Effect of some factors on direct ^{99m}Tc monoclonal antibody labelling efficiency*

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Abstract This study is focused in the influence of some experimental factors on direct ^{99m}Tc monoclonal antibody labelling method. The factors include weak ligands, reducing agent content and reduction time used in labelling. Biodistribution and scintigraphic imaging of animals are also performed. Finally, optimal parameters are supposed.
Keywords Technetium-99m labelling, Monoclonal antibody (MoAb), Ligands, Biodistribution

1 Introduction

Technetium-99m is inexpensive and readily available. It is well-suited for tomography because a high dose can be administered and the gamma energy is optimal for the scintigraphic measurements. Therefore, technetium-99m-labelled monoclonal antibodies prepared with the simple, fast and safe procedure described by Schwarz *et al* [1] have attracted a lot of attention in the past years.[2–5] This study was aimed to evaluate some experimental factors on radiochemical yield and scintigraphic characteristics, such as antibody reduction and transition weak ligand used, for ^{99m}Tc labelling of monoclonal antibody. The availability of ^{99m}Tc labelled antibody without 2- mercaptoethanol (2ME) pretreatment in scintigraphy was also tested.

2 Experimental

The IOR-1, human-IgG and anti-CEA-170 provided by IAEA, and the home-developed monoclonal antibodies, IA5, Ng76 and LC-1 were used in this study. To antibodies, 2 ME in 0.05 mol/L PBS solution (pH 7.4) was added, then swirled and incubated for given time at room temperature (20°C) in nitrogen atmosphere. In order to investigate the effects of reducing agent contents and reduction time on the labelling efficiency, different ratios of the 2 ME to antibody as well as incubation time of 30 and 90 min were chosen. The reduced antibodies were purified on Sephadex G-25 column (1.5 cm x 7.0 cm), previously equilibrated with 0.001 mass fraction BSA in PBS, and eluted with 0.05 mol/L PBS containing 1 mmol/L 2ME monitored by UV detector and purged with nitrogen. The protein containing fractions was filled with nitrogen, after being sampled to measure its concentration, divided into aliquots, frozen immediately at -70°C, and stored ready for use.

A vial of domestic MDP kit (containing 5 mg $SnCl_2$ and 5 mg MDP) was reconstituted with 0.05 ml nitrogen purged saline, 20 μl of it was added to every 0.2 mg reduced (unreduced) antibody (in 0.05 mol/L PBS). ^{99m}Tc-pertechnetate solution of 74 MBq (2m Ci) was added to each test, made equal volume with saline, swirled and incubated for given time (30 and 90 min, respectively) at room temperature (20°C), then purified on Sephadex G-50 column using saline as eluant. Labelling efficiency was analysed by paper chromatography using saline as mobile phase (Fig.1). The labelling efficiencies obtained from reduced antibodies are evidently higher than those from unreduced antibody ($P < 0.01$)(Fig.2). The experimental results give some evidences that labelling efficiency increases with increasing reduction time

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as shown in Table 1, and generally increases with increasing reducing agent content, but over reduction, on the contrary, could decrease labelling efficiency as shown in Table 2, where an extreme case (mole ratio of 2 ME to antibody is 5000:1) is presented.

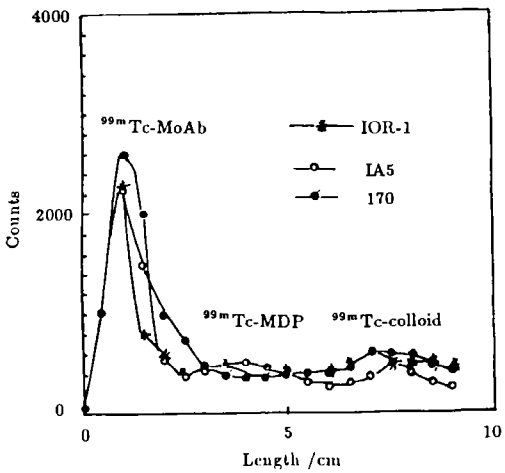

Fig.1 Radiochromatography of MoAbs

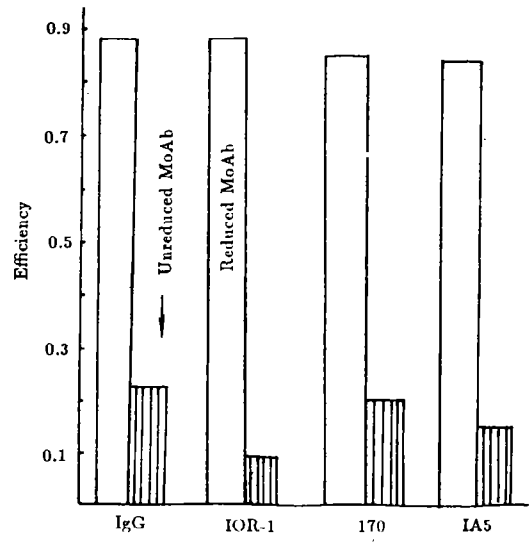

Fig.2 Effect of 2 mercaptoethanol reduction on labelling efficiency

Table 1 Influence of reduction time on labelling efficiency

Antibody	Reduction time/min	Efficiency	*P*
170	90	0.826±0.055	<0.01
	30	0.274±0.094	<0.01

Table 2 Influence of reducing agent content (2ME:MoAb) on labelling efficiency

Antibody	500:1	1000:1	5000:1	*P*
170	0.351±0.063	0.522±0.112	0.274±0.094	< 0.01
Ng76	0.524±0.081	0.933±0.021	0.427±0.176	<0.05

