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LABELING EFFICIENCY OF MONOCLONAL ANTIBODY OF EPITHELIAL ORIGIN TUMOURS USING DIFFERENT CHELATING AGENT (MDP)

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ABSTRACT: The epidermal growth factor (EGF) is a little polypeptide of 6kD MW capable of stimulation the proliferation of epithelial cells and performs this biological activity through a membrane receptor, a 1186 aminoacid glycoprotein of 170 kD MW. This receptor (EGF-R) in high levels have been detected in malignant tumours of epithelial origin, i.e., breast, ovary, vulva, esophagus, colon and lung tumours.

The anti EGF receptor monoclonal antibody, ior-egf/r3 (Cuba) is an IgG2a isotype was labeling with 99mTc. The reduction step was performed with 2-mercaptoethanol at a molar ratio of 1:1000 (2-ME:MAb) The integrity of reduced MAb was checked by gel filtration chromatography on Sephadex G-25 minicolumn (1 X 10 cm). The MAb pyke was submitted to two types of methylen diphosphonate as chelant:Amerscam Medronate II Bone Agent MDP kit (Amersham, UK) and IPEN/CNEN MDP kit. and both were labeled with 1 mCi (37 MBq) of 99m Tc eluted from 99Mo/99mTc generator system (IPEN/CNEN-SP). The purification in ITLC reveals the high grade of labeling monoclonal antibody.

The laboratory tests did not show significant modification in structure of MAb - 99mTc macromolecule in both cases and can be used in immunoscintigraphy.

DESCRIPTORS: Monoclonal antibody ; Chelating Agent; Radiolabelling

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I. INTRODUCTION

It is impossible to relate in detail all of the aspects and all of the centers where monoclonal antibodies (MAbs) is and was used. The first antibodies used were purified polyclonal anti-CEA antibodies raised in goats and labeled with ^{131}I . At the time, little was known on acute and long-term toxicity of goat proteins administered intravenously in patients. Today, we known that this is not a favorable situation for tumour detection with MAbs.

After some years of reluctance and a tendency towards the use of longer-lived radionuclides, technetium-99m has now become established as the radiolabed of choice for imaging with monoclonal antibodies (1-3).

In these applications technetium-99m has almost replaced others radionuclides due to its nuclear properties and its availability from a generator (4).

There are two different methods available for radiolabeling MAb, with ^{99m}Tc: a direct method reacting the reduced antibody directly with reduced technetium (5,6,7) and indirect an method where a bifunctional chelating agent is first conjugated with an antibody (8,9). Direct methods are efficient and adaptable to a kit type of radiolabeling procedure.

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Further more the use of a reducing agent may affect immunoreactivity of the reduced antibody and the labeled antibody could be unstable. The direct method for radiolabeling reduced MAb with ^{99m} Tc in presence of methylene diphosphonate (MDP), developed by Schwarz and Steinstrasser (10) and Mather and Ellison (11) has proved as a good procedure to obtain high labeling efficiency and a stable labeled MAb. The ^{99m} Tc-MDP method uses reduction of disulfite bridges of the immunoglobulin molecule by adding an excess of reducing agent (2-ME). After purification to eliminate the excess of 2-ME in Sephadex G-25 column, the reduced antibody is labeled with technetium via Sn^{2+} reduction of pertechnetate, using MDP as weak competing ligand.

Nevertheless the use of different types of MDP kits is necessary to minimize the damage caused in a whole immunoglobulin macromolecule (or your fragments).

II. MATERIAL AND METHODS

Antibody. The monoclonal antibody ior-egf/r3 is a highly specific murine IgG2a isotype that recognizes the hEGF-r. It was obtained and produced in the Center of Molecular Immunology (Havana, Cuba) by standard hybridoma techniques as previously described (12) in vials containing 1 mL of sterile and apyrogenic solution with an antibody concentration of 5mg/mL were used.

Antibody reduction. 1 mL (5mg/ml) of MAb was reduced by reaction with a molar excess of 2-ME of 1:1000 (4,8 ug of 2-ME: 5 mg of Ab) at room temperature for 30 minutes. The reduced antibody was purified to eliminate the excess of 2-ME on a PD-10 Sephadex G-25 gel filtration column where was applied 1 mL of antibody and washing with saline purged nitrogen and the eluates were collected in 2 mL vial and the protein pike was determined by spectrophotometer UV (Beckman) at 280 nm.

