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ASTATINE-211 LABELING OF A MONOCLONAL ANTIBODY AND ITS Fab FRAGMENT: SYNTHESIS, IMMUNOREACTIVITY AND EXPERIMENTAL THERAPY

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ABSTRACT

An antigastric cancer monoclonal antibody, 3H11 and its Fab fragment, were labelled with a-emitter ^{211}At using p-(^{211}At)-astatobenzoic acid (pAtBA) intermediate, in the yields of more than 30%. The astatinated antibodies were stable in vitro, and had specific immunoreactivity to human gastric cancer cell M85. The therapeutic effect of the astatinated antibodies for subcutaneous xenografts of human gastric cancer were investigated in nude mice by i. p. injection, once every 5 days for 3 times sucessively, with 1. 48 $\times 10^4$ and 2. 22$\times 10^4$Bq/g of ^{211}At-3H11 and ^{211}At-3H11 Fab per injection, respectively. It was showed that the volume and weight of xenografts in all tested groups were much smaller and lighter than that of control group(PBS) from 12. 5 days post first injection. The most evident inhibition was observed in the groups with ^{211}At-3H11 Fab, with tumor inhibility of 67. 5-69. 5% at 15 days and 70. 7-72. 3% at 20 days, respectively.

Keywords: ^{211}At, Gastric cancer, McAb, Fab fragment, Immunoreactivity, Therapy.

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INTRODUCTION

The cyclotron-produced ^{211}At has a half-life of 7.21h and decays by the emission of 6.8Mev mean energy α-particle along two branches. The ranges of α-particle are 55-80μm (about 6-8 cells diameter) on unit density tissue and its mean dose-average LET is 98.84kevμm^{-1}. The latter is very closed to the optium for endoradiotherapeutic effects (approximately 100kevμm^{-1})[1], From a radiobiological viewpoint, ^{211}At is the most attractive nuclide for radiotherapeutic purposes.

^{211}At labelled monoclonal antibodies have been shown to have relatively specific cytotoxic effect to tumor cells in vivo [2] and therapeutic effect to human cancer xenografts in nude mice[3,4]. But intact antibody have a large molecular weight and moved slowly in vivo, and most intact antibodies require 48-72h for maximum tumor uptake[5], whereas the physical half-life of ^{211}At is only 7.21h. In order to localize ^{211}At in tumor tissue rapidly and deposit its most energy in tumor cells, ^{211}At have been used to label Fab or F(ab')$_2$ fragment of antibodies in recent reports[6-8]. In this paper, an antigastric cancer monoclonal antibody 3H11 and its Fab fragment are labelled with a-emitter ^{211}At using p-(^{211}At)-astato-benzoic acid intermediate. Additionally, their immunoreactivity to human gastric cancer cell M85 in vitro , and therapeutic effect to subcutaneous xenograft of human gastric cancer in nude mice are investigated.

MATERIALS AND METHODS

Materials

Antigastric cancer monoclonal antibody 3H11 and its Fab fragment were provided by Beijing Institute for Cancer Research; A gastric cancer strain cell M85(Beijing Institute for Cancer Research, Beijing) and Hela cell(West China University of Medical Sciences. Chengdu) were obtained as in 10%-FCS-RPMI1640 solution.

Nude mice were obtained from Sichuan Industrial Institute of Antibiotics(Chengdu). Subcutaneous tumor xenografts from human gastric cancer were implanted in 4-5 week-end nude mice, weighing from 18 to 24g, by injecting 2×10^6 M85 homogenate in the right flank of each recipient animal. Therapeutic experiments were initiated when tumor xenograhts grew to about 0.5-1.0 cm diameter.

^{211}At was produced by irradiating a Bi-target with 27Mev a-particle via ^{209}Bi(a,2n)^{211}At nuclear reaction in the 1.2M cyclotron of Sichuan University[9]. After irradiation for 3-5h at an α-particle beam current of 10-16μA, ^{211}At was distilled to a silica gel column from the Bi-target at 760℃ in flow of O_2 and N_2, then eluted with 0.1mol/L NaOH. Spectra analysis indicated that the produced ^{211}At is radiopure with a ^{210}Po/^{211}At rate of less than 10^{-8}, and it is suitable for biological or medical use.

