PREPARATION OF ONE VIAL REDUCED ANTI-CEA KIT

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ABSTRACT

A rapid and realiable instant reduced anti-CEA lyophylized kit for labeling with ^{99m}Tc was developed.Each vial contains 0.5 mg of reduced anti-CEA,40 μg of mendronate (MDP),2.75 μg stannous fluoride (SnF_2) and 15 μg p-aminobenzoic acid (PABA). Labeling efficiency and stability were higher than 95% and were determined by instant paper chromatography. Bio-distribution studies were performed in normal isogenic BALB/c mice at 4, 6 and 24 hrs after intravenous administration of the radiolabelled product.The maximum amount of activity was accumulated in the liver followed by intestines and kidneys.

INTRODUCTION

Among the radionuclides currently used in nuclear medicine, ^{99m}Tc is the most suitable element for imaging applications, because it has ideal nuclear properties, i. e., a single photon energy of 140 KeV, a half-life of 6 hrs and its availability from a ^{99}Mo-^{99m}Tc generator. However when used in direct-bonding to an antibody, this radioisotope has major disadvantagens : ^{99m}Tc is linked to the antibody through sites of two differents types : low - affinity, high capacity sites giving unstable *in vivo* compounds which undergo a transchelation process with plasma proteins, and high-affinity , low capacity sites with no transchelation. There are differents routes labeling antibodies with ^{99m}Tc : directly that it is reacting endogenous sulphydryl groups generated within the antibody [1] and indirectly, after attachment to antibodies of an exogenous chelator [2]. Recently more attention has been paid to the direct labeling method owing to the possibility of an instant kit formulation.

Several authors [3], [4], [5], [6] have used controlled reductions to generate free sulphydryl groups (-SH) , through incubation of antibody with a reducing agent (e.g. β-mercaptoethanol).

In most cases the direct method is a combination of two sequential steps : the reducion of the interchain-disulphyide groups of the antibody molecule and the use of a ligand capable of transferring the reduced technetium to the sulphydryl groups of the protein.

This communication describes results obtained using a conventional direct method labeling of anti-CEA monoclonal antibodies with ^{99m}Tc using tin methylene diphosphonate (MDP) kit to reduce pertechnetate. At the same time, heterogeneous phase labeling was undertaken, using stannous ion as reducing agent. Simple, fast and realiable instant reduced anti-CEA lyophylized kits were developed to assess radiolabeling efficiency and ^{99m}Tc antibody stability.

MATERIAL AND METHODS

Monoclonal Antibody : Anti-carcinoenbryonic antigen (whole anti-CEA IOR 1 : CIMAB) was used for developed of the labeling method : Each vial of anti-CEA IOR 1 (5mg/5ml solution in 0.1M phosphate buffer pH 7.4) was concentrated to about 5mg/0.5ml by ultra-filtration at 5-10 °C.

Antibody Reduction : A general schema is shown in Figure 1. Disulphide bridges within the antibody molecule are cleaved by the use of the reductant 2-mercaptoethanol. Following a subsequent purification, the resulting reduced antibody is stored appropriately until required for use. Labeling is performed via Sn^{++} reduction of pertechnetate in the presence of an excess of a low-affinity chelating ligand.

