TUMOR UPTAKE AND THERAPEUTIC EFFECT OF ASTATINATED INTACT McAb 3H11 AND ITS Fab FRAGMENT FOR HUMAN GASTRIC CANCER XENOGRAFT IN NUDE MICE*

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ABSTRACT

The experiments demonstrate that with viewpoint of the pharmacokinetics and therapeutic effect the astatinated Fab fragment is well suited to 7.2 h half-life of ^{211}At, since tumor uptake remains high (~10%/g) from 3 to 14 h after i.v. injection of ^{211}At-3H11Fab, moreover, at the same dose the tumor inhibition with ^{211}At-3H11Fab were 84.4% by weight and 86.4% by volume higher than with ^{211}At-3H11 (67.1% and 67.8%, respectively) on 10 d post last i.p. administration.

Keywords ^{211}At, Monoclonal antibody, Human gastric cancer, Radioimmunotherapy

1 INTRODUCTION

From a rad iobiological perspective, nuclide ^{211}At emitting α-particle is the most suitable radioisotope for certain radiotherapeutic application[1,2]. McAb used as carrier for radioactive isotopes would be expected to accumulate in tumor tissues giving a selectively localized source of radiation. However, the carrier for short half-life nuclide ($T_{1/2}$=7.2 h)of ^{211}At should be characterized not only the possibly highest affinity to tumor tissue but also the rapid pharmacokinetics.

Generally, to accumulate intact McAb in tumor more than 24 h was needed; unfortunately, at which time ^{211}At had decayed to 10% of initial levels. In this paper astatinated intact 3H11 and its Fab fragment was comparison-evaluated via biodistribution and targeted radiotherapy in nude mice bearing subcutaneous xenograft (Xs).

2 EXPERIMENTAL

2.1 Materials

^{211}At was prepared by ^{209}Bi (27 MeV α,2n)^{211}At at 1.2 M cyclotron of Sichuan Union University[3]. ^{211}At-3H11 and ^{211}At-3H11 Fab were labeled by a modified method

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of acylation with *p*-astatobenzoic acid[4]. With initial ^{211}At activity levels of 111~185 MBq, McAb have been obtained at specific activities of 29.6~59.2 kBq/μg (McAb), radioactive concentration of 2.96~5.92 MBq/ml.

HGC (human gastric cancer) line M85, antigastric cancer monoclonal antibody 3H11 and its Fab fragment were all provided by Beijing Institute for Cancer Research, the former is cultured from generation to generation in our Laboratory to obtain M85 homogenate. Nude mice were obtained from Sichuan Industrial Institute of Antibiotics attached to The State Pharmaceutical Administration.

2.2 Nude mice tumor model

Subcutaneous tumor xenograft from HGC was implanted onto 4~5 week nude mice weighing 18~24 g by injecting 2×10^6 M85 homogenate in the right flank of each recipient animal. Biodistribution and/or therapeutic effect was studied when average tumor xenograft diameter was about 0.5~1.0 cm.

2.3 Biodistribution

Groups of 3~4 mice of each time point were given i.v. injection in the tail vein with 2.96×10^5 Bq (8μCi) of ^{211}At-3H11, ^{211}At-3H11 Fab, or Na ^{211}At,respectively, and were sacrificed by breaking off vertebral cervices at 3, 7, 14 and 24 h or approximating the times, at which the administered ^{211}At activity had decayed to 75%, 50%, 25% and 10% of initial levels, and the tissues of interest were removed, washed with saline, weighed, and assayed for ^{211}At. Counting data were corrected for physical decay of ^{211}At. Tissue biodistribution data were expressed as percent injected dose of ^{211}At per gram of tissue, %/g; and tumor/normal tissue ratio, T/NT.

