Effect of ^{188}Re on chromosome aberration in human peripheral blood lymphocytes

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Abstract Human peripheral blood exposed with $^{188}ReO_4^-$ at various radioactivities for 54 h was examined to observe the chromosome and chromatin aberrations at metaphases in the mitosis of lymphocytes. The findings indicate that there is an increasing tendency of the aberration yields of both types with increasing $^{188}ReO_4^-$ concentration and a dose dependency could be obtained. The aberration yield was best fitted by a quadratic model. Chromatin aberrations, aberration cells and chromosome fragments were fitted into a linear-regression model.

Keywords Peripheral blood, Chromosome aberration, $^{188}ReO_4^-$ irradiation-response
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1 INTRODUCTION

Along with the recent development on radiopharmaceuticals, the radionuclides of rhenium are widely used for cure of tumor and pain-reducing of cancer patients with bone metastases. Because of its excellent nuclear property, rhenium-188 have been taken into account much more in nuclear medicine and were used for treatment of cancer. The half-life of ^{188}Re is 16.9 h. Its beta ray energy is suitable for tumor therapy with a range in tissue of 4 mm. Its small amount of low energy gamma ray is appropriate for gamma camera imaging to observe tumor size or locate the position, but not increasing the body dose. ^{188}Re-labelled radiopharmaceuticals can mainly accumulate in tumor tissue to obtain the curative effect through its beta ray. Juweid[1] reported the results of 11 patients with gastrointestinal cancer treated by ^{188}Re-labelled MN-14IgG, including its phmarcokinetics, dosimetry and toxicity etc. After injected 740~2886 MBq of ^{188}Re-MN-14IgG, the most accumulations were found in liver, spleen and kidney tissues in these patients. The biological decay values for ^{188}Re-MN-14IgG in blood and urine were 8.2±4.1 h and 98.0±37.8 h, respectively. The absorbed doses to whole body and red marrow were 0.135±0.014 mGy/MBq and 0.973±0.27 mGy/MBq, respectively. Inhibited red marrow was occurred only when the toxic dose level reached. The tolerated dose was 2.22 GBq. Palmedo[2] treated 22 cases of cancer with bone metastases using ^{188}Re-HEDP and its effective rate was 75%. The dose in treatment was 1295~4440 MBq and

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the maximum toleration dose was 3.33GBq. The most important side effects were the thrombo and leukopenia. Oh[3] studied patients injected with 370 MBq of ^{188}Re-DTPA. Whole body imaging after 28 h showed the radiation absorpation doses of thyroid and kidney were 0.753 mGy/MBq and 0.685 mGy/MBq respectively, but the dose of whole body was 0.0208 mGy/MBq only. We can see that radiorhenium-188 is a kind of safe and effective radioisotope and its widespread application has been explored.

The dose is very important in radiation clinic. Only control the accurate irradiation dose, can the effective therapy be achieved. To get an accurate knowledge of dosage, it is necessary to investigate the biological genetic effects of ^{188}Re. Radiobiological method has the advantage of measuring the dose on a more basic level with high precision. The chromosome aberration is used in this work for indicating the effects of ionizing radiation and other mutagenesis factors on mammalian. In this study, the relation between radioactivity and chromosome aberration yield were fitted with regression equations. Corresponding radiation dose could then be gained from the chromosome aberration rate, thus the use of radiopharmaceuticals would be more precise, effective and safe.

2 MATERIALS AND METHODS

2.1 Materials

$Na^{188}ReO_4$ was obtained from Shanghai Kexing Pharmaceutical Co. Culture medium "1640" was purchased from Japan. Phytohmagglutinin (PHA) was gained from Institute of Radiation Medicine, Shanghai Medical University. Vituline serum was supplied by Shanghai Institute of Cell Biology. Microscope was bought from OLYMPUS, Japan

2.2 Methods

2.2.1 Making up $^{188}ReO_4^-$ solution of different radioactivity

Cultivation liquid was constructed by joining '1640" cultivation radix with 20% vituline serum, and suitable quantity of PHA, penicillium and streptomycin. Radioactive stock liquid was produced by addition of $^{188}ReO_4^-$ into cultivation liquid, and then diluted with none-radioactive cultivation liquid using half-and-half method. Cultivation liquid containing various concentration of $^{188}ReO_4^-$ was prepared and was loaded 5 mL per vial.

2.2.2 Preparation and analysis of chromosome samples

Four cases of venous blood samples were taken from healthy volunteers (2 males and 2 females, respectively). 0.3 mL venous blood was added to aforementioned cultivated vial. Besides control group, seven dose groups were established. Their initial $^{188}ReO_4^-$ radioactivities per vial were 0.137~8.695 MBq, respectively.

They were cultivated for 48 h at 37°C, then were added to colchicine (final concentration was 0.2μg/mL) and were further cultivated for 6 h. Chromosome samples were prepared from the collected cells according to conventional method. Metaphase mitosis of $2n = 46\pm1$ were checked in oil-lens by double-blindness method in term of Buckton standard of chromosome aberration analysis. Experimental data were analyzed using

least square regression and were fitted into activity response curves.