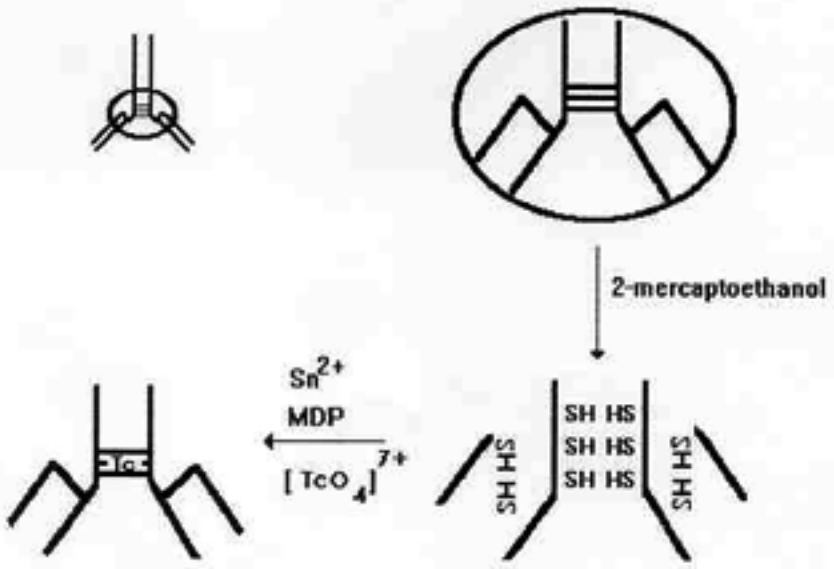


FIGURE 1 - General schema showing outline of labeling procedure

The antibodies concentrated to 5mg / 0.5ml by ultrafiltration on Ultrafree - 20 (Millipore) in neutral phosphate buffered saline (0.1 M PBS, pH 7.4) were reduced with 2 - mercaptoethanol (2 - ME) at molar ratio of 1000 : 1 (2 - ME: Ab), were incubated at room temperature for 30 minutes. The reduced antibodies were purified to eliminate the excess of 2 - ME on a Sephadex G - 50 gel filtration column using cold PBS (pH 7.4) purged with nitrogen as mobile phase. After this 2.5 ml fractions were collected and protein concentration were determined by optical density (O.D.) at 280 nm on a UV / visible spectro- photometer (Perkin Elmer).

Kit Formulation : In order to develop a lyophilized kit reduced antibody to label with ^{99m}Tc for the convenient clinical application of the radiopharmaceutical an instant kit was formulated as follows:

An aliquot of 40 µL MDP bone scanning kit, containing 40 µg of methylene diphosphonate (MDP), 2.75 µg of stannous fluoride (SnF_2) and 15 µg of p-aminobenzoic acid (PABA) was added for each vial containing 500 µg of the reduced antibody in PBS. The mixture was lyophilized for 24 hours.

Radiolabeling and radiochemical quality control : The radiolabeling efficiency of the reduced monoclonal antibody to label with ^{99m}Tc was measured by paper chromatography (Whatman 3 MM) and developed in 0,9 % sodium chloride solution.. An aliquot of 37 MBq (1 mCi) of $^{99m}TcO_4^-$ eluted from the ^{99}Mo / ^{99m}Tc (IPEN / TEC) generator was added to reduced anti-CEA kit. Samples of 5 µl were spotted, dried under nitrogen stream and immediately developed on solvent.

Animal biodistribution studies : The biodistribution studies were performed by injecting 100 µ Ci of ^{99m}Tc - anti - CEA into the tail vein of normal male Balb/c mice weighing 25 - 30 g. Res-pectively at 4 , 6 and 24 hours after injection, groups of mice (n = 6) were killed. The liver, heart, lungs, kidneys, spleen, stomach with contents , intestine , sample of muscle and skeleton were dissected and weighed. Blood was also collected and weighed at each of the times. Samples were counted in a gamma counter and organ activity was expressed as mean of percentage of injected dose per grams of tissue.

RESULTS AND DISCUSSION

The availability of a quick and reliable labeling technique of MoAb with ^{99m}Tc for tumor immunoscintigraphy is important. Among the different methods for radiolabeling MoAb with ^{99m}Tc, those which employ a covalent binding of the radionuclide to the sulphydryl groups of the protein have been fairly extensively used for clinical imaging [7], [8].

In this work we have adapted original direct methods [3], [5] in order to label the monoclonal antibodies anti - CEA with ^{99m}Tc.

Anti-CEA MoAb (IOR-1) were reduced with 2-mercaptoethanol (2-ME) at molar ratio of 1000:1. After 30 minutes of treatment at room temperature the reaction mixture were purified by column chromatography.

The Figure 2 shows the results of fractions obtained with aliquots of 2,5 ml and determined in $O.D._{280\ nm}$.

The gel filtration in Sephadex G-50 of antibody reduced anti-CEA using one chromatographic column is presented in the figure 2 and it is represented the typical elution pattern.

