2007 International Nuclear Atlantic Conference - INAC 2007
Santos, SP, Brazil, September 30 to October 5, 2007
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-02-1

RADIOLABELING OF ANTI-CD20 WITH Re-188: LIQUID KIT

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ABSTRACT

Radioimmunotherapy uses the targeting features of monoclonal antibody to deliver radiation from an attached radionuclide. The radionuclide ^{188}Re is currently produced from the father nuclide ^{188}W through a transportable generator system. Because of its easy availability and suitable nuclear properties ($E_{\beta MAX}$ =2.1 MeV, $t_{1/2}$=16.9 h, Eγ=155 keV), this radionuclide is considered an attractive candidate for application as therapeutic agents and could be conveniently utilized for imaging and dosimetric purposes. The objective of this work is the optimization of radiolabeling of anti-CD20 with ^{188}Re using a liquid formulation. Anti-CD20 was reduced by incubation with 2-ME and purified over a PD-10 column. The number of resulting free SH was assayed with Ellman's reagent. Optimization of radiolabeling was achieved by varying parameters: antibody mass, reducing agent, reaction time and ^{188}Re volume in the liquid kit. Radiochemical purity of ^{188}Re-anti-CD20 was evaluated. An average of 12 SH groups per mol in the reductions was found. The best labeling efficiency (> 93%) was achieved in the following conditions: 1 mg anti-CD20; 82.8 mg sodium tartrate; 1 mg $SnCl_2$; 0.25 mg gentisic acid, 1 mL ^{188}Re and reaction time of 1 hour at room temperature.

1. INTRODUCTION

Radioimmunotherapy (RIT) uses target-specific monoclonal antibodies or fragments labeled with a radioactive isotope to combine humoral and radiolytic functions[1] and has the advantage of targeting not only the cell to which the antibody is bound but also the surrounding tumor cells and microenvironment[2]. The most successful clinical studies of RIT in patients with Non-Hodgkin's Lymphoma (NHL) have targeted $CD20^+$ B-cell tumors[1].

Antibody therapy directed against the CD20 antigen on the surface of B cells is considered one of the first successful target-specific therapies in oncology[3]. CD20 is an hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes. The antigen is expressed on most B-cell NHL but is not found on stem cells, pro-B cells, normal plasma cells or other normal tissues. CD20 is not shed from the cell surface and does not internalize upon antibody binding. Anti-CD20 mAbs react with greater than 95% of B lymphocytes and greater than 90% of B-lymphocyte NHL[4,5].

As targeted radionuclide therapy involves the specific deposition of beta-emitting radionuclides in malignant tumor via labeled ligands that specifically bind to tumor cells to reduce injury to normal tissue[6], the use of rhenium-188 (^{188}Re) with beta emissions with energies of 2.12 MeV (71.6%) and 1.96 MeV (25.1%)[7,8] represents an attractive alternative to other beta-emitter radionuclides for tumor therapy[9]. ^{188}Re ($T_{1/2}$ = 16.9 h) is produced

from beta-decay of the tungsten-188 father ($T_{1/2}$ = 69 d) through the elution of a $^{188}W/^{188}Re$ generator system. In addition to the emission of high energy electrons with average energy of 769 keV, ^{188}Re also decays with emission of gamma photon with energy of 155 keV (15%).

Besides the therapeutic usefulness of ^{188}Re, the emission of the 155 keV gamma photon is an added advantage since the biodistribution of ^{188}Re-labeled agents can be evaluated *in vivo* with a gamma camera. In this manner, the biodistribution *in vivo* can be used to determine target tissue specificity, and the absorbed radiation dose values can be estimated[9,10].

The objective of this work is the optimization of radiolabeling of anti-CD20 mAb with ^{188}Re using a liquid formulation.

2. MATERIAL AND METHODS

2.1. Antibody reduction

The mAb (5 and 10 mg, MabThera/Roche) was reduced by reaction with 5 and 10 μL of 2-mercaptoethanol (2-ME / Sigma) at room temperature for 30 min. The resulting solution was purified on a PD-10 column (Sephadex G-25M, Pharmacia) using phosphate buffered saline (PBS) (pH 7.4) as mobile phase purged with nitrogen and fractions of 1 mL were collected (10 fractions). The concentration of the reduced antibody was determined by optical density at 280 nm on a UV/visible spectrophotometer (Hitachi Instrument).

