THE INHIBITION EFFECT OF ^{211}At—Te COLLOID AND Na^{211}At INJECTIONS ON MURINE EHRLICH ASCITES TUMOR CELLS

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ABSTRACT

Na^{211}At and ^{211}At-Te colloid injections are prepared. It has been demonstrated that the ^{211}At-Te colloid is stable in vivo and vitro, and can be applied in the study of biology and medicine. In the report, the model of Murine Ehrlich Ascites Cells cultured in vivo and vitro is elected for a series of experiments. It has been proved that Na^{211}At and ^{211}At-Te colloid injections possess an inhibition effect on tumor cells. The inhibition effect was expressed in surviving of the mice and inhibiting growth of tumor as well as the changes of enzyme activity. Meanwhile, it was also noticed that Na^{211}At and ^{211}At-Te colloid injections of various dose inhibited the absorb of pyrimidine nucleosides in Murine Ehrlich Ascites Cells. And the effect isn't reversible. It is closely related to the dose administratered and 50% inhibition rate needs about 1.48×10^{5} Bq/ml culture.

Keywords: ^{211}At ^{211}At-Te colloid Absorb Inhibition rate Prolongation rate Enzyme activity

1 INTRODUCTION

In recent times it has been focused on investigation of the α -emitting radionuclide anticancer-drug, which may easier accumulate in the pathological part[1-3]. ^{211}At is a radiohalogen, pure α -emitter. Its physical half-life is 7.21 h, the mean linear energy transfer is 113 keV/μ m, the mean energy is 6.8 MeV and alpha range in soft tissue is 60 μ m[4-6]. It's obvious that ^{211}At is the most suitable nuclide for radiotherapeutic purposes.

In order to facilitate the application of radionuclide ^{211}At in tumor treatment, the radiobiological effect of ^{211}At, especially its kill-effect on tumor cells and possible way of inhibiting tumor growth, was investigated.

2 MATERIALS AND METHODS

2.1 The preparation of ^{211}At and its injections

^{211}At was produced by irradiating Bi-target with 27 MeV α -particles via ^{209}Bi (α, 2n) ^{211}At reaction at cyclotron of INST of Sichuan University[7]. After seperation, spectra analysis indicated that ^{211}At is radiochemical pure, and it can be used in biological or medical study.

^{211}At–Te colloid was obtained by absorbing ^{211}At from 2mol/l HCl solution onto the freshly prepared tellurium particles, the diameter of which was between 3–8 μ m. The stability of ^{211}At–Te colloid both in vivo or vitro was well.

$Na^{211}At$ injection was prepared from the solution of 0.1 mol/l NaOH–0.01 mol/l $NaHSO_3$ adjusting pH to 6–7 with 2mol/l HCl.

2.2 The treatment of tumor strain and tumor cells suspension

The murine Ehrlich ascites tumor (EAT) strain was choosen. By aseptic manipulation, the ascites was catched from a mouse which was planted for 7–9 days and then the ascites was centrifuged, washed and counted. At last the concentration of the tumor cells suspension was 10^6 cells/ml, culture was treated with RPMI 1640 solution.

2.3 The experiment of kill—effect in vitro

There are 6 mice in every group. After the murine Ehrlich ascites tumor cells were planted in RPMI 1640 solution and clutured at 37°C in vitro for 36 h. $Na^{211}At$ injection was added according to 7.4×10^4 Bq/ml culture and the culture was continued for 6, 15 or 22h. These tumor cells (2×10^6 cells/mouse) were inoculated into healthy mice via i.p. and the control groups were inoculated with tumor cells that wasn't treated. The prolongation rate of survival was estimated. In addition the tumor cells were smeared for morphological and enzyme histochemical observation.

