Pharmacokinetics of $^{111}$In-labeled Anti-p97 Monoclonal Antibody in Patients with Metastatic Malignant Melanoma

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ABSTRACT

Twenty-eight patients with metastatic malignant melanoma received anti-p97 murine monoclonal antibody (96.5) infused over 2 h at doses between 1 and 20 mg coupled to either 2.5 or 5.0 mCi of $^{111}$In by the bifunctional chelating agent diethyltriaminepentaacetic acid. Clearance of $^{111}$In from plasma closely fit an open, one-compartment mathematical model ($r^2 > 0.90$). The overall half-life of $^{111}$In in plasma was approximately 31 h and did not appear to be dependent on the total dose of antibody administered. The apparent volume of distribution of the $^{111}$In label approximated the total blood volume (7.8 ± 0.7 liters) at the 1-mg dose and decreased to 3.0 ± 0.14 liters at the 20-mg dose, suggesting saturation of antigenic or other extravascular binding sites at higher antibody doses. The clearance of the murine monoclonal antibody itself from plasma was measured by an enzyme-linked immunosorbent assay. The pharmacokinetics for the murine antibody in plasma also fit an open, one-compartment mathematical model. All pharmacokinetic parameters for unlabeled antibody closely paralleled those found for $^{111}$In-labeled antibody pharmacokinetics. This suggests that the $^{111}$In radiolabel remains complexed to the monoclonal antibody after in vivo administration. The cumulative urinary excretion of the $^{111}$In label over 48 h was between 12 and 23% of the total administered dose and is assumed to represent $^{111}$In-labeled chelate complex unattached to antibody.

Analysis of the $^{111}$In label in spleen, liver, heart, and kidney showed that the concentration of label in liver tissue was reduced with increasing antibody doses and coincided with changes in the apparent volume of distribution.

These studies show that murine monoclonal antibodies are cleared slowly from the circulation in humans and that early, rapid distribution of labeled antibody to the liver can be reduced by increasing the dose of unlabeled antibody. This may be particularly important in limiting hepatic toxicity when administering antibody coupled to drugs, radionuclides, or toxins.

INTRODUCTION

Antibodies directed against antigenic determinants expressed on the surface of tumor cells have the potential to selectively localize in tumors after systemic administration. This property has been successfully exploited due to recent advances in hybridoma technology, which have made available large amounts of purified, high-specificity murine monoclonal antibodies directed against human tumor-associated cell-surface antigens (15). Several studies have described selective surface binding to tumor cells in vitro and in vivo tumor localization of chemotherapeutic agents (2, 14), immunotoxins (4), and radionuclides (3, 5, 6) complexed to antibody carriers.

Radiolabeled antibodies have been utilized by several investigators to locate and visualize a variety of tumors with varying degrees of success (7, 18). Although there are several techniques for radiolabeling proteins, several investigators (9, 10, 22) have covalently bound $^{111}$In to antibodies with bifunctional chelating agents. Recent studies by Hagan and coworkers (8, 11) have suggested that antibodies labeled with chelated $^{111}$In appear to attain higher tumor-blood ratios than antibodies radiolabeled with iodine. This improvement appears to be related to the biological stability of the $^{111}$In complex compared to that of the radiiodinated protein (12). In addition, the binding affinity of the antibody is not apparently altered by this chelation technique using $^{111}$In in contrast to the radiodiodeination procedure.

In concert with a Phase I clinical trial to determine imaging effectiveness and toxicity, we studied the plasma pharmacokinetics and the urinary excretion of $^{111}$In-labeled monoclonal antibody 96.5 reactive against the protein designated p97 found on most human melanoma cells. The pharmacology of the $^{111}$In label was also compared to that of the antibody itself, measured by ELISA assay, to determine whether the $^{111}$In label is stable in vivo. In addition, the relative tissue distribution of the $^{111}$In label was compared in patients receiving various doses of antibody to determine whether observed changes in tissue distribution reflect changes in the pharmacokinetics of the agent.

