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The study on the dose-effect relationship of radiation from α particles of plutonium on certain lung cells (in vivo and in vitro)①

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It is well known that plutonium is one of the most toxic radionuclides and its carcinogenic risk has been seriously concerned. In this study, the dose effect relationship of radiation from α particles of plutonium on certain lung cells (in vivo and in vitro) were investigated[1,2].

The topics of study are as following:

In vivo: deposition and clearance of Pu in respiratory tract, dose-effect relationship of lung cancer induced, histopathological type of lung cancer, primary hemangiosarcoma occurred in thoracic lumph node, radiation effects on Alveolar Macrophage (AM), radiation effect on Natural Killer Cell (NK) and radiation effect on Alveolar Type II (AT-II).

In vitro: radiation effect on the immunological functions of AM, radiation effect on the membrane of AM, possible relationship between cytotoxicity and membranes of AM, effects of radiation (X, α) on the transformation of Wistar rat lung fibroblast cell line (WAL-F1) and protective effect of Se^{4+} against transformation.

IN VIVO

A total of 806 male Wistar rats were involved, 584 were exposed nose only in a specially designed exposure chamber to $^{239}PuO_2$ aerosol (AMAD ≈ 1.1-1.4 μm GSD = 1.9, Concentration 4.42-8.96KBq / L) for 30-60min, 222 rats were observed as control.

1. Deposition and clearance of $^{239}PuO_2$ in respiratory tract [3]

The retention function of $^{239}PuO_2$ in rat lung was fitted to the following equation:

$$R(t) = 79.75e^{-0.693t/22.7} + 21.62e^{-0.693t/373}$$

Translocation of ^{239}Pu from lung to TLN increased gradually with time elapsed after exposure and was a function of the percentage of intial lung burden of ^{239}Pu, plutonium content in TLNs could be expressed as:

$$Y = 1.16 - 1.163e^{-0.0017t}$$

The specific radioactivity of TLNs of rats increased gradually while the specific radioactivity of lung decreased (see Fig. 1), and beyond 378 days post exposure, the former

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became higher than the latter and resulted in a more rapid increase rate of absorbed dose from ^{239}Pu in TLNs than that in lung.

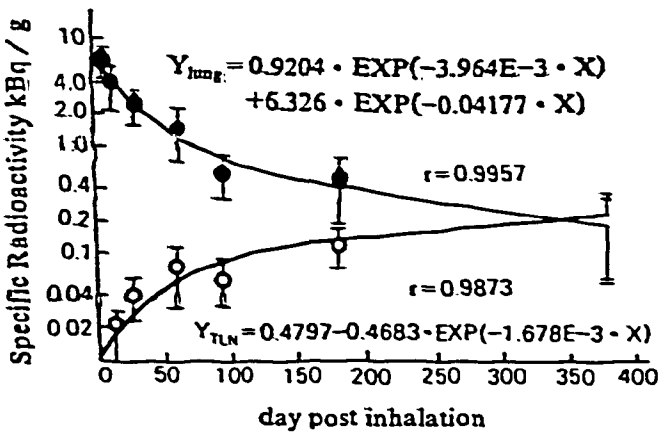

Fig 1. Specific Radioactivity of PuO_2 retained in lung and TLN after inhalation

2.Dose–effect relationship of lung cancer induced by inhaled $^{239}PuO_2$ and its histopathological characteristics[4].

All experimental rats died before 640 days after exposure. The lungs were observed macroscopically and microscopically. The relationship between life time lung cancer incidence and average lung dose contributed by $^{239}PuO_2$ inhaled were examined.

The relationship was fitted with the following exponential function: (see Fig.2).

Based on the above formula, the life time risk of carcinogenesis was estimated, the risk coefficient is 2.152×10^{-6} / c Gy which is well comparable with other authors.

8 types of lung cancer were found in the experimental animals, that included adenocarcinoma, squamous cell carcinoma, papillary carcinoma, mixed type of them etc. In 332 cases of cancers found, adenocarcinoma ranked the first,161 cases; squamous cell carcinoma, 76 cases followed.