Labeling. After reduction of internal disulfite bonds the (2-ME) and purification the reduced antibody was labeled with technetium via Sn^{+2} and MDP kits were reconstituted with 5 mL of saline purged with nitrogen to Amerscan and 3 mL for IPEN/CNEN-SP; 50 uL were added to each mg of reduced antibody and they were labeled with 40 mCi of pertechnetate eluted from ^{99}Mo/^{99m} Tc generator (IPEN/CNEN-SP).

Quality Control . The labeled MAb was submitted to ascending ITLC strips (1 X 10 cm) chromatography like stationary phase and three systems , acetone, saline as mobile phase; that strips were soaked in 1% human albumin and after dried samples of 1 uL were applied at origin. Radioactivity bound to antibody remained at the origin (saline) whereas free pertechnetate and ^{99m} Tc-MDP migrated with the mobile phase. Strips were also cut and counted in a gamma counter.

III. RESULTS

Reduced monoclonal antibody was analyzed on a PD-10 Sephadex G-25 gel filtration column. Absorbance values at 280 nm revealed the protein pike (Table 1). The ability of the reduced monoclonal antibody to label with ^{99m} Tc was assessed and the best conditions were selected to add MDP solution and 1 mCi of ^{99m} Tc; no have different results with different MDP (Table 2).

The labeling efficiency demonstrated the high grade of Tc incorporation to the antibody macromolecule in three different systems of ITLC chromatography.

TABLE 1
D.O. (280 nm) of 2 mL fractions eluted from PD- 10 Sephadex G-25 column

Volumes	D.O.
2 mL	.60
4 mL	.10
6 mL	.06
8 mL	.1 6

TABLE 2
Scheme and out-put in labeling antibody

	Saline
MDP (Amerscan)	97,2%
MDP (IPEN/CNEN-SP)	97,5%

IV. DISCUSSION AND CONCLUSION

The objective of the present work was to evaluate the prepare of the ^{99m} Tc-monoclonal antibody, designed ior egf/r3 (Cuba), by reducing their antibodies as potential imaging agents for various human epithelial producing malignant tumours.

The EGF/r antigen has the potential as a target for imaging and therapy because of its abundance an uniformity of expressions in most EGF-producing tumours. Even though others factors such as the monoclonal antibody characteristic , the antibody delivery, and the tumour biology may be involved in limiting tumour targeting, the antigenic content of tumours has a positive correlation with uptake of monoclonal antibodies in vivo.

The studies with ^{99m} Tc-monoclonal antibodies were hampered by their stability of the ^{99m} Tc labeling and characterized by considerable accretion of activity in the kid-

ney. Advances in chelate technology have resulted in substantial improvement in the stability of the ^{99m}Tc labeling, which has been translated into reduced kidney uptake in vivo (14,15) . Recently, several investigators have demonstrated that ^{99m}-Tc-labeled antibody and antibody fragments can be directly prepared if the antibody is first exposed to reagents such as 2-mercaptoethanol (2-ME) or 2-aminoethanethiol, which reduce intrinsic disulfite bridges . The reduced antibody is labeled with transchelation in presence of stannous ion, using ^{99m}Tc and phosphonate-type bone imaging radiopharmaceuticals.

In this work, we have adapted the original direct method (11) so as to label ior-egf/r3 with technetium. No significant difference existed between MDP kits, because it seems likely, therefore, that the majority of the labeling is mediated through the exposed-sulphydryl groups and not with other amino-acid side chains. The sulphydryl groups on the antibody molecule are bound to the technetium ion. Although transchelation by cysteine solution occurred slowly, this bound may play an important role in both the in vitro and in vivo stability; Is is generally admitted that technetium must be reduced to the +3, +4 or +5 valence state to be incorporated into a protein (16).

In conclusion, the results of these molecule stability suggest that the binding site of ^{99m} Tc is mainly a constant region and ITLC chromatography analyzes showed no monoclonal antibody damage when two different types of transchelation were used whereas they had prevent the oxidation of the reduced antibody and the action of stannous ion was the only reduction of ^{99m} Tc-pertechnetate, and not serve as ligament between ^{99m} Tc and antibody.

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