MATERIALS AND METHODS

The melanoma-associated antigen (p97) is found on over 80% of human melanoma cell lines and tumor extracts (25). It is also weakly expressed on breast tumor lines, melanocytes, and fetal tissues (24) and is similar in structure and function to a portion of the transferrin receptor (1). The antibody to p97 (designated 96.5) was made by immunizing BALB/c mice with a melanoma cell line (ST-Mel-28) and fusing spleenocytes with the NS-1 myeloma cell line to form hybrids as described previously (25). The antibody was further purified from ascites fluid using a Staphylococcus aureus Protein A column (Pharmacia, Uppsala, Sweden). Antibody 96.5 is an antibody of the IgG2a subclass; it is cytotoxic to melanoma cell lines in the presence of complement and can mediate antibody-dependent cell-mediated cytotoxicity in vitro (13).

The antibody was coupled to the chelating agent DTPA using a modification of the method of Kretzchmer and Tucker (18). The details of this method have been described previously by Halpern et al. (11). The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; $V_m$, half-life; $V_m$, volume of distribution; $C \times T$, concentration times time; PBS, phosphate-buffered saline; DTPA, diethyltriaminepentaacetic acid; p97, M, 97,000 glycoprotein.

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labeling yield was consistently 80 to 100% with routine specific activities being 10 mCi of $^{111}$In per mg antibody. Antibody specificity remained stable during this process. Immunoreactivity of this preparation was approximately 50%. Antibody 96.5 was supplied by Hybritech, Inc., in vials already bound to DTPA. Immediately prior to use, the DTPA-conjugated antibody was mixed with $^{111}$In in aqueous HCl and appropriate neutralizing buffer. An aliquot of this labeled antibody corresponding to either 2.5 or 5 mCi $^{111}$In was then added to a sterile 0.9% NaCl solution (saline) (200 ml) containing unlabeled antibody to adjust the total antibody dose.

Subjects. Twenty-six patients with biopsy-proven malignant melanoma received $^{111}$In-labeled p97 antibody. All patients gave written informed consent to the procedure in accordance with guidelines established by the human subjects committee at M. D. Anderson Hospital and Tumor Institute. Each patient received total doses of 1, 2, 5, 10, or 20 mg of 96.5 antibody, which contained either 2.5 mCi (21 patients) or 5 mCi (7 patients) of $^{111}$In. There were 4 to 5 patients studied at each antibody dose level. The antibody/isotope mixture in 200 ml of saline was administered as a 2-h i.v. infusion delivered by infusion pump. Each patient received one infusion at a single concentration of antibody. Total body imaging was performed using a longitudinal, tomographic imager (Phocon 192; Siemens Nuclear Imaging, Des Plaines, IL). In some cases, patients were also imaged on a conventional gamma camera with computer-assisted data storage (Gamma 11; Digital Equipment Corp., Westwood, MA). Background subtraction techniques were not used. The results of toxicity and image analysis following administration of $^{111}$In-labeled monoclonal antibody are presented elsewhere (21). To determine relative tissue distribution of the $^{111}$In label, densitometric analyses were performed with an X-Rite Model 301 densitometer (X-Rite Co., Grand Rapids, MI) on anterior and posterior whole-body scans obtained 4 h after infusion of labeled antibody. Average densitometric readings from several sites were obtained, and the means of readings of anterior and posterior scans were then calculated.

