Tumor Imaging by Monoclonal Antibodies Labeled with Radioactive Metal Ions

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INTRODUCTION

By the establishment of hybridoma technology by Kohler and Milstein in 1975[1], who were awarded Nobel prize of 1984, opened its new era in Nuclear Medicine. Monoclonal antibodies have been utilized in a) the radioimmunoassay of tumor markers, hormones and viruses, b) the radioimmunoimaging of cancer, myocardial infarction and infections, and c) the radioimmunotherapy of cancer.

Monoclonal antibodies are expected to consist of immunoglobulins recognizing tumor specific antigens and have great potential as agents to transport diagnostic and therapeutic quantities of radioisotopes to tumors with minimal deposition in other organs. Thus, radiolabeled monoclonal antibodies have been used for the in vivo detection of cancer. The production and usage of these reagents in oncology, drawing much attention, has expanded dramatically[2]. Up to now, a variety of monoclonal antibodies against human neoplastic cell types have been produced in many laboratories, such as antibodies directed against melanoma, osteogenic-sarcoma, breast, lung, stomach, prostate, and colorectal cancers, and so on.

It has been demonstrated that there are numerous factors which affect the outcome of the radioimmunoimaging of cancer or other diseases[3]. The choice of antigens to be targeted, the choice of radionuclides and radiolabeling procedures to be used, the choice of antibody or antibody fragments, the amount, the time and so on are still remained to be elucidated. In the present study, we will focus on the radioisotopes and radiolabeling procedures for the radioimmunoimaging of cancer, especially on the potential of metallic radionuclides for the labeling of monoclonal antibodies.

Monoclonal Antibodies against Osteosarcoma

Hosoi et al described monoclonal antibodies directed against osteosarcoma-associated antigens[6]. These antibodies were obtained by immunizing mice with freshly resected osteosarcoma

tissue, and evaluated by histological methods for tumor and organ tissue. One of these antibodies (Ost 7) showed a high degree of osteosarcoma specificity. I-131 labeled OST 7 monoclonal antibody specifically accumulated into human osteogenic sarcoma (KT 005) that was implanted s.c. into athymic nude mice, while no elevated radioactivity of radioiodinated OST 7 was found in the testicular or bladder tumor[7]. Osteogenic tumor KT 005 did not adsorb control nonspecific immunoglobulins. Further, by using $F(ab')_2$ fragments prepared with pepsin digestion, implanted tumor could be clearly delineated at 48 hours after the injection, since the clearance of $F(ab')_2$ fragments from the blood was much faster than the intact whole immunoglobulins (Fig. 1). The evidence that scintigrams could be obtained within 48 hours after the administration provided a good basis for the study of labeling with short half-lived radionuclides instead of I-131.

Labeling with Metallic Radionuclides by Using Bifunctional Chelating Agents

For the imaging of tumors, I-131 labeled monoclonal antibodies have been most widely used, although I-131 is clinically undesirable due to the high radiation dose and high energy emission[4]. However, as indicated in Fig. 5, radionuclides with short half-lives can be chosen for the radioimmunoimaging tumor by using antibody fragments, which allow sufficient clearance of the labeled antibody from the blood with enough tumor accumulation at the time of imaging. In contrast to I-131, I-123 has the most ideal nuclear characteristic for in vivo imaging, however, I-123 is quite expensive and shelf-life of I-123 labeled monoclonal antibody is too short for clinical use[3].

The selection of radioisotopes for radioimmunoimaging is based on the same criteria use din

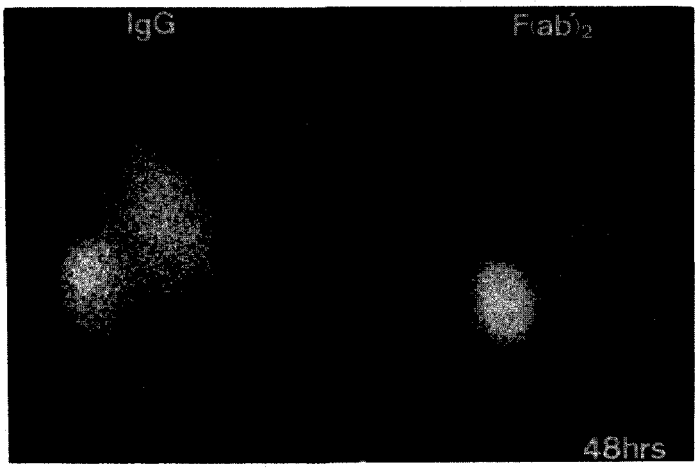

Fig. 1. Scintigrams of mice bearing human osteogenic sarcoma (KT 005). I-131 labeled monoclonal antibody; OST 7 (left) and its $F(ab')_2$ fragment (Right) was injected intravenously and scans were taken at 48 hours after the administration. I-131 labeled $F(ab')_2$ fragement demonsted higher tumor to blood ratio than that of intact whole IgG due to the faster clearance of antibody fragments from the blood. This high tumor uptake within 48 hours after the injection suggested the usefulness ofradioactive metal ions for the labeling of monoclonal antibodies.

