographic studies, $^{99m}\text{TcPPi}$ was found to be concentrated mostly near bone surfaces (endosteal, periosteal, and haversian) at 2 hours post-injection. This is illustrated in Figure 1 where two photomicrographs from the irradiated tibia of

a rabbit 3 months after the single dose show the pattern of uptake of $^{99m}\text{TcPPi}$ near the surfaces of resorbing and forming osteons, respectively. It is interesting to note that at 2 hours post-injection accumulation is about the same on both types of remodeling bone surfaces. The finding that $^{99m}\text{TcPPi}$ localizes on calcified bone surfaces near the blood supply is similar to that reported by others (1,2,5). Deland et al. (2) also found a pattern of uptake of $^{99m}\text{TcPPi}$ around osteocytes. We did observe a light diffuse pattern of $^{99m}\text{TcPPi}$ uptake away from bone surfaces in the cortex (Figure 1), but were unable to locate it near the lacunae of the osteocytes or other structures, perhaps because of the relative thickness of the bone sections. Guillemart et al. (5) reported finding tracer incorporated in giant cells (osteoclasts) adjacent to bone surfaces. In our non-stained sections we could not identify these cells confidently, but it appeared that the uptake we observed on the surfaces of resorbing bone was not due to this cause, because uptake was observed all over the bone surface of the resorption cavity and not merely in the vicinity of Howship's lacunae.

The general distribution of $^{99m}\text{TcPPi}$ observed in this study was qualitatively similar to that described by Rowland (13) for ^{45}Ca in the cortical bone of rabbits and dogs at about the same time post-injection. Both of these agents were mostly concentrated in areas of bone near the vasculature, not uniformly distributed throughout the calcified matrix, and not necessarily concentrated in areas of bone where calcification was in progress. Rowland also observed that the concentration of ^{45}Ca was comparable on the calcified surfaces of resorbing and forming bone at 2-3 hours post-injection, as was reported here for $^{99m}\text{TcPPi}$.

Macroautoradiography. The differences in $^{99m}\text{Tc-PPi}$ deposition between irradiated and nonirradiated tibial shaft in the single dose rabbits as observed in contact autoradiographs are illustrated in Figure 2. Similar, but less marked, differences were observed in the fractionated dose rabbits. The most significant finding was a markedly greater number of discrete concentrations of $^{99m}\text{Tc-PPi}$ in the irradiated than in the nonirradiated tibial cortex at 3 and 6 months

following irradiation with lesser differences observed at 1 and 12 months. The timing of these differences at 3 and 6 months after irradiation corresponds with the increase in the activity ratios of $^{99m}\text{TcPPi}$ for the tibial shaft (irradiated/nonirradiated) as observed in the camera and tissue assay studies (7). The nature of these discrete concentrations is believed to be uptake on the surface of remodeling osteons as demonstrated in Figure 1. The existence of a marked increase in remodeling of the irradiated tibial cortex was observed to follow a similar time sequence in the histological studies (6).

No significant differences were observed in the periosteal concentration of $^{99m}\text{TcPPi}$. In evaluating the autoradiographs of the endosteal surfaces for relative changes in $^{99m}\text{TcPPi}$ accumulation between the irradiated and control bones of the rabbits, no significant changes were observed, except when the plastic had shrunk away from the bone. This resulted in less attenuation of ^{99m}Tc 's high energy internal conversion electrons and low energy X-rays, and a correspondingly

greater impression of the apparent ^{99m}Tc activity on the endosteal surface. Thus, it appears that uptake on the increased surface area of bone undergoing remodeling (6) was one of the major contributors to the second phase of increase $^{99m}\text{TcPPi}$ uptake observed in the camera studies (7).

Relative changes in $^{99m}\text{TcPPi}$ accumulation were difficult to evaluate in trabecular bone of the distal femoral head due to the poor infiltration of the plastic into this region in the time allotted for the process. The impression of an increase in the irradiated bone at 3 months and a decrease at 12 months, was suggested by inspection, however. This agreed with the timing of changes observed by camera (7). In the fractionated dose rabbits, the relative decrease in uptake by the bone of the irradiated side at 12 months was readily apparent (Figure 3). The patchy appearance of the decreased $^{99m}\text{TcPPi}$ uptake as noted in Figure 3 correlated with localized decreases in the vasculature of the region as seen histologically (6).

In the autoradiographs of rabbits sacrificed 1 day after single dose irradiation and five minutes after administration of $^{99m}\text{TcPPi}$ there was a marked difference in localization between irradiated and non-irradiated legs (Figure 4). These rabbits were sacrificed at the time of the largest difference in the camera activity ratios (7). The greatest relative change in these rabbits was found to be in skin, followed by muscle, marrow and bone. This phase of increased relative $^{99m}\text{TcPPi}$ accumulation in the irradiated leg was found to be due to changes in the vasculature (6).

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LEGENDS FOR FIGURES

FIG. 1. Photomicrographs of undecalcified bone sections from the irradiated tibia of a rabbit 3 months following single dose X-irradiation showing $^{99m}\text{TcPPi}$ localization around: (A) Resorption Cavity and (B) Forming Osteon. (500 X before reproduction).

FIG. 2. Composite $^{99m}\text{TcPPi}$ contact autoradiographs of transverse sections of the tibial shafts of rabbits at various times after 1756 rad single dose to left leg. Note greater number of discrete concentrations of $^{99m}\text{Tc-PPi}$ in irradiated than in nonirradiated bone, especially at 3 and 6 months.

FIG. 3. (A) Camera image of hind quarters of a rabbit 3 hours after injection of $^{99m}\text{TcPPi}$ and 12 months after 4650 rad fractionated dose irradiation to left hind leg. (B) $^{99m}\text{TcPPi}$ contact autoradiographs of longitudinal sections from the right and left distal

FIG. 3 femoral ends of the same rabbit as in A. In (cont'd) A and B the irradiated leg is on the right. Note the relatively lesser uptake of $^{99m}\text{TcPPi}$ in the epiphysis of the irradiated as compared with the nonirradiated bone.

FIG. 4. Contact autoradiographs from frozen transverse sections of the mid tibia-fibula region from the hind legs of a rabbit 1 day after the single radiation dose, showing the localization of $^{99m}\text{TcPPi}$ in the bone and soft tissues five minutes post-injection. Note the markedly greater uptake of $^{99m}\text{TcPPi}$ in the irradiated leg (soft tissues and bone).

Fig 7

Figure 2

	NONIRRAD	IRRAD
1MO
3MO
6MO
12MO

Figure 3

A

B

Figure 4