It is worthwhile to indicate that a primary hemangiosarcoma was found in TLN in a rat at 372 days after inhalation of $^{239}PuO_2$. The absorbed dose in the TLN was about 2.2 Gy. So the probability of the occurrence of oncogenic consequence in TLN from inhaling radionuclide should not be ignored.

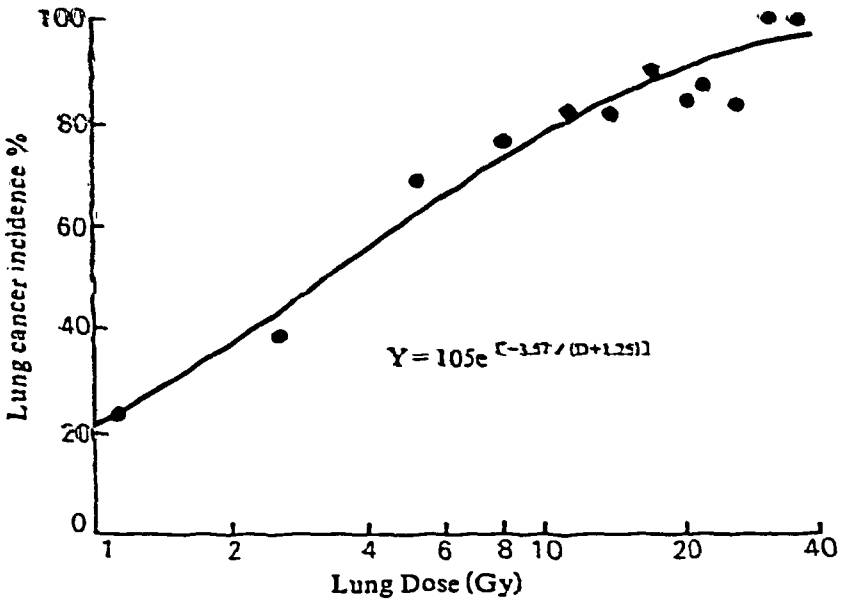

Fig 2. Relationship between life time lung cancer incidence and lung dose contributed by $^{239}PuO_2$ inhaled in rats

3. Radiation effects of Pu on Alveolar Macrophage[5]

Alveolar Macrophages are mononuclear phagocytes that reside in the lung.They are constantly exposed to inhaled pathogenic and / or carcinogenic particulate substances from the environment capable of affecting pulmonory functions. Evidence suggests that AM play a very important role in host defense against neoplasms. So it is important to study the change of functions of AM after inhalation of $^{239}PuO_2$.

Firstly, the percentage of inhaled $^{239}PuO_2$ phagocytized by AM was determined. At different time post inhalation, the $^{239}PuO_2$ particles in lung were lavaged, % of PuO_2 phagocytized by AM were examined , results are shown in Tab 1.

The results indicate that at 1.25hr after inhalation, 85% of Pu inhaled in lung were readily phagocytized by AM, at 24 hr, above 95%, thus giving a high radiation dose to AM. The retention of plutonium R(t) in AM, expressed in % of initial lung deposition, change with time post inhalation, fitted to the exponential function:

$$R(t) = 0.82\exp(-0.693t/3)+0.18\exp(-0.693t/81).$$

Based upon the above metabolic parameters, assuming the Pu particles are monodispersed, by means of Monte-Carlo method, the averaged absorbed dose of nucleus of AM were estimated. The following Table 2 listed the change of proliferative ability of

AM after different dose of radiation[65].

Table 1. Percentage of activity of Pu in AM Lavaged after different time of inhalation of PuO_2 in Rat

Time after inhalation (hr)	% of Pu in AM Lavaged
0.07	75.7
1.25	84.8 ± 0.6
3.0	85.2 ± 1.5
24	95.7 ± 1.8
74	96.1 ± 0.2

Table 2. Functional Changes of AM after Inhalation of $^{239}PuO_2$ in Rats

Dose in AM Nucleus (Gy)	Labelling index(%) mean ± s.e	Mitotic index(%) mean ± s.e
0	4.6 ± 1.2	1.6 ± 0.1
1.0	1.2 ± 0.2	0.2 ± 0.002
1.7	0.9 ± 0.1	0.3 ± 0.1
2.2	1.0 ± 0.1	0.2 ± 0.05
3.2	1.1 ± 0.2	

1.0 Gy of exposure on the nucleus of AM, the labelling cell % and mitotic cell % were significantly reduced to 74–88% of the control.

