CNIC-00528
SMC-0064

电镜放射自显影法研究铀在细胞内亚细胞水平的沉积

STUDY ON THE ACCUMULATION OF ENRICHED URANIUM UO_2F_2 IN SUBCELLULAR LEVEL BY ELECTRON MICROSCOPIC AUTORADIOGRAPHY

原子能出版社

电镜放射自显影研究浓缩铀 UO_2F_2 在体内亚细胞水平的沉积*

朱寿彭　汪源长

（苏州医学院）

摘　要

运用电镜放射自显影术探讨了可溶性浓缩铀 UO_2F_2 在机体内亚细胞水平的蓄积动态。发现浓缩铀 UO_2F_2 在内污染危害早期集中于主要沉积器官肾脏，尤其呈选择性沉积于近曲细管上皮细胞核中和胞质的线粒体内，以及曲细尿管基底膜处，从而可导致近曲细管上皮细胞的变性、坏死和脱落。对肝组织细胞的电镜放射自显影观察表明，浓缩铀 UO_2F_2 开始主要沉积到肝细胞核中以及胞浆中可溶性蛋白质部位，随着观察时间的延长，浓缩铀 UO_2F_2 主要定位于肝细胞的线粒体上，其次是溶酶体中。浓缩铀 UO_2F_2 在骨组织细胞中的沉积是持续增升的，主要沉积在松质骨部位的骨细胞核和破骨细胞核中，而在胞质中，尤其是线粒体中，也有浓集的放射自显影径迹颗粒呈现，且其滞留期长，很难排除。

* 国家自然科学基金资助课题

STUDY ON THE ACCUMULATION OF ENRICHED URANIUM UO_2F_2 IN SUBCELLULAR LEVEL BY ELECTRON MICROSCOPIC AUTORADIOGRAPHY*

Zhu Shoupeng Wang Yuanchang
(SUZHOU MEDICAL COLLEGE)

ABCTRACT

The retentive peculiarity of soluble enriched uranium UO_2F_2 in subcellular level was studied by electron microscopic autoradiography. The early dynamic accumulation of radioactivity in the body showed that enriched uranium UO_2F_2 was chiefly localized in kidney, especially accumulated in nucleus of epicyte of kidney near-convoluted tubule. In liver cells, enriched uranium UO_2F_2 at first deposited in the nucleus and the cytoplasm, then accumulated in mitochondria selectively and lysosome as well. The electron microscopic autoradiographic study showed that the dynamic retention of radioactivity of enriched uranium UO_2F_2 in skeleton rose steadily throughout the exposure. Enriched uranium UO_2F_2 chiefly deposited in nucleus and mitochondria of the osteoblast as well as osteoclast.

* The Subject supported by National Natural Science Foundation of China

INTRODUCTION

Now in the field of radiation medicine, the environmental pollution and damage to human beings by nuclear fuel and its fission products released from nuclear tests and plants are much concerned by the public. Enriched uranium can be applied to nuclear fuel as well as nuclear weapon, so we paid attention to the metabolic peculiarity in organism[1]. In recent years, nuclear power plants are built continually, therefore, the utilization of enriched uranium has reached a rapid development. With an increase in production of enriched uranium, observations of the effect on environment and on human beings become a significant task. So it is necessary to study the radiotoxicology of enriched uranium[2].

The soluble enriched uranium UO_2F_2 was selectively accumulated in kidney, then in skeleton and liver, only a few of them were deposited in other tissues[3]. The dynamic study of its metabolism showed that accumulation of enriched uranium UO_2F_2 in kidney and liver rapidly reached the high peak, then gradualy reduced. However, the accumulation in skeleton rose steadily throughout the exposure[4]. In the environment of production plant, soluble enriched uranium could contaminate the human body through different ways. The detrimental effect showed a close relation with retentive peculiarity in subcellular structure. No such studies were reported yet. So we intended to study the retentive peculiarity of enriched uranium UO_2F_2 in subcellular structure by electron microscopic autoradiography.

