Effect of Tumor Mass and Antigenic Nature on the Biodistribution of Labeled Monoclonal Antibodies in Mice

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ABSTRACT

The effect of tumor mass and antigenic nature on the biodistribution of 111In- and 125I-labeled monoclonal antibodies (MoAbs) was studied using F(ab')2 fragments of three representative anti-tumor MoAbs and SW1116 human colorectal carcinoma grown in nude mice. The 19-9, F33-104 anti-CEA, and 17-1A MoAbs showed specific binding to SW1116 cells. The former two MoAbs recognize circulating CA 19-9 with molecular weights of more than 5,000,000 and CEA of M, 170,000-180,000, respectively, whereas 17-1A reacts with a nonshedding antigen. Both percentage injected dose per gram tumor and tumor-to-blood ratios were inversely proportional to the tumor mass in nude mice administered 111In- and 125I-labeled 19-9, but liver uptake increased as tumor size increased. Analysis of serum samples and tumor homogenates demonstrated the presence of a high-molecular-weight species, probably due to the antibody binding to CA 19-9. In the case of 111In-labeled anti-CEA MoAb, tumor uptake also decreased and liver uptake increased with tumor size, but this effect was less obvious than that of 19-9. In contrast, tumor and liver uptake of 125I-labeled anti-CEA MoAb, 111In- and 125I-labeled 17-1A and control antibodies were independent of tumor mass. The absolute tumor uptake and tumor-to-blood ratios of all 125I-labeled antibodies were lower than those of the 111In-labeled ones. And the effect of tumor mass was also weaker with 125I-labeled antibodies, probably due to in vivo dehalogenation. These results indicate that the effect of tumor size on the incorporation of labeled MoAb into tumors is dependent on the antigenic nature to be targeted and/or radionuclides used for labeling and that high concentrations of circulating high molecular weight antigens may limit in vivo use of MoAb conjugates.

INTRODUCTION

The development of hybridoma technique has made the production of MoAbs feasible, and MoAbs have been employed for a variety of purposes, including the diagnosis and therapy of malignancies in humans (1-8). Radionucleoti and immunotherapeutic agents are conjugated with MoAbs to target cytotoxic agents to tumor cells for direct killing. Such an approach has the advantage of increasing tumoricidal activity and decreasing the systemic toxicity of these cytotoxic agents. These applications require the knowledge of pharmacokinetics and biodistribution of labeled MoAbs after administration to patients, since both the sensitivity of immunonaming and the effect of therapy using MoAb conjugates are dependent on the absolute tumor uptake and/or on the tumor-to-normal tissue ratios of labeled MoAbs (9-18). The outcome of therapy will be estimated from the absolute tumor uptake, the tumor weight and the response to cytotoxic agents. However, the biodistribution of MoAb conjugates are greatly influenced by many factors, such as the nature of antigens to be targeted, antibody or its fragments, radionuclides, coupling methods, the size of tumor and so on (10-18).

Received 8/8/88; revised 1/18/89; accepted 2/21/89.

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2 The abbreviations used are: CEA, carcinoembryonic antigen; MoAb, monoclonal antibody; DTPA, diethyleneetriamine pentacetic acid.

There have been conflicting findings concerning the relationship between the tumor size and the uptake of antibody conjugates administered i.v. (12-15). In the interest of establishing the effect of tumor mass, the nature of antigens and radionuclides on the biodistribution of MoAb conjugates, we have extended this investigation to include well-characterized three MoAbs reactive with gastrointestinal tumors, SW1116-NS-19-9, anti-CEA, and CO 17-1A. These MoAbs recognize different representative cancer-associated antigens, CA 19-9, CEA, and 17-1A, respectively, all of which were expressed on human colorectal cancer cells SW1116 (19-28). F(ab')2 fragments labeled with 111In- and radioiodine were administered to nude mice bearing SW1116 tumors by the same route and at the same dose, to avoid nonspecific binding of Fc portion of MoAbs to Fc receptors and to increase tumor-to-normal-tissue ratios by the rapid clearance from circulation.