4. Radiation effect of Pu on natural killer cells in rat lung (NK).

The natural killer cells in lung and other tissues play an important defense role in the immunological surveillance system against neoplasm. In this study, natural killer cells in lung of rat, 9.5–11 months after inhalation of PuO_2, were used as the effector cell, YAC–1 cell as the target, by means of ^{51}Cr labelling method, the killing ability of NK was determined. The results are shown in Fig. 3.

The above result showed that the inhibitory effect of radiation (about 30 Gy) on killing ability of NK was obvious.

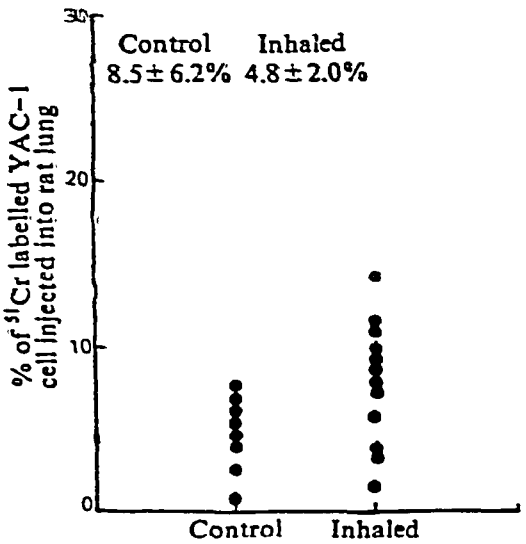

Fig 3. Killing ability of NK cells post inhalation of PuO_2

5. Effect of inhaled $^{239}PuO_2$ on Alveolar type II cell

(1). Microdosimetric study[7]

The alveolar type II cell is one of the critical cell for radiations damage in the lungs after inhalation of radioactive aerosols. With the aid of a Quantimet−970 image analyzer and a VAX−II / 780 computer, the radiation dose to rat alveolar type II cells from α particles emitted by $^{239}PuO_2$ were calculated. A series of dosimetric parameters for type II cells, including: track length distribution, linear energy transfer (LET), values of the specific energy for a single hit of a spherical target (Z1) cellular dose, hit number, and their spatial distribution were calculated according to the following flow chart(Fig. 4).

By comparing the volume density of type II cells and lung tissue with energy deposited in alveolar type II cells, we found that the energy deposited per unit volume of type II cells was larger than that of lung tissue excluding type II cells. The Z1 for spherical targets and the LET across type II cells were less than those in lung tissue excluding type II cells. The neoplastic transformation probability for type II cells in such condition might be higher than that of others. The results suggest that the type II cell is an important target cell in the rat lung for exposure to inhaled $^{239}PuO_2$.

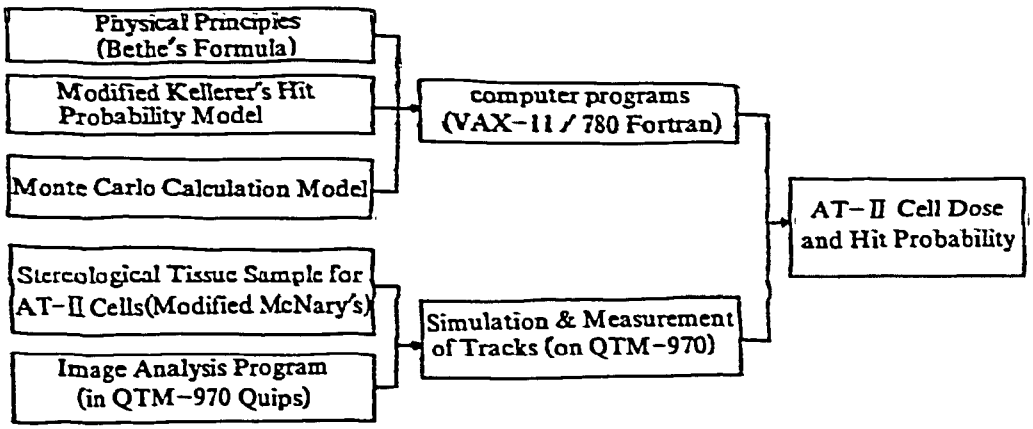

Fig 4. Flow chart of dosimetric study

(2).Stereo-morphometric study [8]