choosing nuclides for other imaging studies[4]. We have selected metalic radionuclides, such as Indium(In)-111, Gallium(Ga)-67 and Technetium(Tc)-99m, for the labeling of monoclonal antibodies.

These radioisotopes have characteristics of a) the useful radiation for imaging, b) convenient short half-lives, and c) the simple and rapid radiolabeling[5].

The most common procedure is radiolabeling using proteins covalently tagged with metal-chelating groups, called "bifunctional chelating agents" which enables to use metal ions for the radioimmunoimaging. Radiolabeling of monoclonal antibody with metal ions consists of 2 steps[5]. In the first step monoclonal antibody is attached to a bifunctional chelating agent and in the second step, just before use, metallic radionuclides is added to form a stable complex. By applying two separate steps for the preparation fo antibody labeling with radionuclides, monoclonal antibodies conjugated with bifunctional chelating agents can be stored in non-radioactive form for several months. In addition, metallic radionuclides are labled with antibody through the chelation by a simple mixing with high efficiency and reproducibility, and purification steps are not necessary, as frequent steps in radioiodination. Therefore these methods will be applied for the preparation of labeling kits.

Bifunctional chelating agents are molecules that contain both a chelating group which binds metal ions and a reactive functional group capable of reacting with protein side chains[5]. Among various bifunctional chelating agents we have studied, the followings were the choice of chelating agents for the antibody labeling with metal ions.

Indium-111 ---------- DTPA Antibody
Diethylenetriaminepentaacetic acid

Gallium-67 ---------- DFO Antibody
Deferoxamine

Technetium-99m -------- DTS Antibody
Dithiosemicarbazone

The obtained antibody-metal conjugates were found to be quite stable both in vitro and invivo, without interfering the antibody reactivity. Monoclonal antibodies labeled with radioactive metal ions offered many advantages over the I-131 labeled antibodies.

Indium-111 Labeling

Among various radioactive metal ions, In-111 has been most extensively investigated for the labeling of monoclonal antibodies[5,8-11], since In-111 has 64 hour half-life which matches well with the maximum tumor concentration and produces a useful energy range for imaging (173 and 247 KeV).

Radiolabeling of monoclonal antibody with In-111 is accomplished through the chelation with deithylenetriaminepentaacetic acid (DTPA), which is used as a bifunctional chelating agent. DTPA is coupled to monoclonal antibody with cyclic DTPA is coupled to monoclonal antibody with cyclic DTPA anhydride. The obtained DTPA-antibody conjugates are stable and can be stored frozen for several month in a non-radioactive form.

In an animal experiment, using monoclonal antibody against α-fetoprotein (AFP) as a model, DTPA conjugation with antibody influenced both the antigen-binding activity and the biodistribution in nude mice transplanted with AFP-producing human testicular tumor. We have found that the number of DTPA molecules incorporated per antibody molecule was critical, which was dependent on thepH, antibody concentration and the added cyclic DTPA anhydride to antibody molar ratio during the reaction of DTPA coupling[11].

For example, In-111 labeled monoclonal antiody containing more than 414 DTPA molecules per antibody molecule, of which the antigen binding activity was only one hundredth of the original antibody, showed low tumor accumulation but high liver uptake. However, In-111 labeled antibody which contained 0.9-1.0 DTPA molecules per antibody molecule showed almost full retention of Ab activity and higher tumor accumulation than I-131 labeled one (Fig. 2). Localization of In-111 labeled antibody in the tumor was 10.9% injected dose/gram tissue, comparing to 4.3% of radioiodinated antibody.

