COMPARISON OF ^{111}IN AND ^{99m}Tc LABELLED MONOCLONAL ANTIBODIES IN NORMAL MICE: BIODISTRIBUTION STUDIES AND ELECTROPHORETIC ANALYSES OF PLASMA SAMPLES*

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SUMMARY: Anti-melanoma monoclonal antibody (Mab) kits were obtained from Sorin, Italy. They were labeled with ^{111}In and ^{99m}Tc. The labeling efficiencies determined by thin-layer chromatography (ITLC) were 97% and 99% for ^{111}In-Mab and ^{99m}Tc - Mab, respectively. Each radiopharmaceutical was I.V. injected to 6 NMRI mice. Those mice injected with ^{111} In-Mab were sacrificed at 24 h and 48 h. The other 6 injected with ^{99m} Tc-Mab were sacrificed at 6 h and 24 h. Some blood and organs were taken out for weighing and radioactivity assay. The maximum percent uptake per whole organ was obtained in the liver (5.65-0.09 % at 24 h and 6.14-0.10 % at 48 h). The maximum % uptake per g organ was obtained in kidneys (21.70-0.40 at h and 16.18-1.64 at 48 h). When ^{99m}Tc-Mab was used the maximum % uptake per whole organ (11.67-0.32 at 6 h, 5.01-0.07 at 24 h) and per g tissue (73.55-3.36 at 6 h, 27.73-0.94 at 24 h) were obtained in kidneys. Electrophoretic analyses of plasma samples indicated that ^{111}In was transferred from Mab to plasma proteins, which explained the observed higher blood radioactivity levels compared to ^{99m}Tc-Mab.

INTRODUCTION

Radioimmunodetection by the use of radiolabelled antibodies against tumor antigens has drawn considerable attention in the diagnosis and treatment of cancer (1-3). Specific antibodies for various tumor antigens have been developed and labelled with radioisotopes such as ^{111}In, ^{131}I or ^{99m}T. For imaging purposes each one has advantages and disadvantages (4-5). ^{111}In is mostly preferred, because of its physical half-life of 2.8 days, since the maximum tumor uptake occurs 2-3 days after administration, and the stability of the label (3). Same antibodies when labelled with different radioisotopes exhibited differences in biodistribution, blood clearance and excretion (6,7). With ^{131}-label stomach, thyroid and bladder were visualized due to catabolism of localized or circulating antibody and liberation of ^{131}I (6). Published results have shown a high accumulation of ^{111}In in the liver, kidney, spleen and blood of animals bearing tumors with only a minimal radioactivity in the tumor tissue (5,8,9).

In the present investigation we compared the biodistribution of ^{111}In with ^{99m} Tc labelled monoclonal antibodies (Mab) against human melanoma antigens in mice and analyzed the plasma samples by electrophoresis to find out about the in vivo stability of the labelled antibodies.

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MATERIALS AND METHODS

Radiopharmaceuticals:

a) Preparation: Indomab-1 and Technomab-1 kits were obtained from Sorin Biomedica S.p.a., Italy. These are anti-melanoma monoclonal antibody [F (ab')$_2$] kits to be labelled with ^{111}In and ^{99m} Tc, respectively, and indicated for immunoscintigraphy. ^{111}In (10 mCi/ml 0.04 M HCl solution) and ^{99m} Tc generators were obtained from Amersham, England. The kits were labelled according to the directions. ^{111} In-Mab was prepared by adding 1 mCI ^{111}In in 0.1 ml solution to the kit vial and adjusting the volume to 1 ml. ^{111}In-DTPA was also prepared: 5 mg diethylene triamine pentaacetic acid (DTPA) was dissolved in 1 ml saline. The pH was adjusted to 5 with 1 N NaOH. The solution was millipored into a strerile vial. 10 uCi ^{111}In was added to the vial ^{99m} Tc-Mab was prepared by adding 4 mCi of pertecnetate to the kit vial. It was left to react at R.T. for 10 min. The solution was passed through a column containing Dowex 1x8 ion exchange resin (supplied by Sorin) to remove unreacted ^{99m} Tc.

b) Quality Control: Chromatographic quality control of the prepared radiopharmaceuticals was performed by the use of impregnated thin layer chromatography (ITLC) plates supplied by Glman Scientific Co. Saline, acetone or aq. Methanol (85%) were used as solvents. In-Mab, ^{111}In-DTPA, ^{99m}Tc-Mab and ^{99m} TcO_{4-} were all analyzed. The motility and the amount of impurities in the prepared radiopharmaceuticals were determined for each batch. The stability of ^{111}In-Mab and ^{99m}Tc-Mab were checked after 4 days and 24 h of

storage at R.T., respectively. The strips were cut into 1 cm segments and assayed for radioactivity in a gamma counter (Berthold: BF 5300, F.R.G.).