The tartarate solution was reconstituted from a vial of the kit (containing 2 mg $SnCl_2$ and 20 mg tartaric acid) with 0.2 ml nitrogen purged saline. The citric solution was made by dissolving 5 mg $SnCl_2$ and 50 mg citric acid in 0.5 ml nitrogen purged saline. And $SnCl_2$ solution without ligand (as control) was also used in parallel with the above MDP solution. Each solution containing the same amount of stannous ions (20 μg) was added to 0.15 mg of above reduced antibody. Finally, 74 MBq (2 mCi) ^{99m}Tc-pertechnetate was added and made equal volume with saline. After 30 min at room tem-

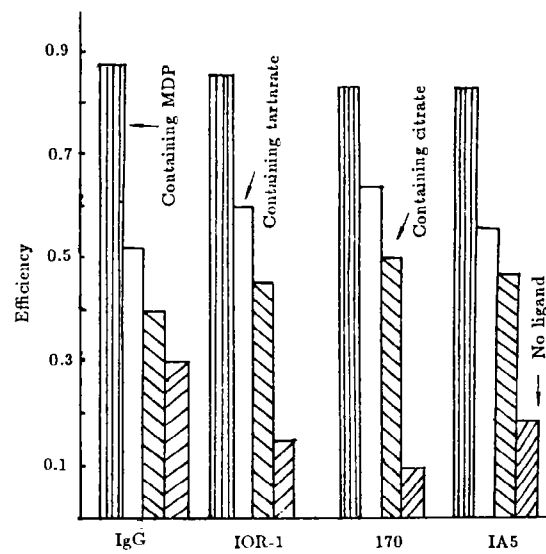

Fig.3 Effect of weak ligands (with $SnCl_2$) on labelling efficiency

perature (20°C) samples were analyzed. The results show that the weak ligands have very significantly enhanced effect on labelling efficiency and MDP is the best among the three ligands, as shown in Fig.3.

The domestic-developed anti-lung cancer monoclonal antibody LC- 1 fragment Fab and anti-melanoma monoclonal antibody Ng76 (used as control) were used to evaluate scintigraphic application of ^{99m}Tc-MoAb labelled with and without 2-mercaptoethanol pretreatment. 200 μl of MDP solution containing 100 μg stannous ion was added separately to 2 mg of LC-1 Fab and Ng76 in 0.05 mol/L saline solution. About 740 MBq (20 mCi) of ^{99m}Tc pertechnetate was added and after incubating of 30 min at room temperature(20°C) the labelling efficiency was ~0.85. The labelled antibodies were i.v. administered separately to lung cancer bearing BALB/C nude mice with dose of 150 μl (containing 80 MBq/each). Biodistribution and scintigraphic imaging of animals were performed on 24 h post injection. The distribution of LC-1 fragment had high tumour uptake over other tissues except kidney and bone. The target/nontarget ratios were 14.0±7.0, 13.0±6.7, 9.8±8.6, 4.2±2.3, 2.5±1.1, 1.1± 0.9 and 1.3±1.2, respectively for muscle, blood, heart, lung, spleen, liver and stomach (Fig.4). The animal imaging also exhibited good tumour concentration, while as control monoclonal antibody Ng76, which had not 2-mercaptoethanol pretreatment, had different distribution and insignificant tumour concentration. The T/N ratios for most parts of organs were less than 1.

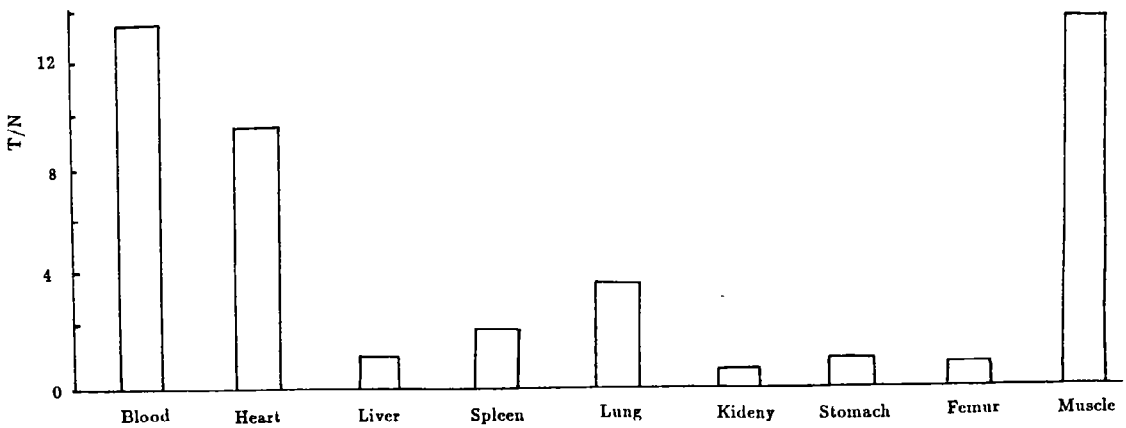

Fig.4 The T/N ratio of ^{99m}Tc-LC-1 in nude mice bearing human lung cancer

3 Conclusion

As shown above, we could come to the conclusion that the direct labelling of proteins with ^{99m}Tc depends on the amount of - SH groups created by the reduction, as pointed out by Mather *et al.*[3] Moreover, according to our experiments on labelling with different reducing agent contents and times it was found that an appropriate amount of -SH groups per molecule of monoclonal antibody is able to produce a high labelling efficiency and a much enhanced reduction which generates too much - SH/molecule during every unit time is, therefore, considered useless and even harmful.

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