Stereo-morphometric changes of rat alveolar type II cells were studied in rats at 8 and 10 months following inhalation of $^{239}PuO_2$ to elucidate the biological role of AT-II cells in the induction of lung tumors. Transmission electronic microscope micrographs of random sections of lung were analyzed qualitatively and quantitatively using an automatic image analyzer. Eighteen morphometric parameters were obtained according to stereological principles. The parameters are: A. related to size of nucleus and cell: Nucleus Feret (μm), Nucleus Volume (μm^3), Cell Volume (μm^3), Nucleus Volume Density (μm^0). B. related to shape of nucleus and cell, Nuc. Axial ratio, Nuc. Regularity. Nuc. Smoothness, Cell Axial ratio, Cell Smoothness, Centroid distance between cell and nuc.(μm). C. related to mitochondria: Feret Diameter (μm), Numerical Density (μm^{-3}), Volume Dinsity (μm^0). D. related to lamellar bodies: Feret Diameter (μm), Numerical Density (μm^{-3}), Volume Density (μm^0). The results showed that: following the inhalation of $^{239}PuO_2$, nucleus enlarged, nuc / cytoplasm increased, regularity decreased.Number of mitochondria increased and number of lamellar body decreased. All these changes of indicated AT-II cells became less differentiated and the metabolism of the pulmonary surfactant in AT-II cells was disturbed.

IN VITRO

1. Radiation effect on the immunological functions of rat AM[9,10].

The inhibitory effect of immunological functions of AM after inhalation of radioactive particles has rarely been studied. In this study, an in vitro model was developed for the investigation of radiation effect of α particles and x rays on AM, nonspecific cytotoxicity index and specific phagocytosis of AM were used as the biological indicators.

Wistar rats were activated by BCG. 2 days later, their lungs were removed and lavaged with 0.02% EDTA in phosphate buffered saline. The AM harvested from BCG activated rats were irradiated with a ^{60}Co γ source, receiving absorbed dose of 0, 100, 300, 500 Gy, respectively.

(1). Nonspecific cytotoxicity of AM

After irradiation in vitro, the suspension of AM (effector cell E) were cultured together with human lung adenocarcinoma (AGZY-83-a) cells and Hela cells (target cells, T) according to a fixed E / T, ^{125}I-udR labelling method was used to determine the cytotoxicity index.

Results are shown in Tab.3.

Table 3. Effect of ^{60}Co-gamma-rays on cytotoxicity index of BCG activated AM against 2 tumor cell lines (means ± SD)

Dose (Gy)	Cytotoxicity Index	
	HeLa cell line	Adenocarcinoma cell line
0	81.3 ± 1.9	94.3 ± 0.3
100	75.5 ± 1.0	92.9 ± 0.9
300	59.0 ± 1.6	80.0 ± 0.1
500	50.9 ± 7.2	60.7 ± 2.6

AM, activated by BCG, were very effective in killing both AGZY83-a cells and Hela-cells. After irradiation, the cytotoxicity index of AM decreased with increasing radiation dose.

(2). Specific phagocytosis of AM

The specific phagocytosis of BCG activated AM was determined by using antibody coated chicken red blood cell in vitro. The percentage of AM of phagocytizing CRBC and the average number of CRBC phagocytized per AM ie. phagocytic index were determined. The results are shown in Tab. 4.