2.4 The absorb experiment of pyrimidine nucleosides

The tumor cells suspension was planted in RPMI 1640 culture medium with aseptic method. After incubated for 12 h at 37°C, ^{211}At radiopharmaceuticals ($Na^{211}At$ or ^{211}At–Te colloid injection) and ^3H–Tdr or ^3H–Ur were added in the culture at the same time. The control groups were divided into both the added equal RPMI 1640 solution and the added no-labelled tellurium colloid injection. All samples were continued to incubate for various times (7–80 h). At last the tumor cells were collected and washed with normal saline and anhydrous alcohol and digested with $HClO_4$. The tumor cell samples of treatment were uncoloured to a bright solution. A scintillation solution (PPO 0.4%, POPOP 0.01%) was added. The radioactivity was measured in a Model FJ2101 Liquid Scintillation Spectrometer.

3 RESULTS AND DISCUSSION

3.1 The injury effects of ionic ^{211}At on the experimental ehrlich ascites tumor cells in vitro

The injury effects on tumor cells induced by ionic ^{211}At in vitro expressed the surviving prolongation of the mice (cf. Table 1) and the inhibiting growth of tumor cells in the experimental mice.

Table 1

Prolongation rate of survival of mice in the experiment

Groups	Survival time (d)		Prolongation rate (%)	*P* value (*t*-test)
	Range	$\bar{X}\pm$S.E.		
At^{211} irrad. 6 h	25-27	26.33±0.33	13.64%	0.01
Control	21-27	23.17±0.60		
^{211}At irrad. 15 h	>300* *	300±0.0	90.9%	0.01
	(35/1 mouse* , 190/1 mouse*)			
Control	25-39	33.0±2.32		
^{211}At irrad. 22 h	>300* *	300±0.0	97.1%	0.01
	(36/1 mouse*)			
Control	27-29	28.17±0.31		

* The tumor and ascites weren't seen in the autopsy * * The mice were sacrified after they survived for 300 d. The tumor and ascites weren't found in anatomy.

It was found that there were a marked degree of injury to tumor cells as evidenced by a swelling of cell in various degree and cellular necrosis. The injury of cellular structures resulted in the decreased activity of enzyme located in the mitochondria and cytosol but increase activity of enzyme located in the lysosome. The degree of functional and structural radiolesion was related to the various irradiation dose and sacrificed time post administration (cf. table 2).

Table 2

The changes of enzyme activity of Ehrlich ascites cells induced by ionic ^{211}At under the culture condition in vitro

Location in tumor cells	Enzyme	The changes of enzyme activity				Final result
		Control	Test (6 h)	Test (15 h)	Test (23 h)	
Mitochondria	MDH	++++	+++	+~++	0~+	↓
	ICDH	++++	++~+++	0~++	0~+	↓
	GDH	+++	++	+	+	↓
	SDH	-	-	-	-	-
	β-oHBDH	+++	++	+~++	0~+	↓
Lysosome	β-GA	±	+~++	++	+	-
	β-Gr	±~+	+	+	±	-
	ACP	±	±	+	±	-
Cytosol	α-GPD	+++	++	+~++	0~	↓
	LDH	++++	++	+	0	↓

3.2 The inhibition effect of various dose At—Te colloid on absorb of pyrimidine nucleosides in murine Ehrlich ascites tumor cells in vitro

The inhibition rate on absorb of thymidine and uridine nucleosides was related to the activity of ^{211}At-Te colloid administered and 50% inhibition rate was found at 1.48

$\times 10^5$Bq/ml culture (*cf.* Fig.1).