MATERIALS AND METHODS

Monoclonal Antibodies. F(ab')2 fragments of murine MoAb 1116-NS-19-9 (IgG1), CO 17-1A (IgG2a), and anti-CEA (F33-104) (IgG1), as well as normal murine immunoglobulin controls were used in the present study (19-26). Both uncoupled and DTPA-coupled F(ab')2 fragments of MoAb 1116-NS-19-9 and CO 17-1A were provided from Centocor (Malvern, PA) through the courtesy of Nihon Mediphysics Co. (Takarazuka, Japan). These three MoAbs are known to be reactive with SW1116 human colorectal carcinoma cells (19, 25-28). F(ab')2 fragments of normal murine immunoglobulin were supplied by Nihon Mediphysics. MoAb 1116-NS-19-9 recognizes a sialylated fucopentaose II carbohydrate determinant, designated CA 19-9 expressed on circulating glycoprotein antigens with molecular weight of more than 5,000,000 (21, 22, 28) and anti-CEA MoAb (F33-104) is reactive with the CEA-specific protein part of CEA with molecular weight of 170,000-180,000 (23, 24). CA 19-9 and CEA are representative cancer-associated antigens released into circulation, whereas 17-1A is reported to react with a nonshedding antigen (25, 26).

Radiolabeling. Antibodies were readily labeled with 111In via chelation with DTPA by simply incubating DTPA-coupled F(ab')2 fragments and [111In]chloride for 30 min, at room temperature, and the labeling efficiency was more than 90% without any further purification steps. The chloramine T method was employed for the radio-iodination of MoAb, as described previously (29). The specific activity of radiolabeled MoAb was between 0.5 and 2 mCi/mg for 111In-labeled antibodies, between 4 and 7 mCi/mg for 125I-labeled antibodies and between 1.5 and 3 mCi/mg for 131I-labeled antibodies.

The immunoreactive fractions of radio-labeled F(ab')2 fragments of MoAb 19-9, 17-1A, and F33-104 were calculated as about 0.60, 0.60, and 0.40, respectively, according to the method of Lindmo et al. (30). The affinity constants for 19-9, 17-1A, and F33-104 to SW1116 cells were determined as 1.5 x 109, 0.5 x 109, and 1.3 x 109/M, respectively, by the Scatchard plot analysis (29).

Tumors. BALB/c nude mice were xenografted with human colorectal cancer cell line SW1116 by implanting 5 x 106 cells s.c. in their left flank (28). Tumors were allowed to grow for 2 to 12 weeks until they reached the desired size. SW1116 tumors usually grew to 1 g in about 5 to 7 weeks after injection and had only very small necrotic zones. Thyroids were blocked with drinking water containing 0.1% w/v iodo-potassium from one day before to the end of the study. The serum of
xenografted nude mice was obtained by retro-orbital puncture two days before the injection of radio-labeled MoAbs. Serum CA 19-9 and CEA concentrations of the mice were determined at dilutions of 1:2 and 1:5 for CA 19-9 and without dilution for CEA, using commercially available kits (CIS, Saclay, France and Daiichi Radioisotope Laboratories, Tokyo, Japan) (24, 31).

Biodistribution and Imaging. Nude mice bearing SW1116 xenografts were given i.v. injection of $^{111}$In- and $^{125}$I-labeled F(ab')$_2$ fragments. The antibody dose was adjusted to 20 $\mu$g per mouse by mixing labeled and unlabeled MoAb. Tumors weighing 0.1-2.5 g were used in the present study. Animals were sacrificed 48 h after the administration and the tissues were weighed and counted. The biodistribution data were represented as a percentage of the injected dose per gram normalized to a 20-g mouse and tissue-to-blood ratios.

Statistical analysis was performed using linear regression analysis and variance analysis.