^{211}At Labeling of antibodies

The generation of diazonium salt of para-aminobenzoic acid (pABA) has been described[10]. pAtBA was prepared by adding 37-111MBq of Na ^{211}At to 2.5×10^{-8}mol of the freshly diazonium salt in 50μl of 4mol/L HCl. After a 30min reaction at room temperature, the mixture was incubated at 55-56°c for 8-10min to destory N_2. The pAtBA was then extracted into ether, the ether was pooled and evaporated to dryness at 38-40°c in a water-bath. The dryed pAtBA was dissolved in 5μl of tetrahydrofuran (THF), and then 50nmol of tri-n-butylmain (TNB) in 5μl of THF followed by 150nmol of isobutylchlorolformate(IBC) in 5μl of THF were added. After a 20-25min reaction in a ice-bath under stirring, 0.5-1.0mg of McAb 3H11 or 3H11 Fab were added and the mixtures were incubated at 0-10℃ for 40-50min. The astatinated antibodies were isolated by Sephadex G50 column using elution with 0.1mol/L PBS (pH=7.0) and analysed by HPLC on a hydrophobic interaction column (Sephe-agel TSK pheyel 5PW, Φ7.5× 75mm, Beckman) using gradient elution with buffer A: 1.0mol/L $(NH_4)_2SO_4$ in 0.1mol/L PBS and buffer B: 0.1mol/L PBS(pH=7.0).

In vitro stability experiments

The stability of astatinated antibodies were examined by subjecting them to solution of PBS and other reagents for several hours. In the experiment test tubes were charged with 1.0ml of (a)PBS(pH=7.0), (b)1.0 mol/L glycine in PBS and (c) 0.05Mol/L borate buffer (pH=9.0). To each test tube was added 0.5ml of PBS elution contatining ^{211}At-3H11 (2.0×10^6 Bq, 50μg) or ^{211}At-3H11 Fab (2.2×10^6Bq, 51μg). The tubes were then incubated at 37℃ for more than 20h. Aliquots were removed periodically and examined by Sephadex G50 columm as described above.

Immunoreactivity assessment experiments

1.11×10^5 Bq of ^{211}At-3H11(3.3μg), ^{211}At-3H11 Fab (3.2μg) and Na ^{211}At were added the test tubes containing 10^5 cells of M85 or Hela, respectively. After incubated at

37℃ for 1h, the mixtures were washed with 3% FCS-RPMI and centrifuged. The precipitates were counted and the binding rates of ^{211}At were calculated.

Therapeutic experiments

40 of nude mice bearing tumor model were divided into 5 groups at random with to weight of mice as well as size of xenograft, and the therapeutic experiment were carried on with ^{211}At-3H11 (groups I received 2.22×10^{4}Bq/g, group III received 1.48×10^{4}Bq/g, and ^{211}At-3H11 Fab (group II received 2.22×10^{4}Bq /g and group IV received 1.48×10^{4}Bq/g) and PBS as a control group(V) by i.p. injection, respectively, once every 5 days for 3 times successively. All the animal were sacrificed for autopsy and histopathological obsvervation at 5 days and 10 days post the last injection. The tumor volume in nude mice and weight of xenografts after the animal sacrifriced were measured by " double blind method". Tumor inhibility of the astatinated antibodies were determined by the following formula:

$$\text{Tumor inhibillity}=\frac{\text{Tumor volume or weight (PBS)}-\text{Tumor volume or weight (Test group)}}{\text{Tumor volume or weight (PBS)}}\times100\%$$

RESULTS AND DISCUSSION

Labeling antibodies with ^{211}At

pAtBA was easily prepared by adding Na ^{211}At to diazonium salt of pABA as reported previously[10], in this study, the yield of pAtBA was 80-85%. By adding antibodies to the mixed anhydride of pAtBA and reaction for 40-50min, 55-60% and 60-65% of the pAtBA were coupled to McAb 3H11 and 3H11 Fab, respectively. The whole procedure was executed in 3-4h and at least 30% of the added ^{211}At were found in the products. With 3.7-11.1$\times10^{7}$ Bq of Na ^{211}At and 0.5-1.0mg of antibodies, the specific activities of ^{211}At-3H11 and ^{211}At-3H11 Fab were 3.7-11.1$\times10^{4}$ and 4.5-13.0$\times10^{4}$ Bq/μgMcAb, respectively.

HPLC analysis of ^{211}At labelled 3H11 McAb befor and after separation by Sephadex G50 column were described in Fig. 1(a-b). By means of hydrophobic interaction column and gradient eluent, it was showed that UV-peak of 3H11 McAb corresponds to radio-peak of ^{211}At-3H11 with a retention time of 13.00±0.05min and separates with other radioimpurities(Fig. 1a), the isolated ^{211}At-3H11 has only a UV-peak and a corresponding radio-peak (Fig. 1b). HPLC elution of ^{211}At labelled 3H11 Fab before and after separation isolation by G50 column seemed that of ^{211}At labelled 3H11 with a retention time of

12.90±0.05min for ^{211}At-3H11. This results also demonstrated that the isolation of astatinated antibodies by Sephadex G50 column is effective.