Dehalogenation in the tumor is considered to be a cause for the low tumor accumulation of radioiodinated antibodies[8]. Although the radioactivity of the liver is relatively high with In-111

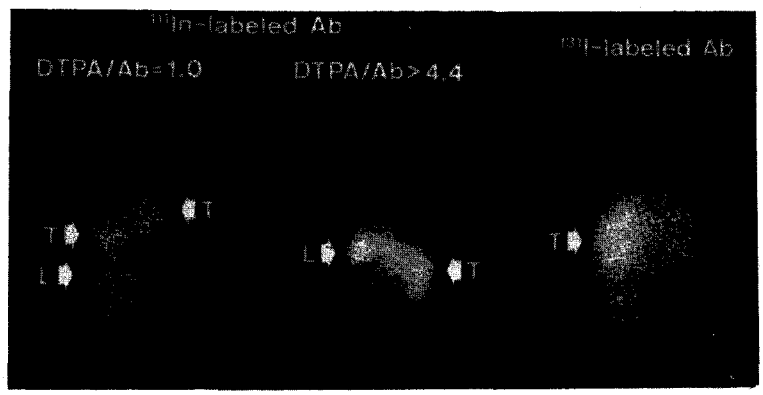

Fig. 2. Scintigrams of mice transplanted with human AFP-producing testicular tumor. In-111 labeled monoclonal antibodies against AFP containing 1.0 DTPA molecule per antibody (left) and containing more than 4.4 DTPA molecules per antibody (center) and I-131 labeled antibody were injected intravenously and scans were taken at 4 days after the administration. In-111 labeled antibody with 1.0 DTPA molecule per antibody, retained its original antibody reactivity, showed higher tumor uptake than that of radioiodinated antibody. However, In-111 labeled antibody containing 4.4 DTPA molecule per antibody molecule, losing its antigen binding activity, showed low tumor uptake, but the radioactivity in the liver was high, reflecting the denaturation of antibody by the heavy conjugation with DTPA.
T=tumor, L=Liver.

labeled antibodies which is a major problem in case of imaging of the abdominal tumor, In-111 labeled antibodies can be prepared with almost full retention of the antibody activity under proper conditions and offer a significant advantage over the iodinated species for tumor imaging. Thus, In-111 labeled monoclonal antibody will be a more suitable radiopharmaceutical for the radio-immunoimaging of cancer.

Gallium-67 Labeling

Labeling of monoclonal antibody with Ga ion has been performed by using Deferoxamine (DFO) as a bifunctional chelating agent[12, 13]. DFO has very high affinity for Fe and Ga ions. But comparing to DTPA or EDTA derivatives, affinity for Ca or Mg ion is very low. DFO is a safe, non-toxic chelating agent and successfully used in the treatment of iron-storage disease.

DFO tagged monoclonal antibody was easily and efficiently labeled with Ga-67 chloride by simple mixing. Both in vitro and in vivo studies indicated that Ga-67 labeled antibody was stable with preserving the original antibody reactivity and transplanted tumor could be visualized by using Ga-67 labeled monoclonal antibody (Fig. 3).

However, background imaging of liver and kidney was higher than I-131 labeled antibody, as was the case in the In-111 complexes. Column chromatography indicated the presence of big molecules, formed during the coupling reaction, which reflected the inter-molecular cross-linkage (Fig. 4 and 5). It is considered that inter- or intra-molecular cross-linkage, which is a major

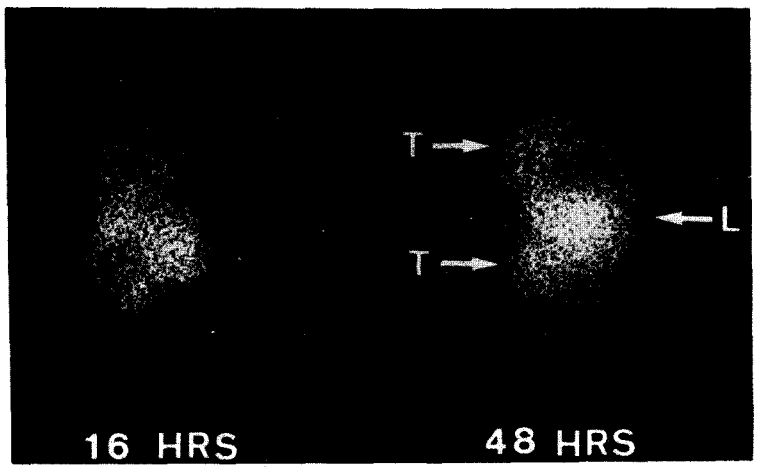

Fig. 3. Scintigrams with Ga-67 labeled $F(ab')_2$ fragment of monoclonal antibody to HCG β-subunit in testicular tumor-bearing nude mice. Forty μCi of Ga-67 labeled antibody was injected into nude mice and scans were taken with a pinhole collimater at 16 and 48 hours after the injection. Implanted tumor could be visualized with Ga-67 labeled antibody, in spite of background imaging of liver, kidney and spleen. Biodistribution of injected antibody in tumor-bearing nude mice was studies at 48 hours after the injection. Organ to blood radios were; tumor 2.61, liver 6.10, Kidney 13.57, spleen 3.70, Stomach 0.29, intestine 0.65, lung 1.04, muscle 0.34 and bone 1.34.
T=tumor, L=Liver.