Animal Studies

a) Biodistribution: 6 NMRI mice (average wt: 30 g) were injected with 100 uCi ^{111}In-Mab (20 ug Mab) in 0.1 ml through the tail vein. They were sacrificed at 24 and 48 h by decapitation. Some blood and muscle, stomach, liver, lungs, kidneys, spleen, intestines, heart and one femur were removed, weighed and assayed for radioactivity in the same gamma counter against a standard prepared from a 1/1000 dilution of the injected solution. 6 NMRI mice (average wt: 30 g) were injected with 0.2 ml (200 uCi, 35 ug Mab) through the tail vein. They were sacrificed at 6 and 24 h post-injection. The same tissues and organs were removed and the sama procedure was followed.

b) Electrophoresis of plasma Samples: The blood samples obtained from mice were centrifuged at 3000 rpm for 10 min and plasma was separated. ^{111}In-Mab, ^{111}In-DTPA, ^{99m}Tc-Mab, $^{99m}TcO_2^-$ and the plasma samples were all analyzed by electrophoresis. Whatman No. 3 mm chromatography paper was spotted with the sample at a point 6 cm from one end. The electrophoresis was performed at 1000 V for 2.5 h in barbitone buffer (0.06 M, pH: 8.5) using a high voltage electrophoresis apparatus (Hormouth-Vetter, Pheograph, Model 64). The strips were dried and cut into 1 cm segments and assayed for radioactivity in the gamma counter.

RESULTS

In all the ITLC systems tested ^{111}In-Mab stayed at origin and ^{111}In-DTPA moved with the solvent front. The labelling efficiency was 97% right after preparation. ^{111}In-Mab was stable at R.T. The amount of ^{111}In-DTPA in the prepared mixture was only 5% up to 4 days after labelling.

In all the ITLC systems tested ^{99m} Tc-Mab stayed at the origin and ^{99m} TcO_4^-moved with the solvent front. The labelling efficiency was about 90% before passage through the column. 10% of the radioactivity was retained by the column. ITLC was repeated and only 0.93-0.82% of $^{99m}TcO_4^-$ impurity was obtained. When the prepared mixture was tested for stability after 24 h of storage at R.T. 44.4% free $^{99m}TcO_4$-was observed.

The biodistribution of ^{111}In-Mab in normal mice is shown in Table 1. The maximum % uptake per whole organ was obtained in liver (5.65+0.09% at 24 h and 6.14+0.10 % at 48 h). The maximum % uptake per g organ was obtained in kidneys (21.70+0.40 at 24 h and 16.18+1.64 at 48 h). This indicated the excretion of the radiopharmaceutical by kidneys.

The biodistribution of ^{99m}Tc-Mab in normal mice is shown in Table 2. The maximum % uptake was observed in kidneys, clearly indicating the excretion of ^{99m}Tc labelled compound by kidneys. Blood radioactivity levels were higher in ^{111}In than in ^{99m}Tc labelled Mab's, indicating slower excretion rate for ^{111}In.

Electrophoretic studies showed that ^{111}In-Mab and ^{99m}Tc-Mab stayed at the point of application. ^{111}In-DTPA moved 14-15 cm towards anode. ^{99m} TcO_4^- moved 22-25 cm toward anode. Plasma 1 obtained from mice after injection with ^{111}In-Mab showed one peak at 5-6 cm that contained 95% radioactivity. Plasma 2 obtained from mice injected with ^{99m}Tc-Mab showed 2 peaks, one at origin containing 60% and the other at 5-6 cm containing 40% radioactivity (Table 3).

Table 1: Biodistribution of ^{111}In-Mab in normal mice.

Organ	24 h	48 h
% Injected dose / Whole organ (mean±SD)		
Stomach*	0.34-0.02	0.25-0.04
Liver	5.65-0.09	6.14-0.10
Lungs	0.47-0.06	0.33-0.04
Kidneys	3.60-0.37	2.82-0.01
Spleen	0.61-0.09	0.59-0.08
Intestines*	1.93-0.44	1.09-0.42
Heart	0.36-0.04	0.27-0.01
Femur	0.27-0.01	0.25-0.01
% Injected dose/g or gan (mean-SD)		
Blood	4.00-0.03	1.36-0.19
Stomach*	2.13-0.16	1.72-0.13
Liver	3.49-0.16	3.85-0.20
Lungs	2.68-0.19	1.82-0.14
Kidneys	21.70-0.40	16.18-1.64
Spleen	4.90-0.12	4.44-0.23
Muscle	0.85-0.01	0.73-0.02
Intestines*	2.68-0.12	2.13-0.13
Heart	2.88-0.19	2.28-0.07
Femur	3.52-0.09	3.41-0.14

*Without contents

Table 2: Biodistribution of ^{99m}Tc-Mab in normal mice.

