A study on indirect radiolabeling of IgG with carrier free ^{188}Re

ZHANG Xiu-Li, WANG Yong-Xian, LI Jun-Ling, YIN Duan-Zhi

(Shanghai Institute of Nuclear Research, the Chinese Academy of Sciences, Shanghai 201800)

Abstract ^{188}Re labeled monoclonal antibodies are potential candidates for use in radioimmunotherapy. S-Bz-MAG$_3$ as a bifunctional chelating agent was used for labeling of IgG with carrier free ^{188}Re by pre-radiolabeling of the chelating approach. The conjugation conditions were optimized. The stability of ^{188}Re-MAG$_3$-IgG *in vitro* was high. The results may be useful to the studies of ^{188}Re labeled MAbs for radioimmunotherapy.

Keywords Carrier free ^{188}Re, IgG, S-Bz-MAG$_3$, Radioimmunotherapy, Bifunctional chelating agent, Indirect radiolabeling

CLC numbers O615.4

1 Introduction

With the advent of monoclonal antibodies (MAbs), there has been a renewed interest in their use for targeted delivery. MAbs that carry particle-emitting radioisotopes offer a powerful approach to cancer therapy in view of their exquisite specificity and targeting capability. They are the potential candidates for the use in radioimmunotherapy (RIT).[1]

A number of β emitting radionuclides have been recommended as being useful to radioimmunotherapy. ^{131}I (β, E_{max} = 0.6 MeV) is a commonly used radionuclide for clinical RIT. However, the low β-energy, de-iodination in vivo of the labeled protein and easy release from target tissue limited its use. Another nuclide, ^{90}Y (β, E_{max} = 2.2 MeV), was often accumulated in liver, spleen, and bone marrow if it was released from the labeled compounds in vivo. This has stimulated the search for more effective radioisotopes for RIT.[2]

^{188}Re (β, E_{max} = 2.12 MeV; 155keV γ–photons, 15%) is a very attractive radioisotope for RIT. Rhenium chemistry is dominated by redox reactions, and perrhenate, like pertechnetate, is a negatively charged anion that is rapidly excreted from living systems, preventing the ultimate metabolite from accumulating in non-target tissues.[3] However, the greatest importance is the availability of carrier-free ^{188}Re-perrhenate by saline elution of a ^{188}W/^{188}Re generator system.[4] The ^{188}Re isotope, with its 16.9 h half-life, is very promising for RIT. The ^{188}W has a half-life of 69 days, which means that such a generator would have an extended lifetime for more than half a year. The high-energy β-particles emitted by ^{188}Re has a longer mean path length (about 2.2 mm), which results in a more homogeneous distribution of the radiation dose.

There are two approaches to the ^{188}Re-labeling of MAbs—direct labeling and indirect labeling. The direct labeling is a convenient and efficient method, but the labeled MAbs are shown to be unstable both in vivo and in vitro.[5,6] The indirect labeling, on the other hand, involves formation of ^{188}Re complex with a bifunctional chelating agent and conjugation of the ^{188}Re-BFCA complex to MAbs in a separate step on the trace level.[6]

S-bezonyl-mercaptoaceyltriglycine (S-Bz-MAG$_3$) has been extensively used in nuclear medicine both as ^{99m}Tc radiopharmaceutical for kidney imaging and as a prosthetic group for labeling antibodies and biomolecules with ^{99m}Tc, ^{186}Re and ^{188}Re.[7-11] MAG$_3$ and related derivatives are attractive ligands for coupling biomolecules[12] in that they provide very stable complexes. The metabolites arising from enzymatic cleavage would be expected to be cleared through the kidney. Furthermore, Tc/Re-MAG$_3$-labeled biomolecules could be used as a matched pair system in radiodiagnostic and radiotherapeutic applications.