Table 4. Radiation Effect on Specific Phagocytosis of BCG Activated AM

Dose (Gy)	Number of Animals	Percentages of AM (%)	Phagocytic Index
0	8	14.2 ± 2.2	2.2 ± 0.2
100	10	9.2 ± 1.4	2.0 ± 0.4
300	11	7.8 ± 2.3	2.2 ± 0.6
500	11	4.8 ± 2.0	2.1 ± 0.5

The above data showed the % of AM with specific phagocytosis decreasing with increasing dose.

2. Damage effect of radiation on the membrane of AM in Vitro.

The membrane of AM plays an important role in maintaining intracellular environ-

ment and manifestation of immunological functions. Membrane damage by irradiation may culminate in enzyme release, so in this study cytoplasmic enzyme–Lactate Dehydrogenase (LDH) and lysosomal enzyme–β–Glucuronidase(β–Glu) released from AM were examined.

AM activated with BCG were exposed to γ–irradiation with doses of 0,100,200,300 and 500 Gy, after 24hr culture, the percentages of LDH and β–Glu in supernatant of AM culture were measured. The results (see in Fig. 5 and 6) indicated that percentage of LDH and β–Glu in supernatant of AM cultures increased with increasing irradiated doses.

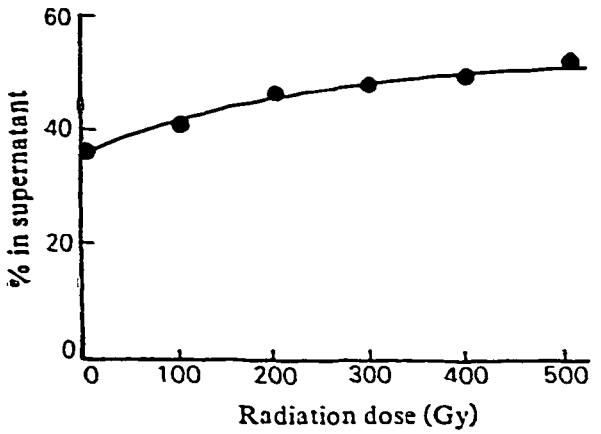

Fig 5. Effect of irradiation on cytoplasmic membrane of BCG–activated AM (in terms of activity of LDH).

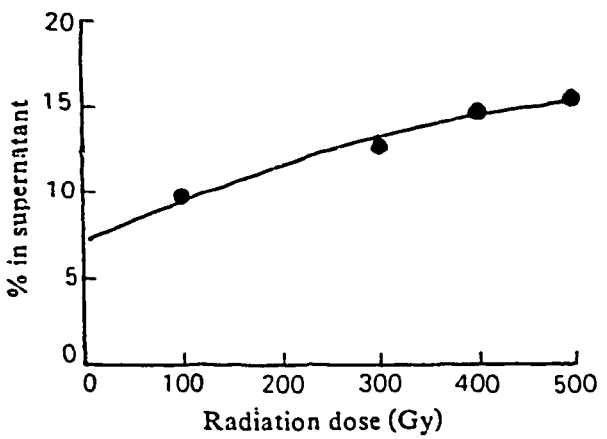

Fig 6. Effect of irradiation on lysosomal membrane of BCG–activated AM (in terms of activity of β–glu).

Morphological changes in irradiated AM

BCG–activated AM observed under the scanning electron microscope showed com-

plex surface structure and cell shape. Most of the BCG-activated AM appeared more flattered, had many extended, thin pseudopods and very prominent surface ruffles. In AM irradiated with dose of 100 Gy , contraction or disappearance of pseudopods, loss of surface ruffles, smooth cells surfaces were most often seen(see Fig. 7). This indicated the cytoplasmic membrane of AM was damaged by irradiation.

