Radiotoxicity induced by Auger electron emitters in human osteosarcoma cell line using comet assay

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Abstract The comet assay (single cell gel electrophoresis assay) was used to evaluate the radiotoxicity of Auger electron emitters in the human osteosarcoma cell line (HOS-8603). After internal exposure to ^{67}Ga-EDTMP, the sarcoma cell has been injured severely. The comet length was longer along with the increase of dose, the appearance of comet tail was different from that with respect to the ^{60}Co γ-ray irradiation. DNA damage of cell was mainly due to the radiation effect of Auger electrons. The ^{67}Ga may be a therapeutic radionuclide with good prospect for tumor treatment and palliation of bone pain induced by metastasis.

Keywords ^{67}Ga-EDTMP, Auger electron emitters, Comet assay (single cell electrophoresis assay), DNA damage, Fluorescence microscope

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1 Introduction

The radionuclide therapeutic agents were used widely to treat the primary tumors and to palliate the bone pain induced by metastasis.[1] The most common used radionuclides are ^{131}I,^{153}Sm,^{186}Re and ^{188}Re, all of which are β^- emitters with higher energy. Although their treatment effects were reliable, bone marrow suppression usually occur during the course of treatment, because the stem cells in the hematopoietic tissue are damaged.[2,3] Recently, some new radionuclides, such as ^{125}I,^{123}I, and ^{111}In,[4,5] which are γ photon emitters as well as Auger electron emitters, have been reported for the treatment of cancer. One of their advantages is higher RBE with less bone marrow suppression if the agents enter the neoplasm cells specifically, which is due to the fact that the Auger electron has lower energy, shorter range and higher LET. ^{67}Ga is not only a gamma decay radionuclide with energies of 93(38%), 185（24%）, 300（19%）和 393（5.3%）keV, but also decays by EC and emits Auger electron. Thus, it may be a potential therapeutic radionuclide. The purpose of this study was to evaluate the radiotoxicity of ^{67}Ga-EDTMP (ethylene diamine tetramethylene phosphonic acid) to human osteosarcoma cell line by comet assay and to determine the possibility for the use in tumor treatment. The comet assay was a single cell gel electrophoresis method that could measure many kinds of DNA damage and repair in individual cells. It may be used to examine the effect of cytoplasmic irradiation and to evaluate the high LET irradiation such as heavy ion beam and α particles.[6]

2 Materials and methods

2.1 Radionuclide therapeutic agents

^{67}Ga-EDTMP was produced by Shanghai Institute of Nuclear Research, the Chinese Academy of Sciences. Before sarcoma cells culture, the ^{67}Ga-EDTMP aqueous solutions were sterilized by ultrafiltration. The activity was 2×10^4 and 8×10^4 MBq/L, respectively.

2.2 Cell line

The human osteosarcoma cell line HOS-8603 was established by Gene Laboratory, Suzhou Medical College, China. The culture medium was modified

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RPMI 1640 （GIBCO）, containing 2 nmol/L L-glutamine, 1×10^5 IU penicillin/L, 100 mg streptomycin/L and 10% foetal bovine serum. The cells were cultured in flasks at 37 °C in 5% CO_2 incubator. The exponentially growing cells were digested by 0.05% trypsin and washed three times with Hank's buffer (without Ca^+ and Mg^+). Before using, the cell suspension was adjusted to 5×10^7 cells/L.

2.3 Internal and external radiation

5000 cell/100μL was added to glass plate (6 cm diameter) with 0.5% solid agar RPMI 1640（GIBCO）medium. At the same time, 2 MBq and 8 MBq/100 μL ^{67}Ga-EDTMP solution was added respectively. The cells were cultured at 37°C in a saturated water vapour incubator with 5% CO_2 for 13 d, and then the cloned cells were harvested, washed and cell suspension was made (5×10^7cells/L) for the comet assay.

For comparing, the cell suspension (5×10^7cells/L) was irradiated by ^{60}Co γ-rays at 1Gy/min dose rate, the dose was 1, 5, 10, 20 and 30 Gy respectively.

2.4 Comet assay

The comet assay was performed under yellow light as described.[6] 100 μL of 0.5% regular agarose (Sigma) was added to the fully frosted microscope slide and a coverslip was set on top at once. After the agarose layer solidified, the coverslip was removed. The 0.7% low melting point agarose of 75 μL and 10 μL of cell suspension (about 30,000~200,000 cells) were mixed and added to the slide. The coverslip was set on the top again and the slide was placed at 4°C for 5~10 min to solidify the agarose. After removing the coverslip, the 0.7% low melting point agarose of 75 μL was added as the third layer, covered again until it was solidified. After removing the coverslip, the slide was immersed in lysing solution (2.5 mol/L NaCl, 100 mmol/L Na_2-EDTA, 10 mmol/L Tris, pH 10, with 1% triton X-100, 1% sodium sarcosinate and 10%DMSO) for 1 h at 4°C.