Organ	6 h	24 h
% Injected dose/Whole organ (mean–SD)		
Stomach*	0.060-0.007	0.30-0.002
Liver	1.90-0.32	0.93-0.059
Lungs	0.095-0.014	0.047-0.002
Kidneys	11.67-0.32	5.01-0.07
Spleen	0.068-0.003	0.049-0.002
Intestines*	0.49-0.06	0.20-0.14
Heart	0.047-0.07	0.015-0.009
Femur	0.026-0.001	0.014-0.001
% Injected dose/g or gan (mean-SD)		
Blood	0.82-0.06	0.24-0.02
Stomach*	0.44-0.01	0.19-0.02
Liver	1.47-0.18	0.57-0.03
Lungs	0.55-0.18	0.25-0.02
Kidneys	73.55-3.36	27.73-0.94
Spleen	0.67-0.05	0.39-0.06
Muscle	0.14-0.01	0.069-0.004
Intestines*	0.46-0.03	0.26-0.06
Heart	0.40-0.05	0.11-0.06
Femur	0.41-0.11	0.19-0.01

*Without contents

Table 3: Electrophoretic mobilities of the variois radioactive components analyzed at 1000 V for 2.5 h, using veronal buffer (0.06 M; pH:8.5).

Analyzed sample	Distance travelled towards anode	% in the main peaks
^{111}In-Mab	0	97
^{111}In-DTPA	14-15	100
Plasma 1	5-6	95
^{99m}Tc-Mab	0	98
$^{99m}TcO_4$	22-25	100
Plasma 2	0	60
	5-6	40

*Without contents

DISCUSSION

Radioimmunoscintigraphy (RIS) by the use of various radioisotopes bound to monoclonal antibodies was introduced to attain high specific concentration in tumor tissue for early in vivo diagnosis and follow -up of tumoral recurrences. However, the goal of obtaining high tumor specific concentration has not been attained. RGIS suffers from the same drawbacks as a tumor imaging agent as the routinely used ^{67}Ga citrate (10).

One of the important points to consider is the in vivo stability of the label. Iodine radioisotopes (^{123}I or ^{131}I) have been used to label antibodies, but it was found later that the iodine label was not as stable in vivo as it was in vitro. There was evidence that prior to the catabolism of antibody molecule dehalogenation occurred (5). ^{99m}Tc label is stable in vivo, but this radioisotope is not ideal for RIS, because of its short physical half-life of 6 h. The maximum accumulation of Mab's in tumor tissue occure 2-3 days after administration (2). ^{111}In with a physical half-life of 2.8 days and photon energies of 173 keV (89%) and 247 keV (94%) is the ideal radioisotope for RIS. However, ^{111}In may also be lost from the antibody in vivo. Ionic indium is bound to transferrin in plasma (11) and thus raises the background activity. In order to prevent the loss of ^{111}In from the antibody a new method of labelling was developed (12) where a strong chelator diethylene triamine pentaacetic acid (DTPA) was covalently coupled to the antibody molecule. This DTPA end of the molecule binds strongly with ^{111}In, forming a metal complex. In case of cleavage of ^{111}In-DTPA from the antibody molecule, a fast excretion of ^{111}In-DTPA via kidneys would solve blood background problem. However, animal experiments reported in the literature (5-7) and also in the present investigation show high blood radioactivity levels. In the present study % injected dose/g blood was 4.00-0.03 with ^{111}In, but only 0.24-0.02 with ^{99m}Tc 24 h after administration. Analysis of plasma samples by electrophoresis showed that the high level of radioactivity was not due to ^{111}In-DTPA. There was one peak at 5-6 cm towards anode and no activity at 14-15 cm. This peak contained both the monoclonal antibody fragments still labelled with ^{111}In and also the plasma proteins, in this case transferrin labelled with ^{111}In. In the case of ^{99m}Tc-Mab the plasma contained 2 peaks, one at origin which is the intact ^{99m}Tc-Mab (60%) and the other at 5-6 cm containing the labelled antibody fragments (40%) (Table 3). So the activity in plasma of animals administered with ^{111}In-Mab is due to ^{111}In-transferrin.

Our study showed that ^{111}In-Mab is not stable in vivo. There is a transfer of label from Mab to plasma proteins in spite of the fact that DTPA was coupled to Mab to make the label more stable.

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