Supported partially by Key Project of Knowledge Innovation Program of the Chinese Academy of Sciences (KJCX1-SW-08)
Received date: 2002-04-16

The use of ^{188}Re labeled antibodies and biomolecules for targeted radiotherapy is, in principle, an attractive concept. We have investigated the feasibility of using S-Bz-MAG_3 as a BFCA for indirect labeling IgG with carrier free ^{188}Re. In our studies, S-Bz-MAG_3 is labeled with ^{188}Re at first, then, the radiolabeled MAG_3 is chemically activated to obtain ^{188}Re-MAG_3-TFP, and the activated ester is coupled to IgG at last (Fig.1).

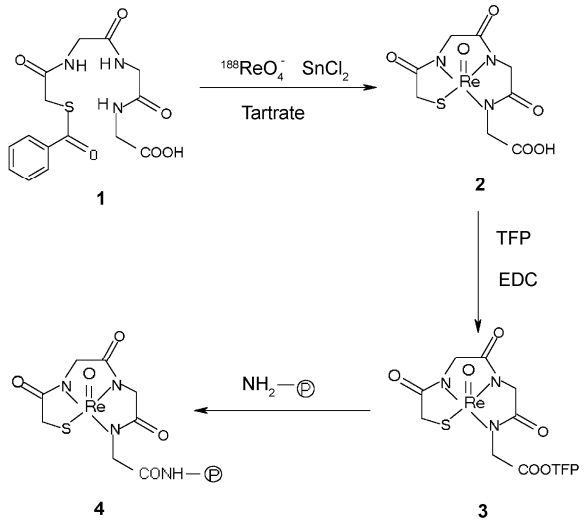

Fig.1 The route of ^{188}Re labeling IgG.

2 Materials and methods

2.1 Materials

^{188}Re was obtained from an alumina-based ^{188}W/^{188}Re generator (Shanghai Ke Xing Pharmaceutical Co.), loaded with the ^{188}W solution supplied by the Oak Ridge National Laboratory (Oak Ridge, TN). Carrier-free ^{188}Re was eluted from the generator with 0.9% NaCl. The nuclear purity of ^{188}Re was more than 99% analyzed by γ-spectroscopy with a high purity germanium (HPGe) detector (GEM-15190, EG & G Ortec, Oak Ridge, TN USA) and the radiochemical purity of $Na^{188}ReO_4$ was more than 95% by paper chromatography developed with 0.9% NaCl.[4] A solid scintillation counter with NaI (Tl) crystal was used for radioactivity measurements. Human IgG-reagent grade (SIGMA); 2,3,5,6-tetrafluorophenol (TFP), 1-ethyl-3-(3-dimethylaminopropyl)-carbidiimide (EDC) (TCI); Sephadex G25 (Pharmacia); Sep-Pak C18 cartridges (Waters). All other chemicals were of guaranteed grade.

2.2 Methods

2.2.1 Synthesis of ^{188}Re-MAG_3 (**2**)

4 mg $SnCl_2 \cdot 2H_2O$, 4 mg ascorbic acid, 1 mg S-Bz-MAG_3 solution, and 0.5mL 37~370 MBq/mL ^{188}Re solution were added in turn to 0.5 mL of 0.1 mol $\cdot L^{-1}$ tartrate buffer solution (pH = 5). The reaction mixture was allowed to react in boiling water for 1 hour. The labeling yield and radiochemical purity of ^{188}Re-MAG_3 were determined by two-strip Xinhua No.1 paper chromatography. The PC strip was developed with tetrahydrofuran: chloroform: acetone = 2:1:1 for the detection of ^{188}Re-MAG_3 (R_f = 0) and hydrolyzed rhenium ($^{188}ReO_2$) (R_f = 0). The other PC with normal saline was for detecting hydrolyzed rhenium($^{188}ReO_2$) (R_f = 0). 1.0 mL ^{188}Re-MAG_3 solution was diluted with 3 mL 1% acetic acid-water, passing through a Sep-Pak C18 column (Waters), which was activated with 10 mL alcohol and 10 mL 1% acetic acid-water in turn, then rinsed with 10 mL 1% acetic acid-water. Remaining water residues were purged with nitrogen, and ^{188}Re-MAG_3 eluted with 2.5 mL 90% acetonitrile. The solution was evaporated under a stream of nitrogen at 100°C.