The slide was set in the electrophoresis reservoirs filled with alkaline buffer (300 mmol/L NaOH and 1 mmol/L Na_2-EDTA, pH 12.0) for 20 min to let DNA unwind and show alkali-labile damage. The electrophoresis apparatus (2DY-2A, Nanjing University) was set at 25V, the current was set to 300 mA by lowering the buffer level. The DNA was electrophoresed for 20 min. The slide was then moved to a staining tray, neutralized with buffer (0.4 mol/L Tris, pH 7.5 in HCl), and washed three times. At last, DNA was stained with 100 μL of 2 mg/L ethidium bromide (Sigma).

The cell comet was observed by a fluorescence microscope with 516~560 nm excitation from a mercury light (Olympus BX-60, PM20 camera, Japan). The comet length of at least 30 cells per slide was measured and the cell comet appearance was observed.

3 Results and Discussion

3.1 Appearance of cell comet

The comet of cell was stained red by ethidium bromide under the fluorescence microscope. The shape of comets was clearly different between ^{67}Ga-EDTMP and ^{60}Co γ-ray irradiation. Fig.1 showed the comet appearance of human osteosarcoma cells irradiated by ^{67}Ga-EDTMP, the tail shape of comet was round and the tail was strongly saturated. However, the comet tail of cell irradiated by ^{60}Co γ-rays was tear-drop-shaped, as shown in Fig.2. The acceptable explanation was that injuries induced by high LET Auger electron in the cytoplasm and nucleus were more complex because of the larger number and size of multiply damaged sites. The shorter range's Auger electrons directly and densely impacted the DNA molecules, the more single strand breaks and double strand breaks were produced. A great number of small fragments due to ssb and more dsb may reduce DNA content and change the appearance of comet. The external ^{60}Co γ-ray radiation may induce more ssb and a few dsb indirectly by free radicals in water, producing some large fragments to change the shape of comet.[6] Although it had better measure comet moment of two kinds of cells by different radiation, it was markedly that the internal irradiation of ^{67}Ga-EDTMP might induce the DNA damage, which was different from that induced by ^{60}Co γ-ray.

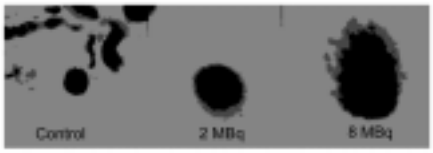

Fig.1 The comet photo of HOS-8603 internally irradiated by ^{67}Ga-EDTMP.

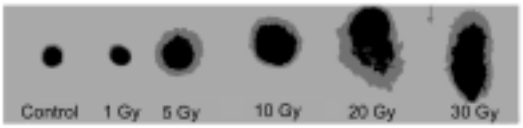

Fig.2 The comet photo of HOS-8603 externally irradiated by ^{60}Co γ-ray.

3.2 Dose-response relationship

The comet length of human osteosarcoma cells was measured for different irradiation doses. Table 1 showed the relationship between the comet length and the doses of ^{60}Co γ-ray external radiation. In the range from 1 to 30 Gy, along with the increase of radiation dose, the comet length was longer significantly. The comet length represented the grade of cell DNA damage. The relation between the comet length and dosage of the internal irradiation of ^{67}Ga-EDTMP was similar. At the 2 MBq dose point, the comet length was longer than control cell significantly, and the comet length at the 8 MBq dose point was significantly longer than that at the 2 MBq dose point (shown in Table 2).

Table 1 The comet length of HOS-8603 cell line induced by ^{60}Co γ-ray ($\bar{X}$ ± SD, n = 20)

	Dose of ^{60}Co γ-ray external radiation (Gy)					
	0	1	5	10	20	30
Comet length (μm)	19.37±0.94	21.25±2.8	40.57±4.90	50.72±4.70	60.19±4.72	111.40±44.03
p-value (student test)		0.32	<0.01	<0.01	<0.01	<0.01

Table 2 The comet length of HOS-8603 cell line induced by ^{67}Ga-EDTMP ($\bar{X}$ ± SD, n = 20)

	Dosage of ^{67}Ga-EDTMP (MBq)		
	0	2	8
Comet length (μm)	31.04±9.76	38.75±6.73	69.13±19.35
p-value (student test)		<0.01	<0.01

Auger electron emitters are used widely in nuclear medicine imaging and internal radiotherapy of cancer in recent years. Although the energy carried by Auger electrons is only a small fraction of the total energy released in the decay, the collective local deposition is very dense,[7] because the range of these electrons is very short (a few nanometers) and the LET is very high. If the decays occurred in the immediate vicinity of critical macro-molecules, the biological effects should be severe. As a therapy agent, it is important to direct the radionuclide to the DNA in the nucleus of cancer cells. This pilot study now showed that ^{67}Ga-EDTMP could enter sarcoma cell nucleus and induce severe DNA damage, which was different from that by ^{60}Co γ-ray external radiation. Further study is necessary to confirm whether any difference exists between ^{67}Ga-EDTMP fractions respectively entering cancer cell and normal cell (especially the blood stem cell).

4 Conclusion

^{67}Ga-EDTMP could enter sarcoma cell nucleus and induce severe DNA damage measured by comet assay. The DNA damage was mainly due to the radiation effect of high-LET Auger electrons. So ^{67}Ga may be a therapeutic radionuclide with good prospect for tumor treatment and palliation of bone pain induced by metastasis.

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