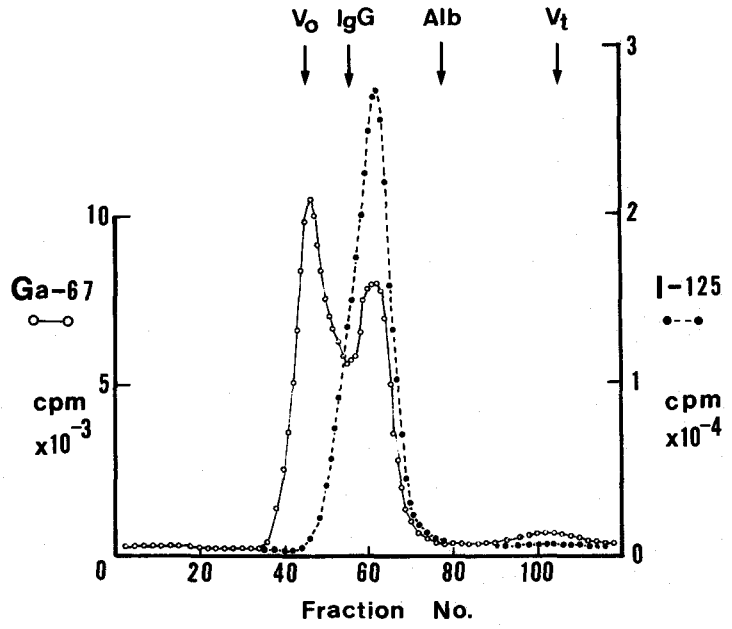

Fig. 4. Sephadex G-150 column chromatography of Ga-67 (o-o) and I-125 (o-o) labeled $F(ab')_2$ fragments of monoclonal antibody to HCG β-subunit. Approximately half of Ga-67 labeled $F(ab')_2$ fragments was eluted at the void volume, probably due to the formation of the inter-molecular crosslinkage in DFO-antibody conjugates. Antibody activities of fractions obtained from the first and second peak of Ga-67 labeled $F(ab')_2$ fragments were examined by the radioimmunoassay and Scatchard plot analysis as shown in Fig. 5.

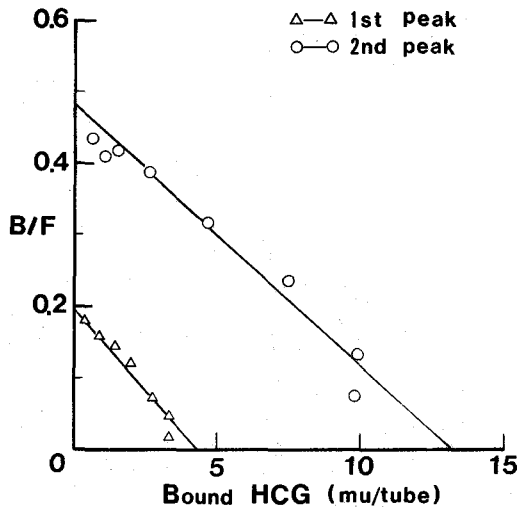

Fig. 5. Scatchard plot of antibody obtained from the first ($\Delta-\Delta$) and second (o-o) peak of Ga-67 labeled $F(ab')_2$ fragment of monoclonal antibody to HCG β-subunit. I-125 labeled HCG, unlabeled HCG and 1st or 2nd peak, obtained from Fig. 4, were incubated, precipitated with anti-mouse IgG antibody and analysed by Scatchard plot. The maximum binding capacity of the 1st peak of high molecular weight was less than one third of the 2nd peak, in spite of having similar affinity constant. These results indicated that inter-molecular crosslinkage formed in Ga-67 labeled monoclonal antibody, would be responsible for the deactivation of antibody-binding activities.

problem when monocloanl antibodies are labeled with metal ions, may be responsible for the high uptake in the liver and also for the loss of antigen-binding activities.

Ga-68 is a positron-emitting agent, produced by the generator system. Ga-68 can be labeled with monoclonal antibodies by using the same method with Ga-67, yielding higher specific antivities due to its very short half-life (68 minutes). Availability of Ga-68 and facilities of positron emitting tomography will make Ga-68 labeled monoclonal antibody a very useful tool for the tomographic imaging of various tumors.