2.2.2 Synthesis of ^{188}Re-MAG_3-TFP (**3**)

The residue of **2** was dissolved in 500 μL water. 200 μL of 100 g $\cdot L^{-1}$ 2,3,5,6-tetrafluorophenol in MeCN/H_2O (9:1) and 50 mg EDC were added. The pH was adjusted to 6 with 0.5 mol $\cdot L^{-1}$ H_2SO_4. The reaction mixture was incubated at room temperature and protected from light for 1 h. The ^{188}Re-MAG_3-TFP ester was diluted with water to a volume of 4 mL and purified on two activated Sep-Pak C18 cartridges. Washed with 10 mL water, 10 mL 20% (*V*/*V*) EtOH/0.01 mol $\cdot L^{-1}$ sodium phosphate (pH = 7.5), 5 mL water and 2 mL diethylether in turn, the active ester was eluted with 2 mL acetonitrile. The purified ester was dried at room temperature by a stream of nitrogen.

2.2.3 Preparation of ^{188}Re-MAG_3-IgG conjugates (**4**)

The purified ester was dissolved in 500 μL 0.9% NaCl, and 0.5 mg IgG (1.0 mg $\cdot mL^{-1}$) was added. The reaction mixture was allowed to react at room temper-

ature for sometime to obtain ^{188}Re-MAG$_3$-IgG. The radiochemical yield of the ^{188}Re-MAG$_3$-IgG was determined on Xinhua No.1 paper using 0.9% NaCl as developing agent. ^{188}Re-labeled IgG remains at the origin, while sodium perrhenate migrates to the top of the strip.[5] The labeled IgG was purified by Sephadex G25, which is packed into 0.5 cm×20 cm, and equilibrated with 0.05 mol • L^{-1} PBS buffer (pH = 7.5). The reaction volume was loaded onto the column and the eluate was collected in plastic tubes for monitoring with a NaI (Tl) scintillation counter.

2.2.4 In vitro stability

0.5 mL radiolabeled IgG, purified by Sephadex G25, was added to 1.0 mL 0.9% NaCl and 1.0 mL 0.05 mol • L^{-1} EDTA, respectively. The solutions were incubated at 37°C for 20 h, sampled at various time points for the determination of ^{188}Re-MAG$_3$-IgG by paper chromatography.

3 Results and discussion

In the synthesis outlined in Fig.1, S-Bz-MAG$_3$ was labeled with ^{188}Re in the presence of tartrate, citrate and gluconate as transfer ligands at pH = 5, respectively. The labeling yield of ^{188}Re-MAG$_3$ was more than 98%. The labeling yield of ^{188}Re-MAG$_3$ appeared highly dependent on the reaction time and the concentration of $SnCl_2$. The influence of pH on the labeling yield is negligible. So almost quantitative yield of ^{188}Re-MAG$_3$ was obtained similar to the method.[12] ^{188}Re-MAG$_3$ is a stable compound in aqueous solution. As a result, 95 ± 6% pure ^{188}Re-MAG$_3$ was obtained after the Sep-Pak C18 column purification step.

^{188}Re-MAG$_3$ could be converted to the corresponding radiolabeled ^{188}Re-MAG$_3$-TFP, using EDC as condensing agent. According to the method,[8] the radiolabeled active ester was separated from the excess of activation agent by using two Sep-Pak C18 cartridges. Impurities were eluted with water, ethanol, etc. To elute the radiolabeled active ester, 2 mL acetonitrile was sufficient. Radiochemical yield of the prepared ^{188}Re-MAG$_3$-TFP was 70~80%.

The radiolabeled active ester could be conjugated with IgG in aqueous solution. The radiochemical yield of ^{188}Re-MAG$_3$-IgG was higher at pH = 6 than other pH values. Fig.2 shows the influence of pH on the radiochemical yield of ^{188}Re-MAG$_3$-IgG. The influence of coupling time on the radiochemical yield of ^{188}Re-MAG$_3$-IgG at pH 6 is shown in Fig.3. The optimized coupling time is 2 h.