1 EXPERIMENTAL METHODS

1.1 Preparation of electron microscopic radioactive specimen and ultrathin sections

Enriched uranium UO_2F_2 containing 18.9% ^{235}U was used at a concentration of 60 mg/mL in this experiment. Enriched uranium UO_2F_2 was injected by i.v. 20 mg/kg into 24 male Wistar rats weighing 185±12 g. Rats were killed by decapitation after 6, 24, 48, and 72 h respectively. The blood was heparinized and centrifuged so as to obtain blood cells. The kidney, liver and spongy bone were removed quickly and cut into pieces about 1 mm^3. The small pieces of tissue were fixed in 1% osmium acid solution which was made up with 0.1 mol/L phosphate buffer at pH 7.4 for 2 h at 4℃. Then the pieces were washed three times with physiological saline at the same temperature for 5 minutes each. The washed tissues were dehydrated with increasing concentration of acetone from 30%, 50%, 70%, 80%, 90% to 100%. Then the tissues were

infiltrated with a miscible liquid of both dehydrating agent and embeding agent, and finally replaced by the pure embeding agent. Epoxy resin 618 was used as embeding agent. The embedded tissues were cut into ultrathin section with 0.05 μm thickness by ultramicrotone from LKB. The sections were mounted on the copper grids which were previously covered with support film of collodion. The water on the grids was absorbed with filter-paper. The grids were placed on the supporting column and transferred to the darkroom.

1.2 Preparation of monolayer emulsion and mounting

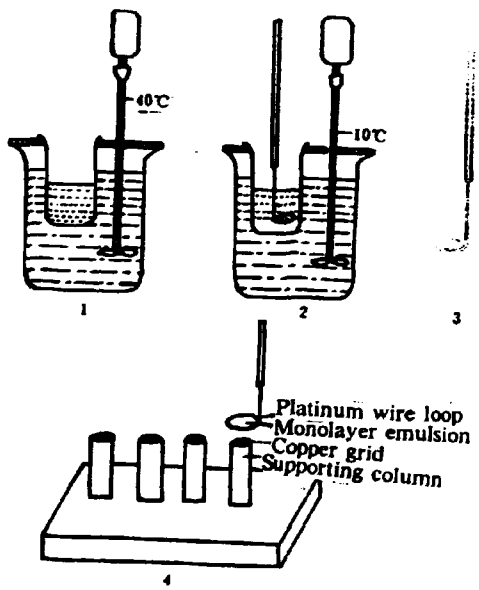

Fig. 1. Preparation of monolayer emulsion by loop method and mounting

1——Emulsion HW-4 was melted in water bath at 40℃ and diluted by 1 : 5(V/V);

2——The diluted emulsion HW-4 was cooled in low temperature water bath at 10℃;

3——The platinum loop was dipped in and withdrawn from the diluted emulsion to form monolayer emulsion;

4——The monolayer emulsion was mounted on the copper grid.

The resolution capacity of electron microscopic autoradiography was very high. The special nuclear emulsion HW-4 was applied. The diameter of silver crystals were about 140 nm. The monolayer emulsion prepared by loop method was then exposed to the tiny radioactive sources in the subcellular structure[6]. By using monolayer emulsion the latent images appeared only in the nearest silver crystals, and not in the other grains. So the cross and overlap images were reduced, and the resolution capacity in-

creased. The monolayer emulsion was prepared as follows: In darkroom under safelight nuclear emulsion HW-4 was diluted with triple distilled water by 1 : 5 (V/V), and was melted in water bath at 40℃ immediately. Then the diluted emulsion was cooled to 10℃ in low temperature water bath. A diameter of 15 mm platinum or steel wire loop was dipped into the melting emulsion, and the loop was withdrawn vertically at lc ' speed. At this conditions the monolayer emulsion over the loop was formed. Then let the monolayer emulsion film already attached to the grid be fallen from the loop to cover the grid wholely (Fig. 1). The mounting grids were put in a labeled specimen box by tweezers lightly. Finally the specimen boxes were placed in a special dryer at 4℃ for exposure.

1.3 Development, stopping, fixation and staining

After exposure, the development of specimen should be carried out in the darkroom. The selected developer was D_{19}. The tiny silver grain was more compact[6]. It is suitable for location of radionuclides in subcellular level[7]. The freshly prepared developer D_{19} was poured into glass bowl. The specimen box was directly dipped into the developer for 2 min at 20℃, then rinsed in distilled water and stopped development in 3% acetic acid solution for 15 s. The specimen was rinsed in distilled water again, then fixed in 24% solution of sodium thiosulphate for 3 min. Later rinsed five times in distilled water for 1 min per time. All prepared solutions should be kept at 20℃[8].

