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PRELIMINARY STUDIES OF TECHNETIUM-99m-LABELED ANTIMYOSIN MONOCLONAL ANTIBODY: DEVELOPMENT OF RADIOPHARMACEUTICAL FOR CARDIAC EVALUATION.

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ABSTRACT

In the acute myocardium infarction, the myocytes cell membrane loses its integrity, allowing the influx of extracellular macromolecules such as circulating antibody into the damaged cell. Specific antibodies to cardiac myosin can therefore bind to the acutely necrotic myocyte, allowing the noninvasive localization and dimension of myocardial infarction. Because of its favorable physical characteristics, low cost, and ready availability, technetium-99m (^{99m}Tc) is the radionuclide of choice for scintigraphy. The purpose of this work was to study the labeling of the antimyosin monoclonal antibody with ^{99m}Tc for development of a radiopharmaceutical with high sensitivity and specificity used in the diagnostic of the myocardial infarction. The intact monoclonal antibody (IgG_1) was reduced by treatment with dithiothreitol (DTT) with the consequent generation of free thiol groups (-SH), responsible for the labeling of the antibody with ^{99m}Tc. The radiochemical yield was determined using Sephadex G-25 column (PD-10). The percentage of ^{99m}Tc-antibody was 90,06% and after purification procedure the radiochemical yield was > 98%. The biodistribution studies showed low uptake in the stomach and thyroid at different times (1, 4 e 24 hours) representing a small amount of unbounded ^{99m}Tc and a good stability of the purified ^{99m}Tc-antibody. The uptake in the normal heart was relatively low as expected. Based on these results, we concluded that the direct labeling procedure applied to the antimyosin monoclonal antibody allowed the easy preparation of the radiopharmaceutical with good stability to be used in the noninvasive diagnostic of the myocardial infarction.

1. INTRODUCTION

Myocardial infarction is characterized by myocyte necrosis manifested by disruption of cellular membrane and leakage of intracellular molecules and ions into the extracellular compartment. As a result, intracellular components, such as myosin, become acessible to the extracellular fluid. The cardiac myosin has provided the basis for the development of specific antibodies that, once labelled with a radioisotope, would identify and localize only necrotic myocardium. Antibody antimyosin localizes in myocardial cells that have suffered irreversible ischemic injury with loss of cell membrane integrity [1]. The concentration of antibody is inversely proportional to blood flow. The location of radiolabeled antimyosin antibody corresponds exactly to histochemically delineated regions of myocardial infarction [2].

The unique receptor specificity of monoclonal antibodies (MAb) combined with the excellent physical characteristics (radiation energy E = 140 keV, half-life = 6.02 h) and ready availability of the radionuclide have made ^{99m}Tc-MAb highly desirable for scintigraphic imaging [3]. This task has been accomplished by two types of methods. In once, a bifunctional chelating agent (BFCA) is first conjugated with an antibody and a radionuclide of choice is added to the conjugate antibody and allowed to complex with the chelating agent. This method is versatile and can be used to chelate radionuclides of polyvalent metal ions. However, the procedures are

lengthy, and depending upon the number of BFCA molecules conjugated per MAb molecule, can alter the immunospecificity and reactivity of an antibody. The second method do not require the use of BFCA, but depend upon the use of sulfhydryl groups obtained after the reduction of the disulfide groups available in the MAb. This method is rapid and may be easily applicable for labeling MAbs with ^{99m}Tc [4,5].

2. MATERIALS AND METHODS

2.1. Materials

Murine antimyosin monoclonal antibody, an antibody (IgG_1) against cardiac myosin, was purchased from Rouger Bio-Tech Ltd. This antibody was reduced by treatment with dithiothreitol (DTT) from Sigma-Aldrich. Reduced antibody was purified on PD-10 Sephadex G25 (Pharmacia, Uppsala, Sweden) size-exclusion columns. ^{99m}Tc was obtained by elution of a molybdenum-technetium generator system (IPEN – CNEN/SP), and the radioactivity was determined in a γ-well NaI(Tl) Packard counter. Stannous chloride was supplied by Sigma, and the various buffer salts by Merck. A UV/vis spectrophotometer (Hitachi) was used to determine the protein concentration estimated by absorbance at 280 nm and the number of thiols estimated by absorbance at 412 nm. Labelling efficiency was measured by chromatography using a Sephadex G-25 column (PD-10). Labeled antibody was purified by PD-10 and injected in *Swiss* mice to biodistribution studies.

2.2. Methods

The antimyosin monoclonal antibody (IgG_1) was reduced by treatment with dithiothreitol (DTT). This reductant cleaves disulphide bridges within the antibody molecule with the consequent generation of free thiol groups (-SH), responsible for the labeling of the antibody with ^{99m}Tc. The antibody (1 mg) was reduced by reaction with DTT at molar ratio of 1:1000 (antibody:DTT) at room temperature for 30 min with continuous rotation (850 rpm). The reduced antibodies were then purified by PD-10 cromatography column and antibody concentration was measured by UV absorbance at 280 nm. The number of –SH groups generated in the protein reduction step was determined using Ellman's method. The absorbance (412 nm) of sample was compared with a standard curve obtained with cysteine standards ranging from 1.0 to 0.25 mM. The reduced antibody was labeled with ^{99m}Tc by direct method, with the reduction of pertechnetate by stannous chloride (Sn^{2+}). Stannous chloride (2.5 μg Sn^{2+}) in 50 μl hydrochloric acid solution (0.1 M) was mixed with the antibody. The mixture was incubated for 60 min at room temperature with ^{99m}Tc-pertechnetate (185 MBq) eluted from a ^{99}Mo-^{99m}Tc generator, the final pH of the mixture was 4.5. The radiochemical yield was determined by chromatography using a Sephadex G-25 column (PD-10). The biodistribution was performed by injecting ^{99m}Tc-antimyosin into the tail vein of the normal mice. Animals were killed at 1, 4 and 24 h post-injection and selected organs were dissected, weighed and the associated radioactivity counted in a gamma scintillation counter. Organ distributions were then calculated as a percentage of the original injected dose/organ and dose/g of tissue.