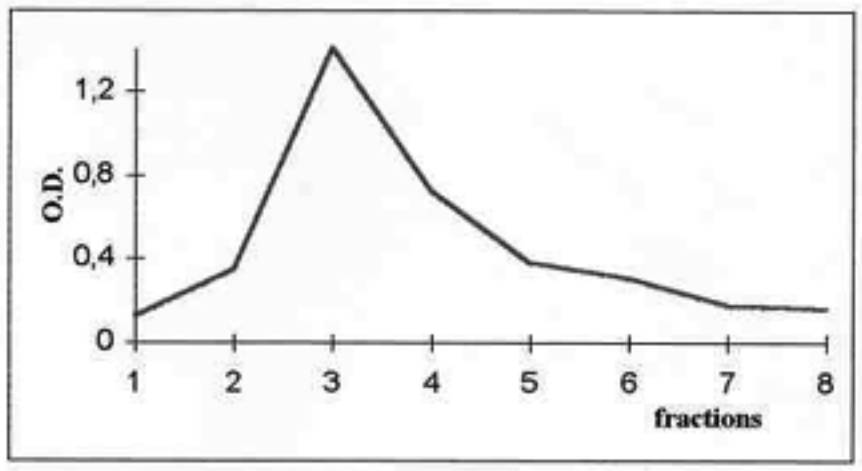


FIGURE 2 - Chromatographic elution of cold reduced anti-CEA using Sephadex gel G-50 fine column and PBS pH 7.4

The labeling efficiency of reduced anti - CEA kit with ^{99m}Tc was determined using ascending paper chromatography and 0,9% sodium chloride solution as mobile phase at 10 minutes after labeling. In this system the anti-CEA-^{99m}Tc remained at the origin (Rf = 0.0 - 0.3) while the free $^{99m}TcO_4^-$ moved with the solvent front (Rf = 0.8 - 1.0).

The figure 3 shows typical chromatograms in Whatman 3MM in saline. Value for labeling efficiency was higher than 95%.

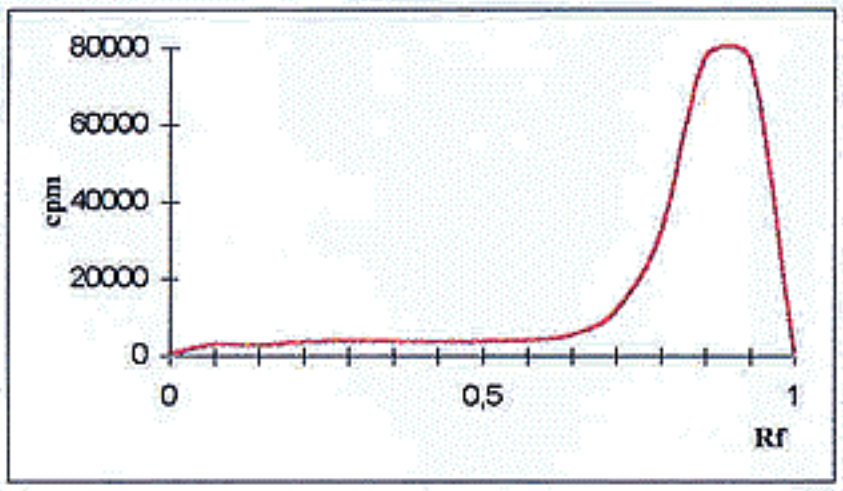


FIGURE 3 - Paper chromatogram (Whatman 3MM) of ^{99m}Tc-CEA, developing solvent : 0.9% sodium choride

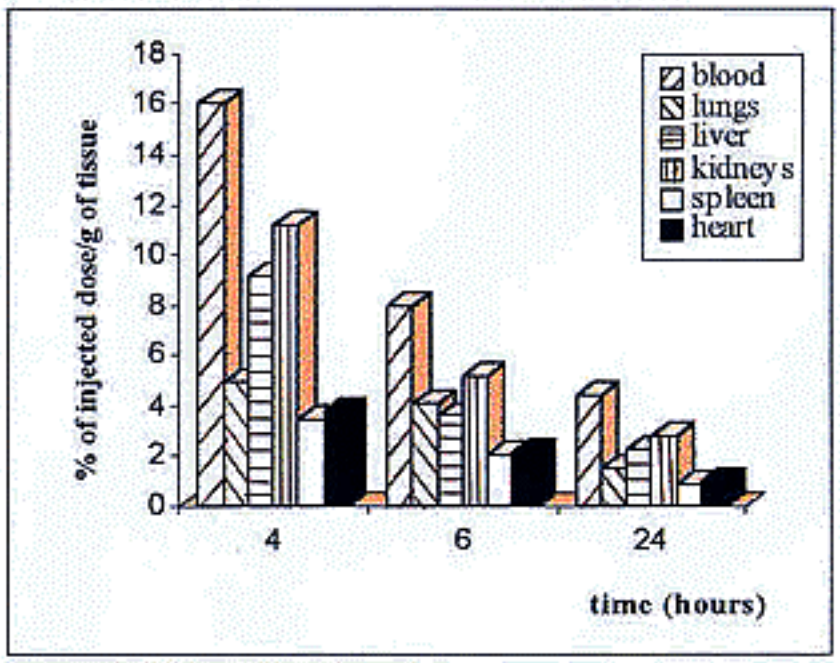
The biodistribution data are summarized in table 1. The results in table 1 showed that in mice the time 4 hr after injection in liver, kidneys and intestine gave: 9.147± 0.321 ; 11.214 ± 0.721 and 3.690 ± 0.631 % D/g respectively , indicating the possibility of *in vivo* metabolism of the labelled antibodies (Figure 4). Blood pool activity was significantly higher, suggesting perhaps that some ligand exchanged had taken place. Biodistribution data in mice at 24 hr after injection did not reveal a specific high accumulation in any particular organ.

