Fluorescence microscopic morphology and inhibition rate studies on apoptosis of osteosarcoma cells induced by ^{153}Sm

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Abstract The apoptosis of osteosarcoma cells treated with irradiation by ^{153}Sm-EDTMP was studied. The morphological changes in osteosarcoma cells were observed by fluorescence microscopy. It was found that osteosarcoma cells exposed with ^{153}Sm-EDTMP displayed significant nuclear fragmentation and marked pyknosis. With the prolongation of observing period, the membrane bound apoptotic bodies formation was observed. It should be noted, that with the lengthening of irradiation time by ^{153}Sm-EDTMP, the inhibition rate of proliferation of osteosarcoma cells increased progressively.

Keywords Fluorescence microscopy, Morphology, Inhibition rate , Apoptosis of osteosarcoma cells, ^{153}Sm-EDTMP

CLC numbers R738.1, R817.5

1 INTRODUCTION

In recent years ^{153}Sm–EDTMP (Ethylenediamine tetramethylene phosphonate) has been used for clinical application in patients requiring analgesia for pain arising from disseminated skeletal metastasis unresponsive to all appropriate conventional treatment modalities[1]. ^{153}Sm–EDTMP is effective not only in alleviating the pain of disseminated skeletal metastasis, but also in the treatment of recurrent pain[2]. But up to date, its mechanism in the treatment of painful skeletal metastasis is still unknown[3]. Therefore, we paid attention to study apoptosis of osteosarcoma cells induced by ^{153}Sm–EDTMP with fluorescence microscopic and inhibition rate observations.

2 EXPERIMENTAL METHODS

2.1 Cell culture conditions

A human osteosarcoma cell line HOS-8603[4] obtained from Cellular Immunological Center of Suzhou Medical College, was harvested and maintained in RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum, 100 u/mL penicillin, 100 μg/mL streptomycin, 7 mmol/L L-glutamine as well as 5×10^{-5} mol of 2-mercaptoethanol, which was known as the complete RPMI 1640 medium. The osteosarcoma cells were kept in an atmosphere containing 5% CO_2 at 37°C and used when in experimental growth[5]. Cells were harvested from exponential-phase maintenance culture using trypsin: versene (0.05%:0.02%)

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treatment of monolayer culture. Thereafter, the osteosarcoma cells were washed three times with Ca^{++} and Mg^{++} free Hanks solution. Simple cell suspension was prepared with complete RPMI 1640 medium and the cells were counted using a hemocytometer. Finally, the suspension was adjusted to a concentration of 2×10^6 cells/mL.

Radioactive and chemical pure ^{153}Sm–EDTMP was used in this study. The osteosarcoma cells suspensions at 2×10^6 cells/mL was added to 1 mL of ^{153}Sm–EDTMP solution with a radioactivity of 3.7×10^2 kBq/mL in complete RPMI 1640 medium in a 5% CO_2 at 37°C for 3, 6, 9, 12 and 24 h.

2.2 Fluorescence microscopic observation

The osteosarcoma cells were suspended in complete RPMI 1640 medium to form suspension at 2×10^6cells/mL. Then 1 mL of the cell suspension was added to 24-well microtitration plate. 1 mL of ^{153}Sm–EDTMP with radioactivity of 3.7×10^2 kBq/mL also in complete RPMI 1640 medium was added to each experimental well. To control wells, only 1 mL RPMI 1640 medium was added. The microtitration plate was then incubated in 5% CO_2 at 37° for different periods. After irradiation with ^{153}Sm for different intervals, the osteosarcoma cells were harvested, and washed 5 times with Hanks solution in order to remove the free radioactivity of ^{153}Sm–EDTMP. After a while , the osteosarcoma cells were suspended in hypotonic fluorescent solution which consists of 50 mg/mL pyridine iodide and 0.1% sodium acetate as well as 0.1% triton X-100[6], in order to examine the morphology of nuclei of osteosarcoma cells.

2.3 Inhibition rate of proliferation

The inhibition rate of proliferation of osteosarcoma cells after internal irradiation with ^{153}Sm–EDTMP was measured by MTT assay[7]. 1 mL of osteosarcoma cell suspension at 2×10^6 cells/mL was added to 24-well sterile microtitration plate. Then 1 mL of ^{153}Sm–EDTMP with radioactivity of 3.7×10^2 kBq/mL also in complete RPMI 1640 medium was added to each experimental well. To control wells, only 1 mL RPMI 1640 medium was added. Thereafter, the microtitration plates were maintained at 37°C in a humidified atmosphere containing 5% CO_2 for 3, 6, 9, 12 and 24h. After internal irradiation with ^{153}Sm–EDTMP for different intervals, the osteosarcoma cells were harvested and washed with Hanks solution. Cell suspension was adjusted to 5×10^5 cells/mL and put to 55-well Elisa microplate of a volume of 100 μL/well. Then 10 μL of MTT solution (5 mg/mL in sterile PBS, filtered through a 0.22μm filter) was added and the microplate was incubated in 5% CO_2 atmosphere at 37° for 4h. Finally 100μL of SDS was added to each well and the microplate was incubated over night . The absorbance (A) was measured on DC-3022A Elasa microplate reader at wavelength of 540 nm.

