ELECTRON MICROSCOPIC AUTORADIOGRAPHIC STUDY ON SUBCELLULAR LOCALIZATION OF FISSION PRODUCT ^{147}Pm IN TISSUE CELLS*

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ABSTRACT

The early risk of internal contaminated accumulation of ^{147}Pm is in blood cells and endothelial cells, especially in red blood cells. Then ^{147}Pm is selectively deposited in ultrastructure of liver cells, such as in nucleus, nucleolus, rough endoplasmic reticulum, mitochondria and microbodies. Dense tracks also appear in mitochondria and lysosome of pedal cells within renal corpuscle, and so does in nucleus as well as in mitochondria and microbodies of epicyte of kidney near-convoluted tubule. With the prolongation of observing time, ^{147}Pm is selectively and steadily deposited in subcellular level of organic component for bone. Substantial amount of ^{147}Pm is taken up into the nuclear fraction of osteoclasts and osteoblasts. Particularly, in organelles ^{147}Pm is mainly accumulated in rough endoplasmic reticulum and in mitochondria. Autoradiographic tracks especially localize in combined point between Golgi complex and transitive vesicle of rough endoplasmic reticulum. In addition, numerous ^{147}Pm deposited in collagenous fibre within interstitial of bone cells is hardly excreted.

Keywords Electron microscopic autoradiography, Accumulation, Fission product, ^{147}Pm, Subcellular level

1 INTRODUCTION

^{147}Pm, one of abundant by-products of nuclear industry, has been considered for use as radioactive heat sources for light-weight electrical power units[1] and as energy source in luminescent paints for watch and instrument dials[2]. Large scale separation of ^{147}Pm from fission product waste and preparation of material for industrial application constitute a potential source of radiation exposure to workers in this field. In order to form a basis for radiation exposure limits and to determine the dose from accidental exposure[2], the transference and accumulation of ^{147}Pm in cellular level were studied [3,4]. However, its micrometabolism, especially its subcellular localization in tissue cells are not reported; but its action and injury effect in organism show a close relation on retentive peculiarity in subcellular structure.

2 EXPERIMENTAL METHODS

2.1 Preparation of electron microscopic radioactive specimen and ultrathin section

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Experiments are carried out on 30 male Wistar strain rats, weighing 100±10g. The accumulation of ^{147}Pm in subcellular level is observed after i.v. injection of 185 MBq/kg $^{147}Pm(NO_3)_3$. Rats are killed by decapitation after 2, 6, 12, 24, 48 and 72 h, respectively. The blood is heparinized and centrifugalized so as to obtain blood cells. The liver, kidney and spongy bone are taken out of the experimental rats quickly and cut into a piece of tissues about 1 mm^3. The small pieces of tissues are fixed in 1% osmium acid solution which is made up with 0.1mol/L phosphate buffer at pH 7.4 for 2 h at 4°C. Then washed three times with physiological saline at the same temperature for 5 min each. The washed tissues are dehydrated with 30%, 50%, 70%, 80%, 90% and 100% acetone one by one. Then the tissues are infiltrated with a miscible liquid with both dehydrating agent and embedding agent. So that the embedding agent could replace dehydrating agent fully. Epoxy resin 618 is exploited as embedding agent. The embedded tissues are cut into ultrathin sections with 0.05μm thick by ultramicrotome LKB. The sections are mounted on the copper grids which are previously covered with support film of collodion. The water on the grids is absorbed with filter-paper. The grids are attached to the holder and transferred to the darkroom.