2.4 Therapeutic effect

Of nude mice bearing tumor, 40 were divided into 5 groups at random with respect to weight of mice as well as size of Xs, and the therapeutic effect were evaluated on ^{211}At-3H11 (group I received 22.2 kBq/g, group III 14.8 kBq/g), and ^{211}At-3H11 Fab (group II received 22.2 kBq/g and group IV 14.8 kBq/g) and group V as a PBS control by i.p.injection, respectively, once every 5 d for 3 times in all. The mice bearing tumor were sacrificed on 5 and 10 d post last injection. To evaluate tumor inhibition in nude mice tumor volume and weight were determined by direct measurement as well as by pathological calculation[5] according to formula: Tumor inhibition% = [Tumor(PBS)-Tumor(T.G)]/Tumor(PBS)×100%

3 RESULTS AND DISCUSSION

3.1 Biodistribution

3.1.1 Uptake of ^{211}At in tumor

Post injection of ^{211}At-3H11 Fab, ^{211}At was more rapidly accumulated in xenograft, the uptake of ^{211}At in tumor reached from 9.48±0.65%/g at 3 h to 8.42±2.38 %/g at 14 h, and decreased to zero until 24 h, while the uptake of ^{211}At-3H11 was lower and maintained basically constant over 24 h, ranging from 4.48±1.65%/g at 3 h to 4.00±1.94 %/g at 24 h. The uptake decreased rapidly with time-course following injection of Na^{211}At,

and was lower than ^{211}At-3H11. It may be suggested that Fab fragment having smaller molecule could result in rapider pharmacokinetics than that of intact 3H11 having larger molecule. Generally, to accumulate in tumor xenograft for intact McAb>24 h was needed, unfortunately, at which time ^{211}At had decayed to 10% of initial levels, but in case of Fab fragment it was 2~ 3 h.

3.1.2 T/NT ratio

It can be seen from Fig.1 that higher tumor/normal tissue ratio could be obtained with ^{211}At-3H11 Fab at all time points, at 14 h T/NT ratios were 5.30±0.53, 4.74±0.47, 3.36±1.15, 3.10±1.30, 1.04±0.4 for blood, liver, bone, intestines, spleen, respectively, T/NT ratios for lung and stomach were less than 1. Taken together, it is very important to point out that the tumor cells would loss reproductive capacity exposed to ^{211}At of 7.4 kBq/ml (0.2μCi/ml) over 14~15 h in our previous experiment[6]; according to theoretical calculation of Bateman W J *et al*[7], when ^{211}At-McAb of 3 Sv was injected in vivo, if T/NT>3, 10^4 tumor cells exposed to ^{211}At, only 1.9 tumor cells could be survived; if T/NT>6, 10^8 tumor cells exposed to ^{211}At, only 3.5 tumor cells could be survived.