Animal images were made using a gamma-camera (Searle, Chicago, IL), equipped with a pinhole collimator at 6, 24, and 48 h following the administration of 100 $\mu$Ci of $^{111}$In-labeled MoAbs.

Chromatographic Analysis. Various samples were analyzed by gel-filtration chromatography in the search for chemical forms of $^{125}$I in the samples. The injectate, serum samples of nude mice and soluble fractions of liver and tumor homogenates of nude mice (supernatants obtained by centrifugation at 100,000 x g for 60 min) were applied to a Sephacryl S-300 column (1.6 x 95 cm) equilibrated in 50 mM PBS, pH 7.5. Fractions were collected and the eluted radioactivity was counted with a well gamma-counter.

RESULTS

Tumor Mass and Serum Antigen Concentrations. In order to establish the relationship between tumor size (weight) and concomitant CA 19-9 and CEA release from SW1116 cells in nude mice, serum samples were obtained from mice bearing SW1116 tumors, and serum CA 19-9 and CEA concentrations were determined as described in “Materials and Methods.” As reported by Klug et al. (28), there was a significant correlation between the size of tumors and serum CA 19-9 values in nude mice. When serum CA 19-9 levels in units/ml was plotted against tumor mass in grams, the correlation coefficient was 0.72, the slope was 59.97, and the y intercept was 16.6, using linear regression analysis ($P < 0.001$) (data not shown). Circulating CEA levels also correlated well with the tumor size ($r = 0.71, P < 0.001$) (data not shown).

Effect of Tumor Mass on Tumor Uptake. $^{111}$In- and $^{125}$I-labeled F(ab')$_2$ fragments of 19-9, anti-CEA, and 17-1A MoAb showed specific localization in SW1116 tumors grown in nude mice. Mean tumor-to-blood ratios of these $^{111}$In-labeled MoAbs were 33.4 ($n = 25$), 5.5 ($n = 10$), and 13.4 ($n = 12$), respectively, at 48 h after the administration. Tumor uptake expressed as percentage injected dose per gram tumor and tumor-to-blood ratios of all $^{111}$In-labeled MoAbs were higher than those of radioiodinated ones (Figs. 1 and 2). As shown in Fig. 1A, in nude mice administered with $^{111}$In-labeled 19-9 and anti-CEA MoAb, the tumor uptake was inversely proportional to the tumor size ($r = 0.65, P < 0.01$ and $r = 0.59, P < 0.01$, respectively). But the effect was more intense with 19-9 (slope of $-6.40$) than with anti-CEA MoAb (slope of $-0.98$), and the tumor uptake of $^{111}$In-labeled 17-1A did not demonstrate a
significant correlation with tumor size. As shown in Fig. 2A, tumor-to-blood ratios of \( \text{\textsuperscript{111}}\text{In} \)-labeled 19-9 and anti-CEA MoAb also decreased as tumors enlarged \( r = 0.45, P < 0.05 \), slope of \(-10.93 \) and \( r = 0.42, P < 0.05 \), slope of \(-1.21 \), respectively) and tumor-to-blood ratios of \( \text{\textsuperscript{111}}\text{In} \)-labeled 17-1A and control antibodies were independent of tumor size.

The effect of tumor size on tumor uptake was also seen with \( \text{\textsuperscript{125}}\text{I} \)-labeled 19-9 (Figs. 1B and 2B). Both the percentage injected dose per gram and tumor-to-blood ratios of radio-iodinated 19-9 decreased as tumor size increased \( r = 0.54, P < 0.01 \), slope of \(-1.11 \) and \( r = 0.47, P < 0.05 \), slope of \(-8.21 \), respectively). But the effect was less than that of \( \text{\textsuperscript{111}}\text{In} \)-labeled 19-9 (slope of \(-6.40 \) and \(-10.93 \), respectively). Tumor uptake and tumor-to-blood ratios of \( \text{\textsuperscript{125}}\text{I} \)-labeled anti-CEA, 17-1A and control antibodies were found to be independent of tumor size.