2.2 Preparation of monolayer emulsion and mounting

The resolution of electron microscopic autoradiography is very high. The special nuclear emulsion HW_4 is applied. Its diameter of silver crystals is about 140 nm. The monolayer emulsion prepared by loop method is exposed by the tiny radioactive sources in the subcellular structure[5]. By using monolayer emulsion so as to do, the latent images are formed only in the nearest silver crystals, and not in the other grains. If so, the cross and overlap of images are reduced, and the resolution is elevated. The monolayer emulsion is prepared as follows: In safelight of darkroom, nuclear emulsion HW_4 is diluted with three-times-distilled water by 1:5(v/v), and melted in water bath at 40°C immediately. Then the diluted emulsion is cooled to 10°C in low temperature bath. A diameter of 15 mm loop made by platinum or steel wire is dippped into the melting emulsion. Be careful takes the loop vertically withdrawn at low speed. At this conditions the monolayer emulsion over the loop is formed. Then attaching the grid against the monolayer emulsion film, which should fall from the loop to cover the grid wholely. The mounting grids are put in a specimen box labeled with number by tweezer lightly. Finally the specimen boxes are placed in a special dryer to expose at 4°C.

2.3 Development, stopping, fixation and staining

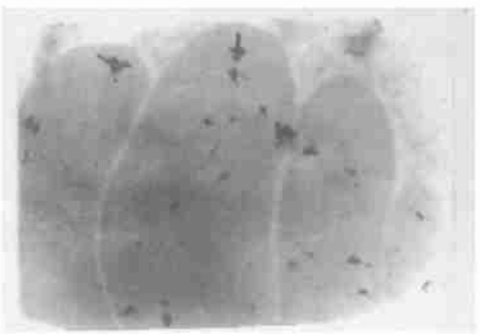
After exposure, the development of specimen should be carried out in the darkroom. The selected developer is D_{19}. As the case stands, that the structure of its tiny silver grain is more compact[6]. It is useful to location of radionuclides in subcellular level[7]. The freshly prepared developer D_{19} is poured into glass bowl. The specimen box is directly dipped into the developer for 2 min at 20°C. After that, rinsed in distilled water and stopped development in 3% acetic acid solution for 15s. Rinsed in distilled water again, then fixed in 24% solution of sodium thiosulphate for 3min. Later rinsed five times in distilled water for 1 min each time. All applicable solutions should be kept at 20°C[5].

The specimen is stained as follows: The experimental copper grids are immersed in 2.5% aqueous uranyl acetate for 3min at room temperature in the darkroom, then rinsed with distilled water thoroughly. After drying, stained in lead citrate solution for 30 min. Rinsed with distilled water again. After drying in dust free air, the grids are stored in dust free container and ready for viewing by the electron microscope H-600.

3 EXPERIMENTAL RESULTS

3.1 Autoradiography of blood cells and endothelial cells

After absorption of ^{147}Pm into blood, it appear rapidly in the formed elements of blood. As shown in Fig.1, especially in erythrocytes, numerous autoradiographic tracks

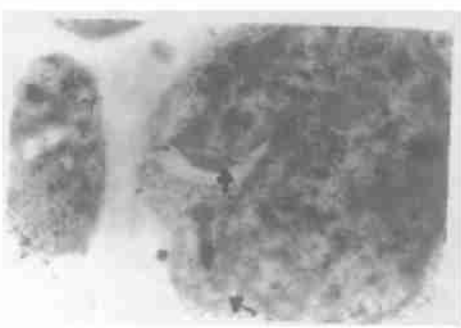


(1) x15000

(2) x12000

Figs. 1–2 Autoradiography of peripheral red blood cell at 2h after i.v. ^{147}Pm (1); of endothelial cell of capillary (2) at 6h after i.v. ^{147}Pm

(3) x10000



(4) x10000

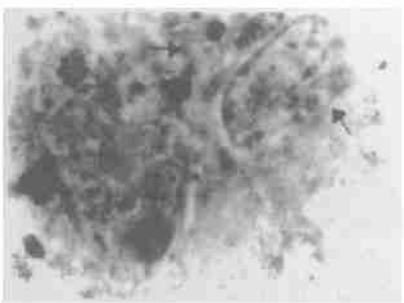
Figs. 3–4 Autoradiography of neutrophil leukocyte at 6h (3); of monocyte at 12h (4) after i.v. ^{147}Pm

appear. At the same time, the incorporation of ^{147}Pm by endothelial cell of capillary (Fig.2) and neutrophil leukocyte (Fig.3) was observed. Fig.2 shows that dense tracks appear within erythrocyte in cavity of capillaries as well as endothelial cell of capillary.