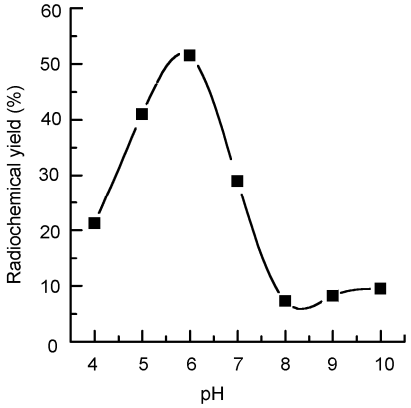

Fig.2 Influence of pH on the radiochemical yield of ^{188}Re-MAG$_3$-IgG.

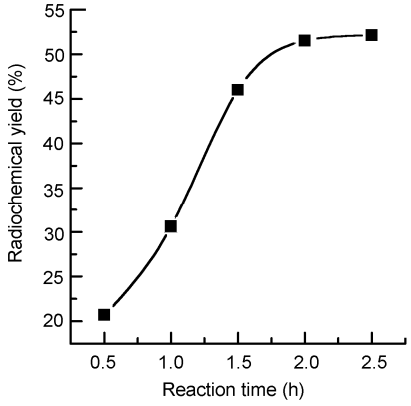

Fig.3 Influence of coupling time on the radiochemical yield of ^{188}Re-MAG$_3$-IgG.

Table 1 Stability of ^{188}Re-MAG$_3$-IgG in different solutions

(%)

t (h)	0.05 mol·L^{-1} PBS (pH 7.5)	Saline	0.05 mol·L^{-1} EDTA
1	99.5	99.0	98.0
3	98.1	97.6	97.2
20	96.2	95.2	92.6

Results of the stability determination of ^{188}Re-MAG$_3$-IgG are shown in Table 1. We have found that ^{188}Re-MAG$_3$-IgG exhibits a good stability

in vitro.

4 Conclusions

S-Bz-MAG_3 as a bifunctional chelating agent was used for labeling of IgG with carrier free ^{188}Re by pre-radiolabeling of the chelating approach. The conjugation conditions were optimized. The stability of ^{188}Re-MAG_3-IgG in vitro was high. The results may be useful to the studies of ^{188}Re labeled MAbs for radioimmunotherapy.

Because the ^{188}Re labeling conditions are influenced by many factors, we did some general studies of carrier free ^{188}Re labeled IgG via S-Bz-MAG_3 as BFCA. This work may be useful to the studies on indirect ^{188}Re labeling of MAbs for radioimmunotherapy.

References

1 Sykes T R, Somayaji V V, Bier S *et al*. Appl Radiat Isot, 1997, **48**: 899-906

2 Griffiths G L, Goldenberg D M, Knapp F F *et al*. Cancer Res, 1991, **51**: 4594-4602

3 Iznago-Escobar N. Nucl Med Biol, 1998, **25**: 441-447

4 Yu J F, Yin D Z, Min X F *et al*. J Labelled Cpd Radiopham, 1999, **42**: 233-243

5 Ding Z G, Yu J F, Yin D Z *et al*. Nucl Tech (in Chinese), 2000, **23**: 762-766

6 Najafi A, Alauddin M M, Sosa A *et al*. Nucl Med Biol, 1992, **19**: 205-212

7 Guhlke S, Schaffland A, Zamora P O *et al*. Nucl Med Biol, 1998, **25**: 621-631

8 Visser G W M, Gerretsen M , Herscheid J D M *et al*. J Nucl Med,1993, **34**: 1953-1963

9 Gog F B , Visser G W M, Klok R *et al.* J Nucl Med, 1996, **37**: 352-362

10 Hjelstuen O K, T ϕ nnesen H H, Bremer P O *et al*. Nucl Med Biol, 1998, **25**: 651-657

11 Gog F B, Visser G W M, Gowrising R W A *et al*. Nucl Med Biol, 1998, **25**: 611-619

12 Fritzberg A R, Kasina S, Eshima D *et al*. J Nucl Med, 1986, **27**: 111-116