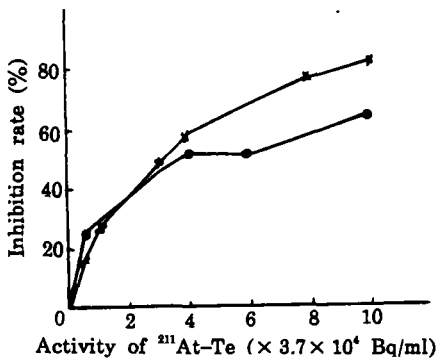

Fig.1 The effect of ^{211}At—Te colloid on absorb of pyrimidine nucleosides

· — · Thymidine absorb × — × Uridine absorb

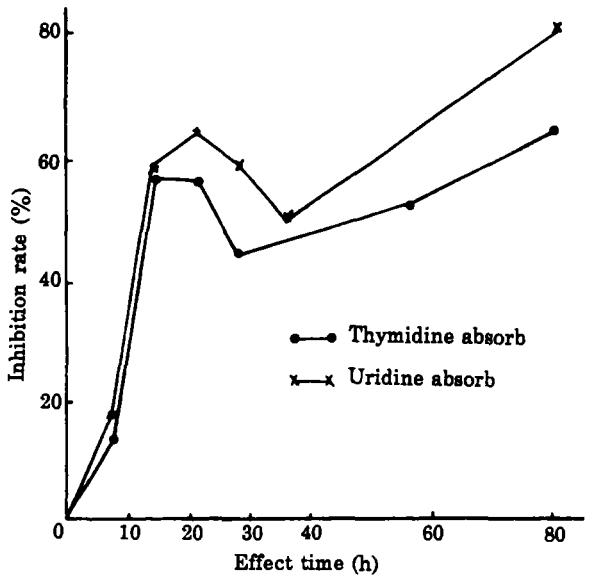

Fig.2 The time effect of inhibition action of ^{211}At—Te colloid on absorb of pyrimidine nucleosides

The suspension of murine Ehrlich ascites tumor cells was incubated with 1.48×10^5Bq/ml culture. At various time the inhibition effect of ^{211}At-Te colloid was observed on absorb of pyrimidine nucleosides. The inhibition rates for thymidine and uridine at 7 h were 18% and 14% respectively. At about 14 h two inhibition rates reached 50%, and the inhibition rates on absorb of these two pyrimidine nucleosides were kept at a higher level later on (*cf.* Fig. 2).

3.3 The reversibility of the inhibition of $Na^{211}At$ and ^{211}At—Te colloid on absorb of pyrimidine nucleosides in murine ehrlich ascites tumor cells

The culture with 1.85×10^1—3.7×10^5Bq/ml $Na^{211}At$ was added in the tumor cells suspension, after the cultures were incubated for 7 h ionic ^{211}At was removed. At same time the inhibition of absorb of pyrimidine nucleoside (thymidine) on the tumor cells was compared with that of the control (ionic ^{211}At wasn't cleaned). It was observed that the inhibition rates in two cases were very near (*cf.* Fig. 3). It was proved that the inhibition effect of $Na^{211}At$ is irreversible.

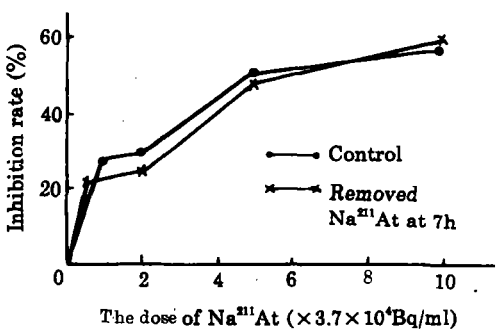

Fig.3 The effect of $Na^{211}At$ injection on absorb of thrimidine nucleoside

The inhibition of ^{211}At-Te colloid on tumor cells is primarily due to α radiation of

radionuclide ^{211}At, 80 hours latter (10 half-life later), although the majority of radio ^{211}At has decayed, it can be still observed that the inhibition rates of absorb of pyrimidine nucleoside stayed at a higher level (cf. Figure 2). This is another evidence of irreversibility of the inhibition of ^{211}At.

4 CONCLUSION

All results showed that Na^{211}At and ^{211}At-Te colloid injections prepared have injury and inhibition effect on tumor. Its inhibition is related to incorporation of pyrimidine nucleosides in tumor cells. The inhibition effect is irreversible between 7–80 hours. As described, radionuclide ^{211}At have violent injury effect on cancer cells in vitro, since the ^{211}At may accumulated in pathological locus (for example McAb) and give a comparatively few damage to surrounding healthy tissues, it will be demanded to be introduced into radiotherapeutical pharmaceuticals.

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