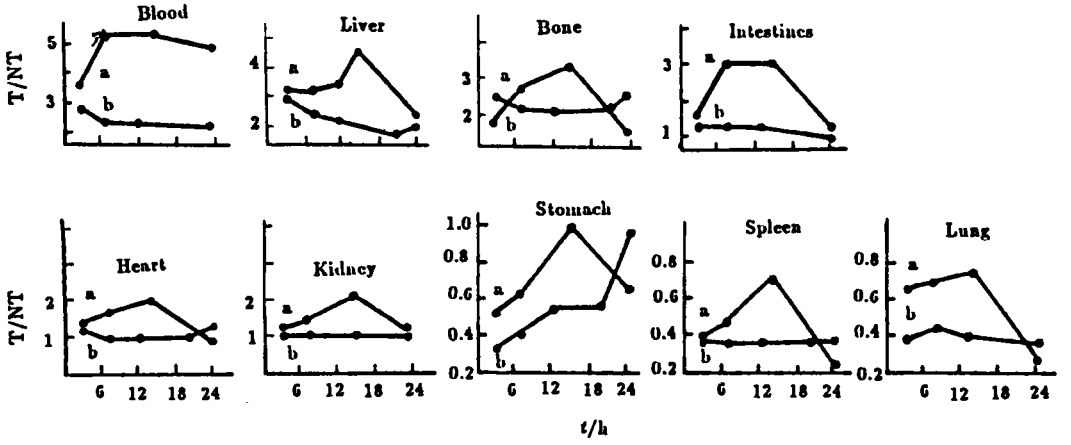

Fig.1 T/NT ratio in nude mice bearing HGC Xs

a. ^{211}At-3H11 Fab; b.^{211}At-3H11

3.2 Therapeutic effect

Table 1 shows that the volume and weight of Xs were much smaller and lighter compared with PBS control; differences in tumor volume and weight were statistically significant (P <0.01, V:I, II, IV) on 5 and 10 d post last injection; therapeutic effect presented in all tested groups due to the immune response between 3H11 and/or its Fab fragment and M85 cells. In brief, the induction of radiation response was related to the dosage, no matter with either ^{211}At3H11 or ^{211}At-3H11 Fab, but ^{211}At-3H11 Fab is obviously preferable to ^{211}At-3H11.

Table 1

Tumor inhibition in nude mice bearing M85 HGC Xs treated by ^{211}At-3H11, ^{211}At-3H11 Fab compared with PBS control

	5 d					10 d				
Group	Direct measurement		Pathological calculation			Direct measurement		Pathological calculation		
	TV /mm^3	IT /%	NA /%	NV /mm^3	IT /%	TV /mm^3	IT /%	NA /%	NV /mm^3	IT /%
I	613.6	73.1	82.1	109.8	87.5	767.4	58.5	76.2	182.6	67.8
II	632.4	72.3	81.4	117.6	86.6	511.5	72.3	84.9	77.2	86.4
III	975.4	57.3	88.8	109.2	87.5	960.3	48.1	77.5	216.1	61.9
IV	738.2	67.7	85.1	110.0	87.5	540.9	70.7	76.2	128.7	77.3
V	2282.8	–	61.6	876.6	–	1849	–	69.3	567.6	–
Group	TW /mg	IT /%	NA /%	NW /mg	IT /%	TW /mg	IT /%	NA /%	NW /mg	IT/%
I	517.5	74.5	82.1	92.6	88.1	660.6	57.6	76.2	157.1	67.1
II	570.0	72.0	81.4	106.0	86.4	492.5	68.4	84.9	74.4	84.4
III	835.0	59.0	88.8	93.5	88.0	875.0	43.8	77.5	196.9	58.8
IV	592.5	70.9	85.1	88.3	88.7	466.5	70.0	76.2	111.0	76.8
V	2035	—	61.6	781.4	—	1557.5	—	69.3	478.2	—

Notes: TV-Tumor volume, TW-Tumor weight, IT-Inhibition, NA-Necrotic area, NW-Necrotic weight, NV-Necrotic volume

4 SUMMARY

^{211}At; as an α-particle emitter characterized by a high LET (98.84,keV/μm) optimal for an endoradiotherapy, a short half-life of 7.2 h and a mean range of 60$\sim$65μm in a tissue, once rapid and selective incorporation with cancer cells due to the properties of carrier (McAb), will deposit most of its energy in cancer cells, and therefore, should significantly increase therapeutic effect without damaging normal tissues surrounding the tumor. So that, from a radiobiological perspective as well as from a pharmacokinetical one, ^{211}At, as a labels of monoclonal antibody fragment, may be a practically attractive for some type of radioimmunotherapy, such as the treatment of micrometastatic disease and scattered cancer cells, or combined with other therapeutic means.

REFERENCES

1 Brown I, Carpenter R N. Radiochimica Acta, 1989; 47:143
2 Vaidyanathan Ganesan, Affleck Donna, Zalutsky Michael R. Nucl Med Biol, 1994;21(1);105
3 Zhou Maolun, Jin Jiannan, Zhang Shuyuan *et al.* Sichuan Univ: Nat Sci Ed, 1986; 23(3):84
4 Liu Ning, Zhang Shuyuan, Jin Jiannan *et al.* J Nucl Radiochem (in Chinese), 1992; 14(3):159
5 Luo Deyuan, Zhou Maolun, Zhang Shuyuan *et al.* Chinese J Oncology, 1993; 15(1):23
6 Wang Xizhong, Zhou Maolun, Zhang Shuyuan *et al.* Sichuan Univ: Natural Science Edition, 1989; 26(2):240
7 Spiegelberg H L, Weigle W O. J Exp Med, 1965; 121:323

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