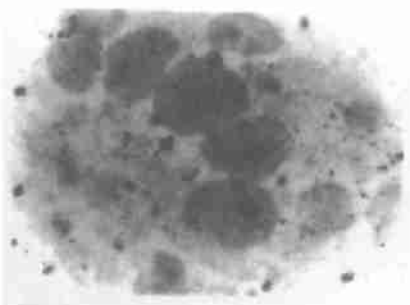
Fig.3 shows that less tracks appear within cell; while dense tracks do within some erythrocytes in cavity of blood vessel. Fig.4 show more numerous autoradiographic tracks in nucleus, rough endoplasmic reticulum, mitochondria and ribosome in monocyte.

3.2 Autoradiography of liver cells

The autoradiographic studies show that at first ^{147}Pm is mainly accumulated in Kupffer's cell, where autoradiographic tracks appear in nucleus, lysosome as well as mitochondria (see Fig.5). With the prolongation of observing time, ^{147}Pm is mainly deposited in lysosome within the cytoplasm as well (Fig.6). Fig.7 shows dense tracks of ^{147}Pm appear in nucleus of liver cell as well as in rough endoplasmic reticulum, mitochondria and glycogen within cytoplasm. As shown in Fig.8 the autoradiographic tracks of ^{147}Pm selectively localized in two nucleolus of liver cells. At the same time more numerous tracks of this radionuclide show in mitochondria (Fig.9).



(5) x10000

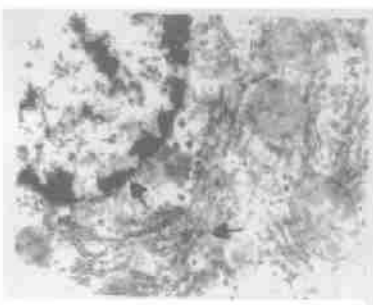


(6) x15000

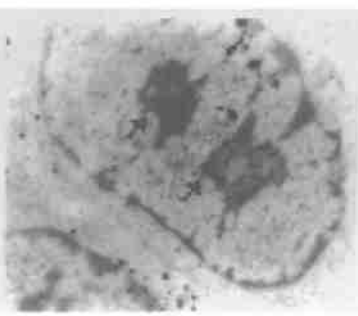
Figs. 5–6 Autoradiography of Kupffer's cell at 6h (5), 12h (6) after i.v. ^{147}Pm

3.3 Autoradiography of bone cells

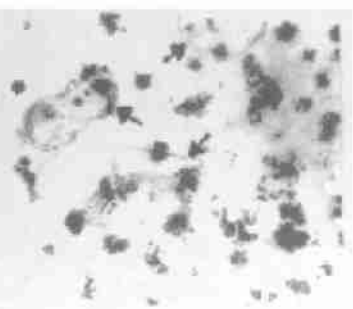
The intake of ^{147}Pm in the body was mainly deposited in spongy bone. Fig.10



(7) x15000



(8) x15000



(9) x15000

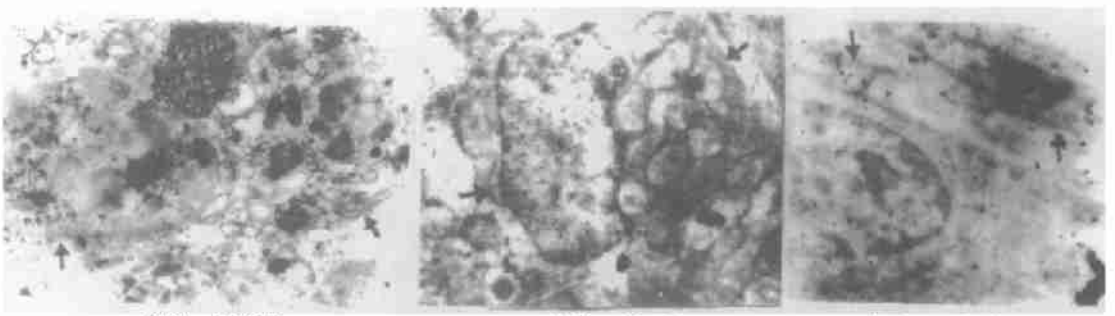
Figs. 7–9 Autoradiography of liver cell at 24h after i.v. ^{147}Pm (9)