**Effect of Tumor Mass in Normal Organs.** In normal organs including the intestine, stomach, lung, muscle, and bone, with the exception of kidney which excreted radiolabeled metabolic products, \( \text{\textsuperscript{111}}\text{In} \) and \( \text{\textsuperscript{125}}\text{I} \)-labeled F(ab\(^\prime\))\(_2\) fragments of 19-9, anti-CEA, and 17-1A MoAb showed neither specific localization nor tumor size dependency (data not shown). However, \( \text{\textsuperscript{111}}\text{In} \) and \( \text{\textsuperscript{125}}\text{I} \)-labeled F(ab\(^\prime\))\(_2\) fragments of 19-9 were incorporated into liver and spleen more than those of the other three antibodies. The accumulation of \( \text{\textsuperscript{111}}\text{In} \)-labeled 19-9 and anti-CEA MoAb in liver increased with tumor size \( r = 0.81, P < 0.001 \), slope of 14.50 and \( r = 0.50, P < 0.05 \), slope of 1.60, respectively) (Fig. 3A). Liver-to-blood ratios of \( \text{\textsuperscript{111}}\text{In} \)-labeled 19-9 also increased as tumors enlarged \( r = 0.59, P < 0.01 \), slope of 48.09), showing greater effect than that of anti-CEA MoAb \( r = 0.55, P < 0.01 \), slope of 3.66) (Fig. 4A). This effect of tumor mass on liver uptake was less with \( \text{\textsuperscript{125}}\text{I} \)-labeled MoAb than with \( \text{\textsuperscript{111}}\text{In} \)-labeled MoAbs (Figs. 3 and 4). A similar effect of tumor mass on spleen uptake was also seen (data not shown).

**Scintigrams.** Scintigrams of nude mice following i.v. injections of \( \text{\textsuperscript{131}}\text{I} \)-labeled F(ab\(^\prime\))\(_2\) fragments of 19-9, F33-104, and 17-1A MoAbs confirmed the results of the biodistribution studies (data not shown). All three MoAbs visualized the SW1116 tumors xenografted in nude mice at 24 and 48 h after administration. High liver uptake was observed in nude mice administered with 19-9, but not with either anti-CEA or 17-1A MoAbs.

**Chromatographic Analysis.** Various samples including \( \text{\textsuperscript{125}}\text{I} \)-labeled F(ab\(^\prime\))\(_2\) fragments of 19-9, serum of nude mice, and liver and tumor homogenates were evaluated by Sephacryl S-300 gel-chromatography (Fig. 5). A radiolabeled high molecular weight peak appeared in the void volume in serum samples, and liver and tumor homogenates. More high molecular weight form was demonstrated in tumor homogenates than in liver homogenates, indicating the presence of an antigen-antibody complex. In addition, the majority of radioactivity in liver homogenates was not the labeled antibody in the injected form but a lower molecular weight species.

**DISCUSSION**

The principal object of this study was to examine the effect of tumor mass, radionuclide and antigenic nature on the tumor
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uptake, and biodistribution of labeled MoAbs. SW1116 human colorectal carcinoma cell line transplanted in nude mice was selected as a tumor model, because it expressed three representative cancer-associated antigens, CA 19-9, CEA, and 17-1A (19, 25-28). Nonimmunological factors that would affect the entry and handling of antibodies by tumor, such as vascularity, necrosis, lymphatic drainage, and extracellular space (32), would apply equally to tumor-specific 19-9, anti-CEA, and 17-1A MoAbs and control antibody, and the selective uptake of the former three MoAbs by the tumor seemed to be mainly attributable to the antigen-binding properties of the MoAbs.