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

2 EXPERIMENTAL RESULTS

2.1 Autoradiography of kidney cells

After enriched uranium UO_2F_2 was absorbed in the body, its retention in subcellular structure of kidney was ununiform. At first it was mainly deposited in epicytes of renal proximal convoluted tubule. Fig. 2 showed dense autoradiographic tracks in nucleus and mitochondria. Some autoradiographic tracks appeared in basement membrane of renal tubule (Fig. 3) and relative a few tracks in glomeruli as well as in other portions of renal tubule.

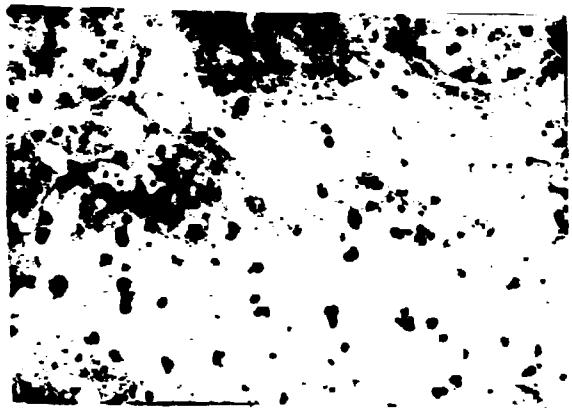

Fig. 2. Electron microscopic autoradiography of epicytes of renal proximal tubule at 6 h after i. v. injection of enriched uranium UO_2F_2 showed more numerous autoradiographic tracks in nucleus and mitochondria, × 10000

Fig. 3. Electron microscopic autoradiography of the basement membrane of renal tubule at 24 h after i. v. injection of enriched uranium UO_2F_2 showed the retention of autoradiographic tracks, × 20000

2. 2 Autoradiography of liver cells

The autoradiographic studies showed that at first enriched uranium UO_2F_2 was mainly accumulated in the nucleus of liver cells, where dense autoradiographic tracks appeared (Fig. 4). At the same time, some autoradiographic tracks combined with soluble proteins appeared in the cytoplasm.

Fig. 4. Electron microscopic autoradiography of liver cells at 6 h after i. v. injection of enriched uranium UO_2F_2 showed dense autoradiographic tracks in nucleus and soluble proteins within the cytoplasm, ×12000

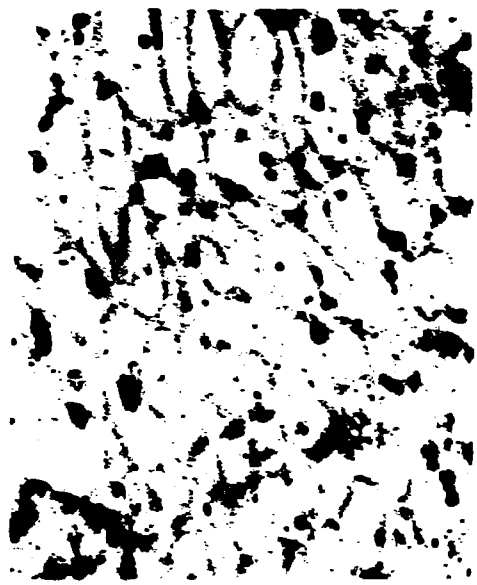

Fig. 5. Electron microscopic autoradiography of liver cells at 24 h after i. v. injection of enriched uranium UO_2F_2 showed more numerous autoradiographic tracks in mitochondria, ×10000

With the prolongation of observing time, enriched uranium UO_2F_2 was mainly deposited in mitochondria where more numerous autoradiographic tracks appeared (Fig. 5). Autoradiographic tracks simultaneously appeared in lysosomes of liver cells as well (Fig. 6).