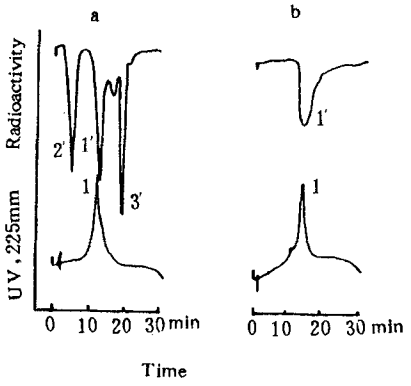

Fig. 1 HPLC elution profile of ^{211}At labelled 3H11 McAb (Gradient elution as: 0-2min, 100% buffer A; 2-7min, 100-0% A; 7-27min, 100% buffer B; 27-29min, 100-0% B. Flow rate: 1ml/min)

a. Before separation by Sephadex G50 column

b. After separation by Sephadex G50 column

1. 3H11 McAb 1'. ^{211}At-3H11 2'. ^{211}At$^-$ 3'. pAtBA

Early attempts to label proteins with ^{211}At were performed by direct methods like radioiodination of proteins[11-12]. Unfortunately, proteins labelled with ^{211}At by these approches are deastatinated rapidly in vitro and in vivo[13]. In order to increase the in vivo stabilities of ^{211}At labeled proteins, Friedman et al[14]. put forward a method for astatinating protein via p-(^{211}At)-astato-benzoic acid intermediate. Ever after the method was modified by Harrison et al[10]. The procedure described here differs from that previoisly published[10,14] in two respects. Firstly, in order to decrease the side products such as para-hydrobenzoic acid (pOHBA) which could compete for antibodies, resulting in decreasing yields of astatinated antibodies, or phenols which could compete for the unreacted ^{211}At, resulting in an astatinated product that would be less stable than p-astatobenzoic conjugates, the added amount of diazinuim salt of pABA for preparation of pAtBA was minimized to 2.5×10^{-8}mol. Hence the pAtBA may not be isolated by HPLC befor conjugated with antibodies as described by Harrison A et al.[10], and same results were obtained. Additionaly, hydrophobic interaction HPLC was used to analyse and identify the astatinated antibodies.

In vitro stability

The in vitro stabilities of ^{211}At-3H11 and ^{211}At-3H11 Fab were found to be high. The deastatination of less than 2% were noted when they were incubated with 0. 1Mol/L PBS or 1. 0Mol/L glycine for 24h, and deastatinations of 3% and 6% for ^{211}At-3H11 and ^{211}At-3H11 Fab respectively were noted in basic 0. 05Mol/L borate buffer(pH=9. 0) for 24h, respectively.

Evalution of immunoreactivity

The binding rates of ^{211}At-3H11, ^{211}At-3H11 Fab and Na ^{211}At to M85 cell or Hela cell were given in table 1. It is showed that the binding rates of ^{211}At-3H11 and ^{211}At-3H11 Fab to human gastric cancer M85 are 6. 70 and 5. 98 times than that of Na ^{211}At, respectively. Whereas their binding rates to Hela cell are not obviously different from that of Na ^{211}At. This results demonstrated that the astatinated antibodies have specific immunoreactivities to human gastric cancer. In our recent work[15], the immunoactivity of ^{211}At-3H11 was certificed by the means of ELISA method, this result implied that ^{211}At-3H11 Fab also preserves the immunoactivity of unlabelled 3H11 Fab.

Table 1 Comparison of the binding rate with cells on ^{211}At-3H11, ^{211}At-3H11 Fab and Na ^{211}At.