An inverse correlation between tumor mass and uptake in the tumor, as well as a proportional increase in liver and spleen uptake with tumor size has been demonstrated in nude mice injected with $^{111}$In- and $^{125}$I-labeled 19-9, and $^{111}$In-labeled anti-CEA MoAbs, while these effects were not observed with the 17-1A or control antibodies. The major difference between the former two MoAbs (19-9 and anti-CEA) and the 17-1A MoAb and control antibody, and the selective uptake of the former three MoAbs by the tumor seemed to be mainly attributable to the antigen-binding properties of the MoAbs.

The effect of tumor mass was also observed with $^{111}$In-labeled anti-CEA MoAb, but less obvious than with 19-9. CA 19-9 is a mucin with molecular weight of more than 5,000,000 (21, 22), while that of CEA is $M_c$, 170,000–180,000 (23, 24). The antigenic nature to be targeted, such as a release into the circulation system or the difference of molecular weight, may be one of the reasons for different effects of tumor mass on the biodistribution of labeled MoAbs. However, other factors, such as crossreactivity of 17-1A with normal tissues (19), tissue permeability and so on, could influence the biodistribution in some way.

Similar effect was observed with $^{111}$In-labeled anti-CEA MoAb, but not with $^{125}$I-labeled one. It may be presumably due to rapid deiodination or the release of radioiodine from the MoAb, which would also explain why the tumor size effect of $^{111}$In-labeled 19-9 was stronger than with $^{125}$I-labeled one (13, 33).

In the present study, instead of intact MoAb, we employed F(ab')$_2$ fragments of four antibodies to avoid a nonspecific uptake caused by the Fc receptor, and a high tumor-to-normal-tissue ratio was reached by a rapid blood clearance of the radioactivity (17). Furthermore, we administered 20 $\mu$g of antibodies to each nude mouse bearing SW1116 tumor. In recent clinical trials, 20 to 100 mg of antibodies were used by adding unlabeled MoAbs for the diagnosis and therapy of tumors, since the tumor detection rate increased as the antibody administered increased (1, 2, 8). The 20 $\mu$g of antibody per mouse was equivalent to this range.

Fig. 3. Relationship between SW1116 tumor size and liver uptake of percentage injected dose per gram liver of $^{111}$In- (A) and $^{125}$I- (B) labeled F(ab')$_2$ fragments of 19-9 (top left), anti-CEA (top right), 17-1A (bottom left) and control (bottom right) antibodies.
Clinically, high liver deposit is often considered to be problematic in the use of \(^{111}\text{In}\)-labeled antibodies (11). However, surprisingly, liver uptake of labeled MoAbs varied, although some properties of the \(^{111}\text{In}\) radiolabel itself were similar. The highest liver uptake was seen with 19-9, as previously reported (11) and low with anti-CEA and 17-1A MoAbs. Liver metastasis was detected in some patients with colon cancer using labeled anti-CEA and 17-1A MoAbs (7, 8), though it may be masked by an intense liver uptake of labeled 19-9 MoAb.

Therapeutic applications of MoAb conjugated with radionuclides, toxins, or antineoplastic drugs will be also dependent on the results shown here. For example, the radiation dose estimate for tumor and normal tissues is directly proportional to the percentage injected dose per gram of tissue (34, 35). Yttrium-90, which is labeled for MoAbs through the chelation with DTPA, has been described as one of the best radionuclides for tumor therapy when conjugated with MoAbs (36, 37). In the case of 19-9 and DTPA-coupled anti-CEA MoAb, one would anticipate that smaller tumor could be given more radiation doses per unit tumor size with less toxicity to liver. Furthermore, if serum human anti-mouse antibody titer of a patient is high due to repeated injections of MoAb conjugates, the administered MoAb may be removed in the form of immune complexes from circulation, resulting in less tumor uptake (38). These results indicate that the effect of tumor size on the incorporation of labeled MoAb into tumors is dependent on the antigenic nature to be targeted and/or radionuclides used.
for labeling and that high concentrations of circulating high molecular weight antigens may limit in vivo use of MoAb conjugates.

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