3 RESULTS

Various types of chromosome aberration were thus produced from human peripheral blood lymphocytes irradiated by $^{188}ReO_4^-$ of various doses (Table 1). Both of the stable and unstable aberration of chromosome were increased obviously with increasing $^{188}ReO_4^-$ radioactive concentration, the chromatin deletion was also increased. Ringed chromosome or chromatin exchange were not analyzed because of their aberration rate was too low. Relations between aberration and radioactivity of other aberrations were all fitted by different mathematical models. The numbers of aberration of cells, fragments and dicentric plus centric rings were best fitted by quadratic models (Table 2).

Table 1 Various chromosome aberration yields in human peripheral induced by $^{188}ReO_4^-$ (percent$\pm SE$)

$^{188}ReO_4^-$ /MBq	No. of cells	Chromosome aberration					Chromatin aberration		Aberration of cells /%
		Dicentric	Acentric ring	Centric ring	Fragment	Inter-change	Chromatin deletion	Chromatin exchange	
Control	1100	-	-	-	4	-	2	-	5
		-	-	-	0.36±0.002	-	0.18±0.001	-	0.45±0.067
0.137	2495	5	-	-	23	-	22	-	46
		0.20±0.001	-	-	0.92±0.002		0.88±0.002		1.84±0.003
0.274	2282	3	-	-	30	-	27	-	48
		0.13±0.001	-		1.31±0.002		1.18±0.002		2.10±0.003
0.544	2467	7	-	-	49	1	29	-	76
		0.28±0.001	-	-	1.99±0.003	0.04±0.001	1.17±0.002	-	3.08±0.004
1.088	1849	18	-	-	64	3	55		105
		0.99±0.002	-		3.46±0.004	0.16±0.001	2.97±0.004	-	5.68±0.005
2.176	1344	21	2	1	84	7	51		133
		1.56±0.003	0.15±0.001	0.07±0.001	6.25±0.007	0.52±0.002	3.79±0.005	-	9.90±0.005
4.348	1455	103	14	1	297	14	127	3	354
		7.08±0.007	0.96±0.003	0.07±0.001	20.41±0.007	0.96±0.003	8.73±0.017	0.21±0.001	24.33±0.011
8.695	730	151	19	2	459	36	152	7	410
		20.68±0.017	2.60±0.006	0.27±0.002	62.88±0.007	4.93±0.008	20.82±0.46	0.96±0.004	56.16±0.018

Table 2 Results of fitting mathematical models for various aberration yields Y in peripheral versus activities A of $^{188}ReO_4^-$

Types of aberration	Fitting model	Results	Statistic meaning value of F
Aberration cell	$Y=a+bA+cA^2$	$Y=1.17+0.77\times10^2\mathrm{A}+7.75\times10^2A^2$	$35.01>F_{0.01(2,5)}$
	$Y=a+bA$	$Y=-0.66+2.34\times10^2A$	r=0.9950
Dicentric + centric ring	$Y=a+bA+cA^2$	$Y=0.14+8.82A+3.61\times10^2A^2$	$189.42>F_{0.01(2,5)}$
Fragment	$Y=a+b\mathrm{A}+cA^2$	$Y=0.58+0.67\times10^2A+8.32\times10^2A^2$	$356.83>F_{0.01(2,5)}$
	$Y=a+bA$	$Y=-2.86+2.58\times10^2\mathrm{A}$	r=0.98
Chromatin aberration	$Y=a+bA$	$Y=-0.066+88.78A$	r=0.9920

4 DISCUSSION

In our study of the rates of the chromosome aberration in human peripheral blood lymphocytes induced by $^{188}ReO_4^-$ irradiation, some obvious features were seen on the radiation-response curves of the aberrations, but had not been reported previously. These radiation response curve offered a microscopic biogenetic foundation for the determination of the ^{188}Re dosage applied. Compared with external irradiations, the characterstics of ^{188}Re induced aberrations and their radiation response curve can be summarized as follows.

1. Both chromatin deletion and chromatin exchange were increased obviously with increasing ^{188}Re dosage from Table 1. They were different from those caused by irradiation with low LET X or γ-rays, in the latter case lymphocytes irradiated were only those in the period of G_0. Whereas $^{188}ReO_4^-$ was a sustaining internal radiation source in whole irradiation period, so not only lymphocyte of period of G_0, but also the lymphocytes of periods S and G_2, which splitted under the excitation by PHA, showed chromatin aberration. In addition to the irradiation, chemical factor could be another cause of aberration.

2. For the dicentric plus centric ring in the double hit products, caused both by low LET rays and by ^{188}Re, the relation between the induced aberration with the quantity of radiations was best fitted by a quadratic model. On the other hand, for single hit products, the quadratic model seemed better than a linear one to those induced by ^{188}Re; for low LET rays induced single hit products, only linear-regression could be used. When a linear-regression $Y = a + bA$ was used to fit the yields of cell aberrations, chromosome fragments and deletions, parameter a were always negative (r=0.99, p <0.001).

3. The yield of chromosome fragment were 3~5 times more than the yield of centric, no matter how much radioactivities (the quantity of radiations) of ^{188}Re were used, which was connect to the nuclear properties of ^{188}Re: 85% β rays of energy 2.12 MeV, 15% γ rays of energy 155 keV only, much lower than the energy of ^{60}Co γ-rays, or the 8 MeV X rays from the linear-accelerator and 14 MeV neutrons[4,5].

These results will be useful for clinical application of rhenium-188 labelled radiopharmaceuticals.

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