Technetium-99m Labeling

Tc-99m is the almost ideal radionuclide not only from its nuclear properties but from its ready availability from a generator. However little has been reported on the labeling of monoclonal antibodies with Tc-99m, since the bonding between Tc-99m and antibody is vulnerable to oxidation and transchelation and suitable bifunctional chelating agents for the radiolabeling of antibody were not reported.

Recently we have found that p-carboxyethylphenylglyoxal bis (N-methylthiosemicarbazone) (CE-DTS) is the choice of chelating agent, which contains di-thiosemicarbazone (DTS) as the chelating site on one side and a carboxyl group as a reacting group with monoclonal antibodies[14]. CE-DTS-antibody conjugates retained its original antigen-binding activity and chelated with Tc-99m in the acetate buffer in the presence of very low $SnCl_2$.

The obtained Tc-99m labeled antibody was as stable as radioiodinated antibody both in vitro and in vivo and comparative studies with I-131 labeled ones indicated a similar disappearance from the blood (Fig. 6). Further the uptake of radioactivity in the stomach was elevated with

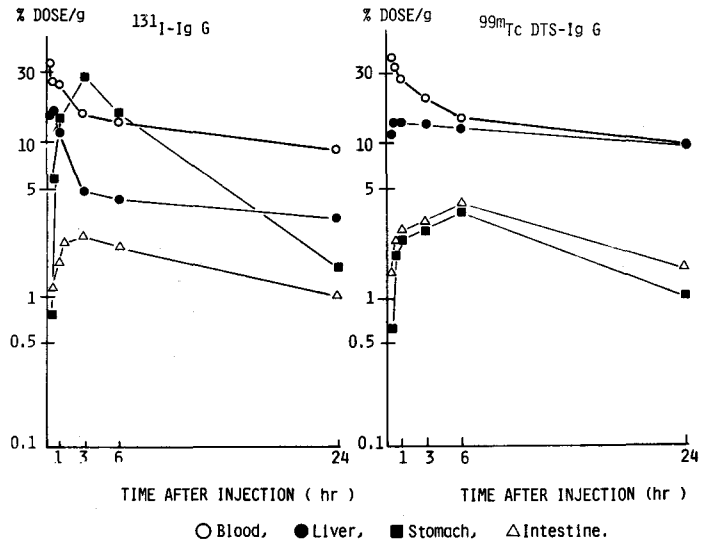

Fig. 6. Comparative distribution of I-131 (left) and Tc-99m (right) labeled antibodies in normal mice. Both preparations showed a very similar clearance from the blood. Radioactivity of Tc-99m in the stomach was lower than that of I-131 labeled antibody, indicating the in vivo stability of Tc-99m complexes.

radioiodinated antibody but not with Tc-99m complexes, indicating that in vivo breakdown to free pertechnetate and/or in vivo ligand exchange with other serum protein seemed negligible. The radioactivity of the liver, in contrast to radioiodinated antibody, was again high with Tc-99m, which may be independent of the metal ions used for radiolabeling and remains a big obstacle to be overcome.

Labeling of monoclonal antibody with Tc-99m using CE-DTS as a bifunctional chelating agent seemed to be satisfactory and the method is applicable for the preparation of labeling kits. Therefore, in the near future, the Tc-99m labeled products with desirable nuclear properties will be hopefully used in the community hospital as well as in the research centers.

SUMMARY

Monoclonal antibodies have become widely investigated in the Nuclear Oncology, especially in the radioimmunosassay of tumor markers and in vivo radioimmunoimaging of cancer. However, there are numerous factors as to whether radioimmunoimaging will ultimately successful. For imaging of tumors, metallic radionuclides such as In-111, Ga-67, Tc-99m have favorable nuclear properties than widely used I-131. These radioistopes have characteristics of the useful radiation for imaging, convenient short half-lives and the simple and rapid radiolabeling of monoclonal antibodies by using bifunctional chelaing agents[5]. The obtained chelate-tagged antibodies are quite stable both in vitro and in vivo, without interfering antibody activities and animal experiments provided a good basis for its clinical applicability for the radioimmunoimaging of cancer. Much attention has also been given to the possibility, only beginning to be exploited, of the specific treatment of malignant neoplasms with these agents[2]. Although specific antibody has not been developed that is uniquely specific for cancer alone[2] and there are still many questions to be answered and problems to be overcome before radioimmunoimaging can be successfully used in ptients with cancer[3], these methods can be applied to the coupling of monoclonal antibodies with anti-neoplastic drugs or radionuclides suitable for internal radiation therapy of cancer[15].

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