ELISA Assay for Murine Monoclonal Antibody In Human Serum. To 96-well microtiter plates (Costar, Cambridge, MA), a 50-µl aliquot of 50 µM bicarbonate buffer (pH 9.6) containing 30 µg of goat anti-mouse IgG antibody (Cappel, Cochranville, PA) per ml was added to each well. The plate was incubated overnight at room temperature and washed 4 times with 0.05% Triton X-100. A 50-µl aliquot of patient serum, PBS, or antibody standards in serum were diluted (1:2) with PBS and added to the plate in serial dilutions. The plates were incubated for 3 h at room temperature with shaking and then washed 10 times with 0.05% Triton X-100. A 50-µl aliquot of goat anti-mouse IgG conjugated to horseradish peroxidase (Cappel; diluted 1:8000) was added, and the plates were further incubated for 2 h at room temperature with shaking. The plates were washed 10 times with 0.05% Triton X-100. Two hundred µl of 0.05 M phosphate:0.25% w/v citrate buffer (pH 5.0) containing 0.4 mg of o-phenylene-diamine dihydrochloride (Sigma Chemical Co., St. Louis, MO) per ml and 0.012% H$_2$O$_2$ were added to each well, the plates were incubated in the dark for 30 min, and the reaction was stopped by the addition of 50 µl of 2.5 M H$_2$SO$_4$. The plates were read at 490 nm in a Dynatech Model MR580 ELISA reader. Results from triplicate analysis of antibody standards were subjected to linear regression analysis for generation of standard curves. Values obtained from patient samples are the result of triplicate assays on dilutions within the dynamic range of the assay system.

Radiological Methods for $^{111}$In Measurement. Whole-blood samples (2 ml) were obtained from patients during $^{111}$In infusion, at infusion end (0), and at 1, 5, 10, 30, 60, 120, 180, 1320, and 2760 min after the end of infusion. Blood samples were collected in 3-ml tubes containing sodium heparin anticoagulant. An aliquot (0.5 ml) of the $^{111}$In-labeled antibody infusion solution was also obtained to serve as a standard and as an isotope decay control. Whole-blood samples were centrifuged at 1500 rpm (Sorvall Model GLC-2B centrifuge), and duplicate 100-µl aliquots of plasma were added to glass 13-x 100-mm disposable test tubes. The tubes were loaded into plastic carriers, and $^{111}$In activity was assessed using a Packard $\gamma$ scintillation spectrometer (Model 5360).

Urine samples were collected for 48 h following the infusion of antibody in 4- and 8-h aliquots. The total urine volume was measured, and duplicate 100-µl aliquots were assayed for $^{111}$In activity as described above. All analyses of $^{111}$In activity were adjusted for isotopic decay.

RESULTS

Pharmacokinetics of $^{111}$In-labeled Monoclonal Antibody. The results of nonlinear regression analysis of $^{111}$In plasma disappearance curves for 7 patients who received 20 mg of $^{111}$In-labeled (5 mCi) monoclonal antibody are shown in Chart 1. The label was cleared from the plasma monoeoxponentially and closely fit ($r^2 > 0.9$) an open, one-compartment mathematical model. A summary of plasma pharmacokinetics of 28 patients who received monoclonal antibody at total doses of 1, 2, 5, 10, and 20 mg with $^{111}$In at doses of 2.5 or 5.0 mCi is shown in Table 1. Plasma half-lives for the $^{111}$In label varied from 27 ± 9 h for the 1-mg dose to 39 ± 9 h for the 20-mg dose; the overall
mean plasma $t_w$ was 32 ± 2 h. Although there appeared to be some variability in the calculated $t_w$ for the various dose levels, these differences were not statistically significant and did not appear to be dependent upon either the total dose of antibody or upon the dose of $^{111}$In label.

The apparent $V_d$ shown in Table 1 varied with the dose administered from 7.8 ± 0.7 to 3.0 ± 0.9 liters at the 1- and 20-mg doses, respectively. At the 1- and 2-mg doses, the apparent volume of distribution approximated the total blood volume (7.5 liters). However, at the 5-mg dose, the $V_d$ decreased sharply to 4 liters, approximating the total plasma volume.

The area under the concentration curve ($C \times T$) ranged from 357 ± 127 to 653 ± 106 Ci/ml times min at the 1- and 10-mg doses. Adjusting for the increase in $^{111}$In dose administered from 2.5 to 5.0 mCi at the 20-mg dose level, the $C \times T$ was within the range noted at the lower doses.