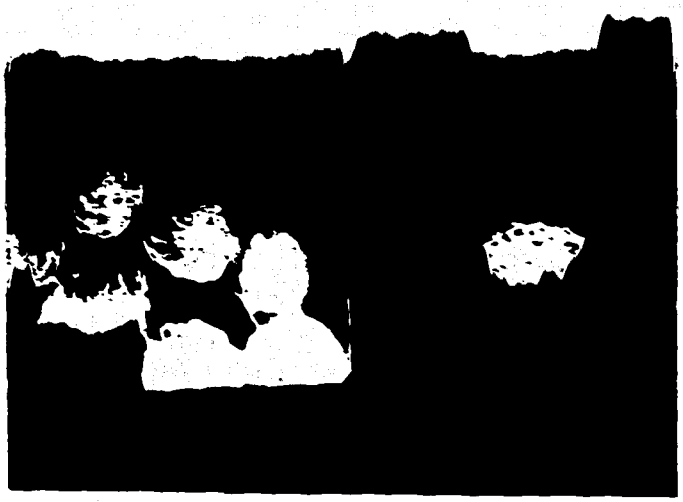

Fig 7. Surface morphology of control and irradiated AM observed by SEM

3. Possible relationship between cytotoxicity and the integrity of membrane of AM.

The functional integrity of AM is essential for the accomplishment of the cytotoxicity against tumor cells, and the manifestation of cytotoxicity of AM relies on cell membrane integrity. For illustrating the possible relationship between the inhibitory effect on cytotoxicity and damage of the integrity of membrane of AM caused by irradiation, we used the data described in the foregoing paragraphs to analyse the relationship between these two indicators.

Fig.8. and Fig.9. and their relative figures indicated the more severe the damage of the membrane, the lower the CI observed. These results did verify the above supposition that there was definite correlation between cytotoxicity and integrity of cytoplasmic and lysosomal membranes of AM. In other words, for the manifestation of nonspecific cytotoxicity (or tumoricidal), an integral membrane is an essential factor.

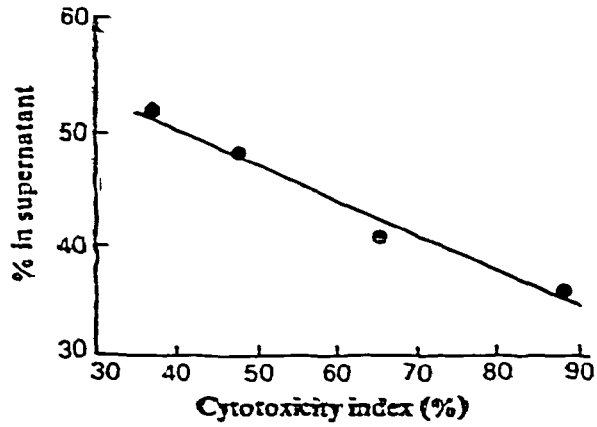

Fig 8. Relationship between cytotoxicity and nonintegrity of cytoplasmic membrane of AM

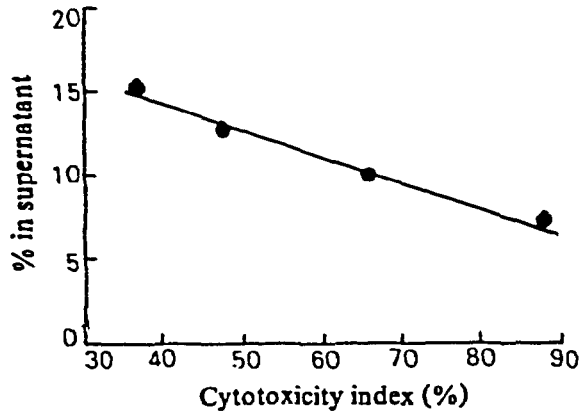

Fig 9. Relationship between cytotoxicity and nonintegrity of lysosomal membrane of AM

4. Effect of radiaition (X rays, α particles) on the transformation of fibroblast cell line of rat (WAL–F1)[11,12].

Studies on cell transformation by radiation in vitro were initiated with Golden hamster cells by Borek et al. in 1966. Since then, extensive studies have been carried out, but relatively little information was available concerning transformation by α–particles. In this study,

both the effects of X rays and α particles emitted by ^{239}Pu on the transformation of cell lines were investigated and comparative examinations were made.