TABLE 1 - Biodistribution of 99mTc-CEA IOR 1 in normal BALB/c mice (% D/g)

ORGAN	TIME (HOURS)		
	4	6	24
HEART	3.753 ± 0.690	2.196 ± 0.209	0.983 ± 0.158
LUNGS	4.923 ± 1.480	4.128 ±0.600	1.534± 0.661
KIDNEYS	11.214± 0.721	5.172± 0.550	2.804 ± 0.397
LIVER	9.147 ± 0.321	3.613 ± 0.486	2.282 ± 0.167
SPLEEN	3.475± 0.255	2.013 ± 0.271	0.851 ± 0.160
STOMACH	1.664± 0.544	0.805 ± 0.105	0.237 ± 0.047
INTESTINE	3.690 ± 0.631	1.164 ± 0.153	0.798 ± 0.116
MUSCLE	0.544 ± 0.094	0.545 ± 0.130	0.092 ± 0.047
SKELETON	1.317 ± 0.430	1.126 ± 0.037	0.361 ± 0.068
BLOOD	16.046 ± 1.102	7.998 ± 0.600	4.413 ± 0.507

Blood volume = 7% of body weight : muscle dose = %ID sample muscle x 0.4 of body weigth : skeletal dose = % ID femur x 0.1 of body weight
mean values (n = 4 - 6)

(a)



(b)

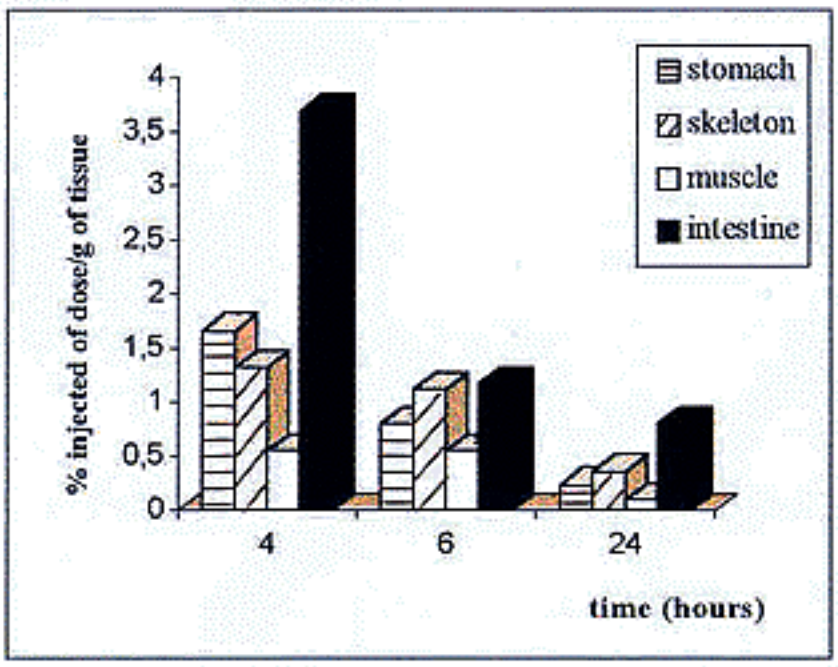


FIGURE 4 (a,b) - Biodistribution of 99mTc-CEA IOR 1 in normal BALB/c mice (%D/g)

CONCLUSION

The goal of our study was to investigate the direct method of the antibody anti-CEA labeling with ^{99m}Tc using the 2-mercaptoetanol reduction . An instant anti-CEA lyophilized kit was formulated with high and reprodutive labeling efficiency higher than 95%. Biodistribution studies in normal mice at 4, 6 and 24 hours after intravenous administration of ^{99m}Tc-anti-CEA did not reveal a specific high accumulation in any particular organ. The maximum amount of activity was accumulated in liver, kidneys and intestine, suggesting its two route excretion through renal as well as intestinal.

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