3. RESULTS AND DISCUSSION

Murine IgG contains only 6 interchain and 12 intrachain disulphide bonds giving an absolute maximum of 36 –SH groups per antibody molecule[19]. Following the determination by Ellman's method, the reduced antibody was found to contain 14.81 –SH groups per molecule, thus proving the efficiency of the reduction. However part of the measured thiols groups can

be related to the presence of dithiothreitol not completely removed in the filtration step. The radiochemical yield determined using Sephadex G-25 column was 90.06 ± 1.53 % (Table 1). Radiochemical impurities were removed by passage of the final reaction mixture through the Sephadex G-25 column (PD-10) to remove unbounded ^{99m}Tc ($^{99m}TcO_4^-$) and hydrolized technetium (TcO_2). After purification procedure, the radiochemical yield was > 98%.

Table 1. Radiochemical Yield of ^{99m}Tc-Labeled Antimyosin Monoclonal Antibody.

ANTIMYOSIN MONOCLONAL ANTIBODY	
Labeling	**Radiolabeled Antibody (%)**
1	89.31
2	89.99
3	87.80
4	89.78
5	92.10
6	91.40
Average	90.06 ± 1.53

In the biological study, the ^{99m}Tc-antimyosin showed a biodistribution pattern similar to the obtained by other authors [4-6, 12]. The biodistribution study (Table 2 and Table 3) showed low uptake in the stomach and thyroid at different times (1, 4 e 24 hours) representing a small amount of unbounded ^{99m}Tc and a good stability of the purified ^{99m}Tc-antibody. High uptake in the liver demonstrates that the radiopharmaceutical is metabolized in this organ. The uptake in the normal heart was relatively low as expected.

Table 2. Biodistribution of ^{99m}Tc-antimyosin in *Swiss* Mice (n=4).

ORGAN	% INJECTED DOSE / ORGAN		
	1 hr	**4 hr**	**24 hr**
BRAIN	0.150 ± 0.019	0.100 ± 0.022	0.048 ± 0.012
THYROID	0.397 ± 0.161	0.573 ± 0.079	0.184 ± 0.038
LUNG	1.054 ± 0.097	0.698 ± 0.117	0.376 ± 0.055
HEART	0.351 ± 0.025	0.251 ± 0.027	0.156 ± 0.034
SPLEEN	1.070 ± 0.136	0.553 ± 0.227	0.402 ± 0.109
LIVER	23.263 ± 1.877	14.599 ± 4.122	8.494 ± 2.262
STOMACH	2.125 ± 0.187	2.070 ± 0.591	0.707 ± 0.176
MUSCLE	0.084 ± 0.002	0.049 ± 0.009	0.029 ± 0.004
KIDNEY	4.564 ± 0.381	4.244 ± 0.421	2.537 ± 0.346
SMALL INTESTINE	4.091 ± 0.286	5.540 ± 0.907	3.590 ± 0.515
LARGE INTESTINE	1.392 ± 0.146	3.015 ± 0.583	5.775 ± 0.589
BLOOD	0.265 ± 0.010	0.160 ± 0.030	0.058 ± 0.008

Table 3. Biodistribution of ^{99m}Tc-antimyosin in *Swiss* Mice (n=4).

ORGAN	% INJECTED DOSE ORGAN / GRAM		
	1 hr	4 hr	24 hr
BRAIN	0.351 ± 0.036	0.234 ± 0.075	0.120 ± 0.030
LUNG	5.312 ± 0.497	3.739 ± 0.816	1.569 ± 0.230
HEART	4.173 ± 0.836	2.490 ± 0.571	1.352 ± 0.302
SPLEEN	11.890 ± 1.514	8.585 ± 2.760	6.022 ± 1.403
LIVER	20.882 ± 2.233	12.936 ± 3.833	7.522 ± 1.046
STOMACH	7.949 ± 0.831	7.229 ± 1.928	2.569 ± 0.501
MUSCLE	0.008 ± 0.000	0.005 ± 0.001	0.003 ± 0.001
KIDNEY	15.746 ± 0.345	15.317 ± 0.893	8.663 ± 0.225
SMALL INTESTINE	3.647 ± 0.660	4.842 ± 1.605	3.416 ± 0.895
LARGE INTESTINE	2.099 ± 0.395	5.291 ± 1.260	8.772 ± 1.893
BLOOD	0.128 ± 0.002	0.081 ± 0.017	0.029 ± 0.005

3. CONCLUSIONS

Based on these results, we concluded that the direct labeling procedure applied to the antimyosin monoclonal antibody allowed the easy preparation of the radiopharmaceutical with good stability to be used in the noninvasive diagnostic of the myocardial infarction. The used method of labeling allowed attainment of a product with high radiochemical yield.

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