The clearance of murine monoclonal antibody from human plasma measured by ELISA assay is shown in Chart 1 for the 20-mg dose level. As shown, the clearance of monoclonal antibody from plasma paralleled the clearance for the $^{111}$In label also shown in Chart 1. Table 2 shows the summary of pharmacokinetic parameters calculated from nonlinear regression analysis of murine antibody clearance from plasma at the 2- and 20-mg dose levels. At both dose levels, clearance of monoclonal antibody fit ($r^2 > 0.9$) an open, one-compartment mathematical model. The calculated half-lives for monoclonal antibody were similar to half-lives calculated for the $^{111}$In label at the same dose levels. At the 2-mg dose level, the apparent volume of distribution calculated for the monoclonal antibody was smaller than that calculated for the $^{111}$In label. The calculated $V_d$ was identical to that found for the $^{111}$In label.

The urinary excretion of $^{111}$In radiolabel is shown in Chart 2 for the 20-mg (5.0 mCi) dose level. Most of the radiolabel (9% of the administered dose) appeared within 8 h of infusion. Thereafter, only approximately 5% of the administered label was excreted over the next 40 h. The 48-h cumulative urinary excretion of the $^{111}$In label for all dose levels is shown in Table 1. Total excretion of the radiolabel over 48 h was between 12 and 23% of the administered dose and did not appear to be affected by the dose administered. The antibody-labeling technique for $^{111}$In leaves approximately 10 to 15% unreacted $^{111}$In radiolabel coupled to antibody-free DPTA. The excretion of $^{111}$In early after administration therefore probably represents this antibody-free $^{111}$In label. Further urinary excretion of the label over 42 h may represent release of the $^{111}$In-DPTA complex from the antibody by metabolic processes.

The initial tissue distribution of the $^{111}$In label can markedly affect calculated pharmacokinetic parameters, such as the apparent volume of distribution. Therefore, the earliest available (4-h) whole-body scans were examined for relative distribution of $^{111}$In in order to determine the organ systems responsible for the change in the $V_d$ with increasing dose. As noted in Table 1, there was a dramatic decrease in the apparent $V_d$ in the 5-mg dose compared to that found for the 2-mg dose (4 compared to 7.5 liters). The relative tissue distribution for the $^{111}$In label 4 h after infusion of labeled antibody is shown in Table 3. With increasing antibody doses, the ratio of radiolabel found in liver compared to label in heart decreased dramatically from the 2- to the 5-mg dose and thereafter decreased slightly from the 5- to the 20-mg dose. The ratio of label in kidney compared to aorta decreased slightly from the 2- to the 20-mg dose. The ratio of label in spleen compared to that found in heart tissue did not significantly change.

**DISCUSSION**

To date, there have been several studies examining the utility of monoclonal antibodies for radioimmunomaging of human proteins. The use of $^{111}$In-labeled monoclonal antibodies has been shown to be effective in several tumor models. However, the pharmacokinetic parameters of these antibodies can vary significantly with different doses and routes of administration. The use of $^{111}$In allows for sensitive detection of small changes in immune clearance and metabolism, providing valuable information for the development of new therapeutic strategies.
tumors in both animal models (20) and in clinical trials (9, 19). However, there have been no comprehensive studies of the pharmacokinetics of murine monoclonal antibodies in humans.

The current study shows that the pharmacokinetic behavior of murine monoclonal antibodies follows nonlinear, dose-independent pharmacokinetic behavior. Although we have demonstrated that the clearance of both the 111In label and the monoclonal antibody itself from plasma closely fits an open, one-compartment mathematical model, Larson et al. (17) have shown that 131I-labeled p97-specific Fab fragments are cleared from patient plasma biphasically. In addition, the plasma half-life of the 131I label was found to be approximately 83 min compared to approximately 32 h for the 111In-labeled intact antibody found in this study. The reasons for the wide differences between the 111In-labeled intact antibody and the 131I-labeled Fab may be both the smaller molecular size of the Fab fragment, allowing relatively rapid adsorption from plasma into tissue sites, and the possibility of dehalogenation reported by various investigators (11, 12, 23). Halpern et al. (10, 11) have shown that the labeling of monoclonal antibodies with 111In avoids this dehalogenation problem. In addition, 111In-labeled antibodies were shown to maintain the same in vivo stability and distribution kinetics as endogenously labeled 75Se-antibody.