2.2. Sulphydryl groups

The number of resulting free sulphydryl groups was assayed with Ellman's reagent (Sigma); 50 μL of the sample of reduced antibody was mixed with 50 μL of the solution containing 0.3 mg/mL of 5,5′-dithiobis (2-nitrobenzoic acid) and diluted to 3 mL with 0.1 mol/L phosphate buffer pH 8. The mixture was incubated at room temperature for 5 min and coloration (yellow color) measured in a UV/vis spectrophotometer at 407 nm. The number of thiols was determined by comparison with a standard curve obtained by the assay of a series of cysteine standards ranging from 0.25 to 1.0 mmol/L. The result was expressed as sulphydryl groups per antibody molecule.

2.3. Preparation and labeling of ^{188}Re-anti-CD20 liquid kit formulation

The studies were first done with a liquid kit of the anti-CD20 that contained: 1 mg anti-CD20; 82.8 mg sodium tartrate; 1 mg $SnCl_2$ and 0.25 mg gentisic acid. Perrhenate ($Na^{188}ReO_4$) eluted from the $^{188}W/^{188}Re$ generator (Polatom) in 0.9% NaCl was added (336.7 – 806.6 MBq). The liquid kit was then incubed for 1 hour at room temperature. The pH was between 5.0 – 6.0.

2.4. Radiochemical control

The labeling efficiency was evaluated by instant thin-layer chromatography (ITLC, Gelman Science Inc.). The paper was cut into 1.5 x 10 cm strips and activated by heating for 30 min, at 110°C. Both 0.9% NaCl and acetone were used as the mobile phases to separate free perrhenate ($^{188}ReO_4^-$). ^{188}Re-tartrate and $^{188}ReO_4^-$ moved with the solvent front (R_f=1) when 0.9% NaCl was used, whereas radiocolloid ($^{188}ReO_2$) and ^{188}Re-mAb remained at the origin (R_f=0). In acetone, ^{188}Re-mAb, ^{188}Re-tartrate and $^{188}ReO_2$ stayed at the origin and $^{188}ReO_4^-$ moved to the solvent front. Human serum albumin (5%)-impregnated ITLC-SG strips were used as the stationary phase and ethanol:NH_4OH:water (2:1:5 [v/v]) as the mobile phase to separate $^{188}ReO_2$ that remained at the origin while the ^{188}Re-mAb, ^{188}Re-tartrate and $^{188}ReO_4^-$ moved with the solvent front.

2.5. Optimization of radiolabeling

Optimization was achieved by varying parameters, including antibody mass (0.25, 0.5, 1.0 and 2.5 mg), reducing agent ($SnCl_2$) (0.5, 1.0, 1.67, 3.0 and 5.0 mg), reaction time (0.5, 1.0 and 2.0 hour) and ^{188}Re volume (1.0 and 2.0 mL) in the liquid kit.

3. RESULTS AND DISCUSSION

3.1. Antibody reduction and sulphydryl groups

The average recovery of the protein was 99.3 ± 1.2% (n=3). An excellent linear correlation was found between the cysteine concentration and absorbance (r=0.9986). The number of SH groups generated per molecule of antibody was calculated based on the cysteine standard curve and after reduction with 2-ME was 11.9 ± 1.0 (average±SD, n=3). The result was better than the one obtained by Leyva et al.[11], 4–5 groups for the same method of reduction.

3.2. Radiolabeling studies

Fig. 1 shows the results of the labeling yield of ^{188}Re-anti-CD20 using the liquid kit, when the concentration of reducing agent was varied.

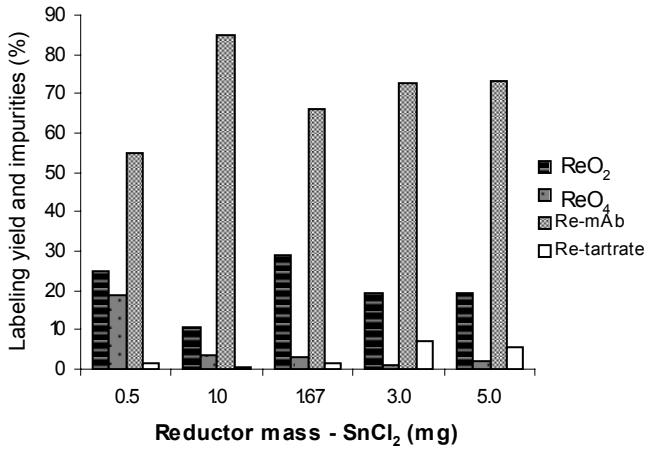

Figure 1. Labeling yield with the variation of the reducing agent mass

Oliver et al.[12] have used 1.67 mg of stannous fluoride and obtained a good labeling yield, while that in this work the best yield was obtained with 1 mg de stannous chloride. From this point on, the mass of stannous chloride used in the liquid kits was 1 mg. It is always desirable to use the minimum mass of the reducing agent due to its potential toxicity.