Old Wistar rat lung fibroblast cell line (WAL-F1) was established in our laboratory and used in this study. α particles source consisted of a 14 cm^2 stainless-steel disk on which 2.6×10^7Bq of ^{239}Pu had been electroplated evenly across its surface. The dose rate delivered by the ^{239}Pu source was estimated by Monte-Carlo method based on physical parameters of ^{238}Pu and 8μm thickness of monolayer cell line and geometrical parameters of irradiation. The rate was 0.93 Gy / min. The cells were exposed to α particles for 7-105 sec.The absorbed doses estimated were 0.01,0.05,0.25,0.5,0.75, 1.0 and 1.5 Gy respectively. 180 KV X ray machine was used as the X-rays source, the dose exposed to the cell line were 0.5,1.0,3.0and 5.0 Gy with dose rate 64.8R / min.

The biological indicators of the irradiated cells examined were proliferation ability (growth rate, doubling time and mitotic index), karyotype analysis, morphology and growth (crisis cross,piling up etc.), transformation rate etc. After the appearance of morphological transformation, the following tests were examined: ConA agglutination text, colony formation in semisolid agar medium and transplatation test. Results are shown in Tab.5.

Tab 5. Comparative study on the effects of X rays and α particles on the transformation of WAL-F_1

Effect	X rays	α particles
Model of dose-Survival curve	Single hit and Multiple target	Single hit and single target
D_0 (Gy)	1.8-2.2	0.17
Proliferative ability of Transformed cell (% of control)	350-380(p.4)*	150-260(p.9)
Mitotic Index (% of control)	120(p.4)	280-500(p.11)
Chromosome aberration (Dose-effect relationship)	Y = -1.985+12.4D	Y = 1.999 ± 22.2D
Con A test (Conc. of Con A)	+ (~100μg / ml)	+ (<100μg / ml)
Colony formation (max. %)	4.4 (3.0Gy)	9.97(0.25Gy)
Transplantation test Fibrosarcoma formation test	3 months, 2 / 5** (3.0Gy)(p.54)	104day, 1 / 3 (0.5Gy)(p.21)

* p in ( ) denotes passage of cell line

** time of appearance of tumor, X / Y denotes tumor occurred in X rats in Y transplanted rats

From the above results, it could be seen that there were some differences between the transformation effects induced by X-rays and α particles, if we expressed it in terms of transplantation test, and adopt X rays' efficiency as 1, then the RBE of α particles should be 6.

5. Protective effect of Se[9,13]

It is well known that selenium, as an integral component of glutathione peroxidase (GSHPx), can promoted the primary function of GSHPx in vivo, for example reduciton of H_2O_2 and other organic hydro-peroxides. It also has been shown the unsaturated fatty acid or lipids of cell memebrane forms peroxides when irradiated in aqueous system. So the addition of Se to the irradiated system of AM, the membrane of AM should be protected form peroxidation, the integrity of memebrane could be maintained. If there is correlation between the cytotoxicity and integrity of cytoplasmic and lysosomal memebranes, then inhibitory effect on cytotoxicity should be prevented in the presence of Se. So this experiment was carried out. (see Tab. 6).

Tab 6. Protective effect of Se^{4+} on the cytotoxicity effect of AM.

Group	Index of cytotoxicity (%)	
	average	S.D
Control	67.9	1.3
300Gy	14.8	17.4
300Gy+Se^{4+}	43.3	6.9
Se^{4+}	54.2	4.9

From the data in Tab.6, it can be found that Se showed protective effect on the cytotoxicity of AM (from 14.8% increased to 43.3%).

Se^{4+} as a scanvenger against free radicals, it might play some protective role in the neoplastic transformation of cell line under the exposure of radiation. So the protective effect of Se^{4+} on the transformation of WAL-F1 after radiation of α particles was also observed. The results are shown in Tab.7.

From the data listed above, five biological indicators observed, espeically the transplantation test, all showed positive results, in other words, Se^{4+} might have protective effect against the transformation induced by irradiation.

Tab 7. Protective effect of Se^{4+} on the transformation of WAL-F_1 after irradiation with α particles

Effect	Control	irradiated	irradiation and Se^{4+}
Survival rate %	2.34	0.1	2.2
Chrom aberr.	1.7	12.8	3.5
ConA test	−	++	±
Colony formation rate %	0.9	4.1	1.5
Transplantation test	−	Tumor (104 days)	−

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