In the current study, the clearance kinetics of the 111In label are almost identical to the clearance kinetics for the monoclonal antibody itself, suggesting relative in vivo stability of the 111In label on the protein. However, of interest is the lack of correlation in the apparent volume of distribution with increasing dose of antibody calculated by measuring monoclonal antibody. This is in contrast to the decrease in Vd with increasing antibody dose calculated by measuring the 111In label. The reasons for this lack of correlation are unclear but may reside in the variance of the ELISA assay at low antibody concentrations or may suggest that the binding of unlabeled antibody to extravascular sites may be different than that of 111In-labeled antibody. The decrease in the apparent volume of distribution calculated using the 111In label is particularly noteworthy. A sharp, statistically significant decrease in the Vd was noted between the 2- and the 5-mg dose levels. This may suggest saturation of extravascular antigenic sites at the 5-mg dose level. Analysis of the relative distribution of 111In label in tissues showed that the decrease in liver content of label at the 5-mg dose paralleled the sharp decrease in the calculated apparent volume of distribution noted at the 5-mg dose level. These data suggest that the hepatic uptake of 111In-labeled monoclonal antibody is at least partially saturable by increasing the dose of unlabeled antibody. The marked change in the Vd between the 2- and 5-mg dose levels may be partially accounted for by the change in distribution of labeled antibody to the liver. This is particularly important for immunologically directed radio-

and chemotherapy, since these data suggest that the liver may be partially protected by increasing the antibody dose. In addition, these data suggest that changes in the calculated pharmacokinetic parameters may accurately reflect changes in disposition of the agent to tissue sites.

In a previous study (21), we described radioimmunoimaging of 31 patients with malignant melanoma using this 111In-labeled monoclonal antibody. Of a total of 100 metastatic sites documented previously, 50 imaged for an overall reactivity of 50%. However, we found that, by increasing both the total dose of 111In and the total antibody dose, the overall percentage of tumor sites imaged increased to 81%. In the current study, there were no statistically significant differences in the apparent volume of distribution, plasma t1/2, or urinary excretion of 111In between patients with a large tumor burden and patients with relatively small tumor mass. In addition, we could find no significant pharmacokinetic differences between patients whose tumors imaged and those who did not. This is not surprising, since studies (11) suggest that acquisition of antibody by the tumor occurs slowly compared to nontarget organs, such as liver and spleen. Furthermore, the relatively small amounts of antibody slowly adsorbed even by large tumors may not make a significant contribution to the overall pharmacokinetic parameters calculated due to the more rapid and extensive contribution of major organs, such as liver, kidney, and spleen.

The extensive, rapid uptake of intact monoclonal antibodies by "nontarget" or normal organs such as liver, spleen, and kidney is particularly important since this may complicate the use of antibodies as carriers of therapeutic agents. The reasons for antibody uptake in these organs could include presence of low amounts of tumor antigen or presence of Fc receptors in these tissues. Finally, since the p97 antigen may circulate in plasma of these patients, the presence of labeled antibody in the organs may represent clearance by the RES of 111In-antibody:antigen complexes. These particular problems with monoclonal antibodies will be the subject of future studies, since their resolution may be critical to limiting the uptake of monoclonal antibodies bound to therapeutic agents, thus preventing or reducing toxicity to nontarget sites.

In summary, the studies show that the clearance of 111In-labeled monoclonal antibodies occurs slowly with a t1/2 of approximately 31 h. Clearance of the murine antibody itself paralleled the clearance of the 111In label, suggesting biological stability of the 111In-antibody complex. The apparent volume of distribution of the 111In-antibody can be modified by increasing the total antibody dose, thereby decreasing distribution of the antibody to the liver. When utilizing monoclonal antibodies as carriers of chemotherapeutic agents, toxins, or radionuclides, the dose of antibody utilized may be important in limiting distribution of the
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agent to the liver and thereby reducing or preventing hepatic toxicity.

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