The effect of the variation of the antibody mass is shown in Fig. 2.

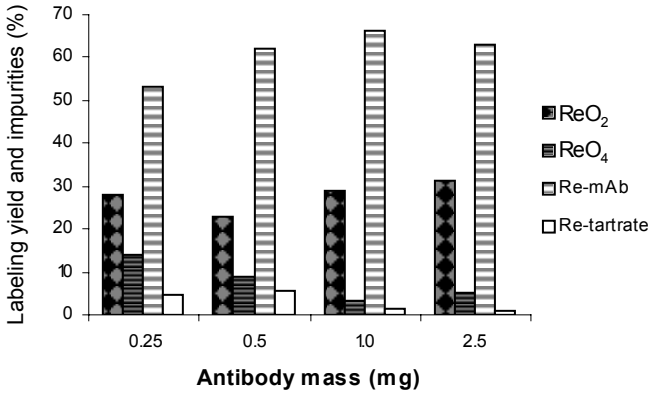

Figure 2. Labeling yield with the variation of antibody mass

The best result was found with the mass of 1 mg of antibody, in accordance with Oliver et al. [12]. It is also important to try to reduce the mass of antibody to minimize the competition in the tumor sites and also by economical reasons.

Fig. 3 shows the labeling yields obtained at the different reaction times employed in this work.

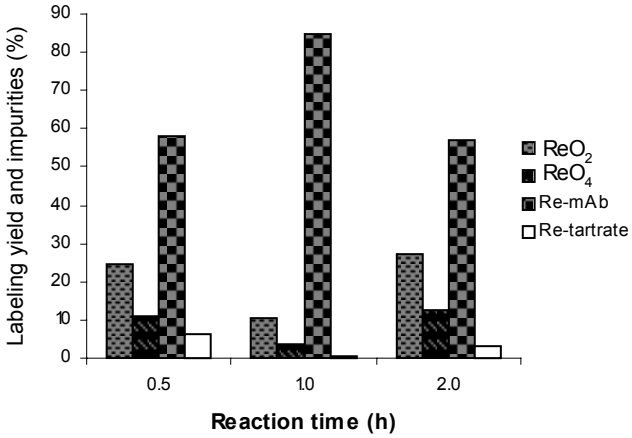

Figure 3. Labeling yield with the variation of reaction time

The best reaction time was 1 h, the same as used by Oliver et al.[12]. It is important to find a minimum reaction time that gives a good yield in order to reduce the loss of ^{188}Re due to its decay.

Also the effect of the volume of ^{188}Re used for the labeling of the antibody was studied, as it can be seen in Fig. 4.

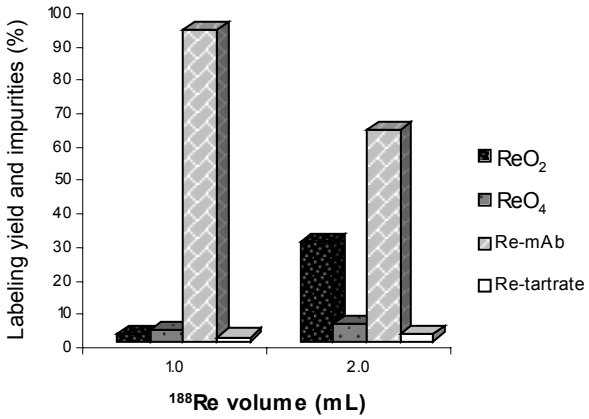

Figure 4. Comparison of the labeling yield with the variation of the volume of ^{188}Re

The volume of 1 mL of ^{188}Re revealed to be superior to the volume of 2 mL concerning the labeling yield.

In summary, the best labeling efficiency of the liquid kit (> 93%) was achieved for the following conditions: 1 mg of anti-CD20; 82.8 mg of sodium tartrate; 1 mg of $SnCl_2$; 0.25 mg of gentisic acid, 1 mL of ^{188}Re and reaction time of 1 hour at room temperature.

4. CONCLUSIONS

The reduction of the antibody anti-CD20 was efficient with high recovery of the reduced protein. The preliminary results showed a high labeling yield of the antibody but further studies will be carried out in order to improve the labeling yields and consequently the specific activity of the product.

ACKNOWLEDGEMENTS

The authors thank the support from the CNPq – Conselho Nacional de Pesquisa for granting a fellowship for this study.

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