Drug	Activity		Binding rate of ^{211}At(%)		Binding ratio	
	x10^{4}Bq	μg	M85	Hela	M85/Hela	/Na ^{211}At
^{211}At-3H11	11. 1	3. 3	19. 5	2. 99	6. 52	6. 70
^{211}At-3H11 Fab	11. 1	3. 2	17. 4	3. 15	5. 52	5. 98
Na ^{211}At	11. 1		2. 91	3. 02	0. 96	1

Therapeutic effect

The change of tumor volume in nude mice bearing tumor model treated by ^{211}At-3H11, ^{211}At-3H11 Fab and PBS were presented in table 2, and the tumor weight of xenografts in the 15 days and 20 days post the first injection were descrbed in Fig. 2. It was showed that the volume and weight of xenografts in all tested groups were much smaller and lighter than that of PBS control group. At 15 days post first injection, the tumor volume of PBS control group was 1. 85±0. 97cm^3, whereas the volumes of group I — IV were 0. 65±0. 10, 0. 56±0. 18, 0. 88±0. 28, 0. 58±0. 33cm^3, respectively. At the same time point, the tumor weights of group I — V were 0. 52±0. 06, 0. 57±0. 05, 0. 84±0. 17, 0. 59±0. 25 and 2. 04±1. 28g, respectively. There were statistically significant differences in tumor volume and weight of group I, II and IV compared

with PBS control group($P<0.01$) at 15-20 days post first injection. As summarized in table 3, the most evidnt inhibition was observed in the groups with ^{211}At-3H11 Fab with tumor inhibility of 67.5-70.7% with 1.48×10^{4}Bq/g and 69.5-72.3% with 2.22×10^{4}Bq/g (analysed with tumor volume), or 70.9-69,3% and 72.0-68.4% (analysed

Table 2 Tumor volume in nude mice bearing tumor model treated with ^{211}At-3H11 ,^{211}At-3H11 Fab and PBS($n=8,\bar{X}\pm SD$)

Group	Tumor Volume(cm^3)					
	0	5d	10d	12.5d	15d	20d*
I	0.081±0.018	0.16±0.049	0.41±0.048	0.60±0.11	0.65±0.10	0.77±0.11
II	0.10±0.026	0.23±0.057	0.48±0.11	0.54±0.13	0.56±0.18	0.51±0.21
III	0.083±0.033	0.23±0.095	0.60±0.23	0.78±0.21	0.88±0.28	0.96±0.25
IV	0.088±0.034	0.15±0.063	0.37±0.22	0.51±0.33	0.60±0.33	0.54±0.35
V	0.092±0.022	0.24±0.060	0.70±0.28	1.19±0.56	1.85±0.97	1.85±0.39

* $n=4,\bar{X}\pm SD$

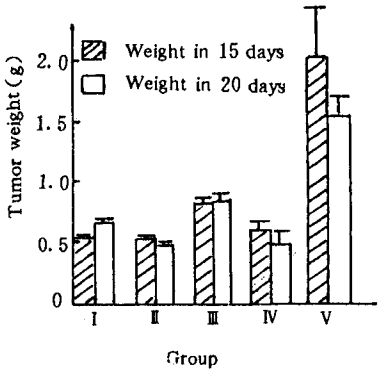

Fig. 2 Tumor weight of xenografts in nude mice bearing tumor model at 15 and 20 days post first injection($n=4,\bar{X}\pm SD$)

Table 3 The tumor inhibility in nude mice bearing M85 cells treated with ^{211}At-3H11 and ^{211}At-3H11 Fab(analysed with tumor volume).

Group	Tumor inhibility(%)						
	5d	10d	12.5d	15d	20d	15d*	20d*
I	30.5	41.4	49.2	64.6	58.5	74.5	57.6
II	1.4	31.8	54.6	69.5	72.3	72.0	68.4
III	1.4	14.5	34.6	52.4	48.1	59.0	43.8
IV	35.2	47.0	56.8	67.5	70.7	70.9	69.3

* analysed with tumor weight

with tumor weight) at 15 days and 20 days post first injection, respectively. It was demonstrated that there is a relatively specific guided therapeutic effect in tested groups. Eventhough the immunoreactivity of ^{211}At-3H11 to M85 cell was some stronger than that of ^{211}At-3H11 Fab in vitro, but the therapeutic effect of ^{211}At-3H11 Fab for subcutaneous xenografts in nude mice was preferable to ^{211}At-3H11, this was possibilly related to quicher accumulation of Fab fragment in tumor tissue in vivo[15].

CONCLUSION

It is well known that a-emitter ^{211}At is suitable as a possible therapeutic radionuclide. This paper described a modified procedure for astatination of antibodies using pAt-BA intermediate. The astatinated antibodies are stable in vitro, and have obvious immunoreactivities to human gastric cancer cell M85. The tumor inhibition of ^{211}At-3H11 Fab were more evident than that of ^{211}At-3H11, it is expected that ^{211}At is used to label monoclonal antibodies, especially label fragment, may bring a hoper outlook for tumor treatment, if combined with other therapeutic means, but there were many investigation should be explored.

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