3 RESULTS

3.1 Fluorescence microscopic study

Fluorescence microscopic appearance of PI-stained nuclei of the control osteosarcoma cells after 12h incubation in RPMI 1640 medium alone was shown in Fig.1. The cells showed heterogeneous nuclear chromatin. While osteosarcoma cells, internally irradiated with ^{153}Sm–EDTMP, displayed marked nuclear fragmentation and pyknosis in nuclei of osteosarcoma cells as shown in Fig.2.

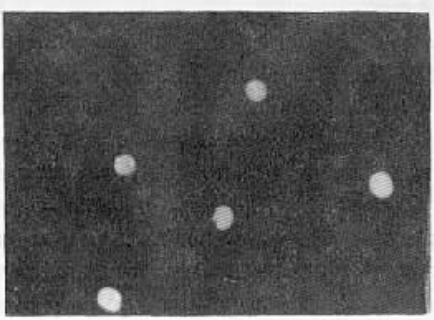

Fig.1 Fluorescence microscopic appearance of PI-stained nuclei of control osteosercoma cells after 12 h incubation in RPMI 1640 medium alone
The cells show heterogeneous nuclear chromatin (×1250)

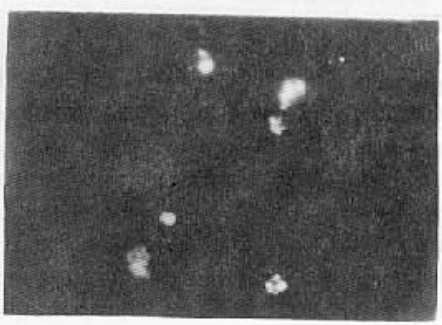

Fig.2 Fluorescence microscopic appearance of PI-stained nuclei of osteosarcoma cells after 24 h internal irradiation with ^{153}Sm–EDTMP
The cells nuclei show marked fragmentation and pyknosis (×1250)

3.2 Study on inhibition rate of proliferation

From experimental results, the inhibition rate of proliferation of osteosarcoma cells after internal irradiation with ^{153}Sm–EDTMP for different intervals is shown in Table 1. The inhibition rate of proliferation of osteosarcoma cells increased progressively with prolonging the time of ^{153}Sm-EDTMP internal irradiation. Especially, the inhibition rate of proliferation in osteosarcoma cells increased significantly after treatment with ^{153}Sm-EDTMP for 6 h to 24 h.

Table 1 The inhibition rate of proliferation in osteosarcoma cells after internal irradiation with ^{153}Sm-EDTMP for different intervals

Time/h	MTT A540 ($\overline{x} \pm s$)[3]		Inhibition rate/%
	Control	^{153}Sm-EDTMP	
0	0.45±0.07	0.45±0.07	-
3	0.57±0.01	0.53±0.02	7.1
6	0.62±0.02	0.54±0.08	12.9[1]
9	0.65±0.03	0.50±0.14	23.1[2]
12	0.71±0.05	0.43±0.12	39.4[2]
24	0.78±0.03	0.29±0.04	62.8[2]

[1]$p < 0.05$, [2]$p < 0.01$, [3]$N = 5$

4 DISCUSSION

Our previous study provided strong evidence that osteosarcoma cells underwent apoptosis when they encountered^{153}Sm–EDTMP. The internucleosomal fragmentation of DNA in osteosarcoma cells , which resulted in a ladder type pattern comprision 180~200 base pair intervals in agarose gel electrophoresis[8]. The microautoradiographic study showed that ^{153}Sm could permeate through cell membrane and displayed membrane-seeking condensation in osteosarcoma cells. Thereafter, ^{153}Sm could be phagocytized and distributed in cytoplasm and nucleus in the form of phagosome. At the same time, the membrane-bound apoptotic bodies were observed[9]. In the present study, the apoptotic changes in these cells displayed significant nuclear fragmentation and marked pyknosis in nuclei, as well as apoptotic bodies formation were observed by fluorescence microscopy. Results indicated that the progression of apoptosis in osteosarcoma cells induced by ^{153}Sm-EDTMP was dependent on the ^{153}Sm exposure time. It should be noted, while osteosarcoma cells were internally irradiated with ^{153}Sm, the apoptotic changes in these cells showed, that the internucleosomal fragmentation of DNA in osteasarcoma cells resulted in a ladder type pattern comprision 180 base pair intervals in agarose gel electrophoresis. Our study indicated that progression of apoptosis of osteosarcoma cells by ^{153}Sm-EDTMP was dependent on the ^{153}Sm exposure time. At the same time, the inhibition rate of cell proliferation was also elevated with prolongation of ^{153}Sm irradiation time.

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