2. 3 Autoradiography of bone cells

The intake of enriched uranium UO_2F_2 in the body was mainly deposited in spongy bone. Figure 7 showed the most numerous autoradiographic tracks were accumulated in

Fig. 6. Electron microscopic autoradiography of liver cells at 24 h after i. v. injection of enriched uranium UO_2F_2 showed some autoradiographic tracks in lysosomes, ×9000

Fig. 7. Electron microscopic autoradiography of osteoclasts from epiphysis of the tibia at 72 h after i. v. injection of enriched uranium UO_2F_2 showed dense autoradiographic tracks in nucleus, and more numerous tracks appeared in mitochondria within the cytoplasm as well, ×10000

Fig. 8. Electron Microscopic autoradiography of osteoblasts at 48 h after i. v. injection of enriched uranium UO_2F_2 showed selective retention of autoradiographic tracks in whole nucleus, and some tracks appeared in thin cytoplasm as well, ×16000

the nucleus of osteoclasts from epiphysis of the tibia. More numerous autoradiographic tracks were retented in mitochondria within the cytoplasm. As shown in Fig. 8 the autoradiographic tracks selectively accumulated in whole nucleus of osteoblasts within the lacunae, and more numerous autoradiographic tracks also appeared in the thin cytoplasm around nucleus.

2. 4 Autoradiography of blood cells

After absorption of enriched uranium UO_2F_2 into the blood, it appeared rapidly in the forming elements of blood. As shown in Fig. 9. especially in erythrocytes, where more numerous autoradiographic tracks appeared. A few autoradiographic tracks appeared in neutrophils.

Fig. 9. Electron microscopic autoradiography of blood cells at 6 h after i. v. injection of enriched uranium UO_2F_2 showed more numerous tracks in erythrocytes, only a few tracks appeared in neutrophils, ×10000

3 DISCUSSION

At present, the possibilities of the contamination by the soluble enriched uranium UO_2F_2 to the human bodies is increasing. Enriched uranium UO_2F_2 was mainly accumulated in kidney cells at early stage. The electron microscopic autoradiographic studies showed that enriched uranium UO_2F_2 was selectively deposited in epicyte of proxi-

mal convoluted tubule of kidney, resulting in denaturation, necrosis and shedding of epicyte of the proximal tubule, finally causing anuria.

In liver cells, at first enriched uranium UO_2F_2 was mainly deposited in nucleus and soluble proteins of cytoplasm. Later, it was selectively located in mitochondria, then in lysosomes, may resulting in disorder of biological oxidation and the functional reduction of hydrolytic enzyme, finally causing liver-kidney syndrome.

The dynamic retention of radioactivity of enriched uranium UO_2F_2 in skeleton steadily rose throughout the exposure. Enriched uranium UO_2F_2 was chiefly deposited in nucleus and mitochondria of osteoblasts as well as osteoclasts within spongy bone. Its retention period was quite long, and it was difficult to excrete, so as to induce harmful effect to the bone cells may resulting ultimately carcinogenesis and teratogenesis[8~10].

REFERENCES

[1] Zhu Shoupeng. Radiotoxicology (In Chinese). 1st ed. Beijing: Atomic Energy press, 1983. 123~130

[2] Morrow P. et al. Health physics. 1982(43). 6: 859

[3] Zhu Shoupeng. Industrial Health and Occupational Diseases. 1987(13). 6: 371

[4] Zhu Shoupeng et al. Study on the Radiotoxicology of Enriched Uranium (In Chinese). CNIC-00086. Beijing. 1987. 1~10

[5] Zhu Shoupeng and Zhang Landseng. Isotopic Tracers in Medicine (in Chinese). 1st ed. Beijing: Atomic Energy press. 1989. 131~144

[6] Rogers A W. Practical Autoradiography. Amersham, England: The Radiochemical Center. 1979. 57~67

[7] Zhu Shoupeng, et al. Study on the metabolism of contamination of radionuclides in organism by autoradiographic techniques (in Chinese). CNIC-00233. Beijing. 1988. 1~9

[8] Durbin P W, et al. Metabolism and effects of uranium in animals. ERDA-93. London. 1975. 84~115

[9] Zhu Shoupeng, et al. Nuclear Techniques. 1987(10). 6: 53

[10] Фишмисов Л. Г и др. Радиобиология. 1978(18). 3: 400

电镜放射自显影研究液锶
铀 UO_2F_2 在体内亚细胞水平的沉积
原子能出版社出版
（北京 2108 信箱）
原子能出版社激光照排中心排版
北京市海淀区三环快速印刷厂印刷
☆
开本 787×1092 1/16 ·印张 1·字数 10 千字
1991 年 9 月北京第一版·1991 年 9 月北京第一次印刷
ISBN 7-5022-0541-1
TL·302

CHINA NUCLEAR SCIENCE & TECHNOLOGY REPORT

ISBN 7-5022-0541-1
TL • 302

P.O.Box 2103
Beijing, China

China Nuclear Information Centre