shows dense tracks in nucleus of osteoclast. Tracks also appeared in mitochondria, in combined point between Golgi complex and transitive vesicle of rough endoplasmic reticulum. ^{147}Pm dominantly incorpolate in osteocyte. As shown in Fig.11 where more

numerous tracks appeared in nucleus, in rough endoplasmic reticulum and simultaneously in mitochondria. Fig.12 shows autoradiographic tracks in collagenous fiber within mesencchymal of osteocyte.

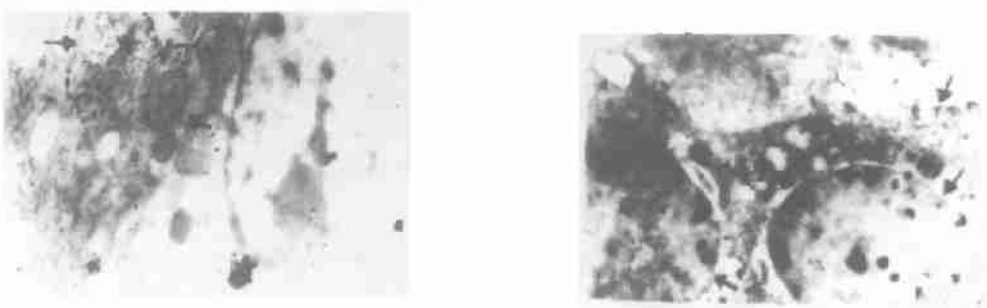
3.4 Autoradiography of kidney cells

The localization of ^{147}Pm in subcellular structure of kidney is ununiform. At first it is mainly deposited in foot cell within corpuscle. Where appeared more numerous tracks in mitochondria and lysosome (Fig.13). With the prolongation of time, ^{147}Pm was selectively deposited in epithelial cell within renal proximal tubule. Fig.14 shows more dense tracks in nucleus, as well as in mitochondria and microbodies.



(10) x10000 (11) x10000 (12) x15000

Figs. 10–12 Autoradiography of osteoclast at 48h (10); of osteoblast at 72h (11); of osteocyte at 48h (12) after i.v. ^{147}Pm



(13) x15000 (14) x12000

Figs. 13–14 Autoradiography of foot cell within corpuscle at 6h (13), of epithelial cell within renal proximal tubule at 12h (14) after i.v. ^{147}Pm

4 DISCUSSION

In view of the exceptional important role of the fission product ^{147}Pm in economic construction, the possibility of this radionuclide to contaminate the body is growing. It is well known, that the nature of the action of radioactive substances in organism and the development of their effects is close linked with their transference and localization

in the body. Therefore study on the relationship between the retentive peculiarity in subcellular level of radionuclides and the injurious effects is certainly a very important problem.

It should be noted that fission product ^{147}Pm was selectively accumulated in liver cells at early stage after contamination. The electron microscopic autoradiographic studies showed that ^{147}Pm was mainly localized in nucleus, lysosome as well as mitochondria. Thus resulting in disorder of biological oxidation and the functional reduction of hydrolytic enzyme.

The dynamic retention of radioactivity of ^{147}Pm in skeleton steadily rose through time of exposure. This radionuclide was chiefly deposited in nucleus and mitochondria of osteoblasts as well as osteoclasts within spongy bone. Its retention period was long enough, and it was difficult to excrete, thus resulting in the late injury effect of bone cells such as carcinoma and teratogenesis[8−9].

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