Interferon-α-2b Immunoconjugate for Improving Immunoscintigraphy and Immunotherapy

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A pretreatment with a single dose of an immunoconjugate (IC) that promises to enhance tumor uptake and decrease liver uptake of radiolabeled monoclonal antibodies (MAbs) might be of use in radioimmunodetection and radioimmunotherapy (RIT). We have shown previously that an interferon (IFN)–MAb (1:1) immunoconjugate (IC) enhances tumor uptake by a factor of 2 or more and reduces liver uptake by 50% in nude mice bearing human tumors. The aim of this study was to determine whether IFN modulates antigenic expression and to ascertain the most effective route of its administration, the optimal quantity to be administered, and the optimal duration of time to lapse between the administration of IC and the radiolabeled MAb. **Methods:** IFN-α-2b and anticarcinoembryonic antigen–F6 (IgG2a) MAb were conjugated (1:1), and $F(ab')_2$ of the MAb was labeled with ^{99m}Tc. Human colorectal tumors were grown in nude mice by implanting 5×10^6 LS174T confluent cells grown in culture. Mice, 5 in each group, received 20×10^3 IU intravenously, intramuscularly, or intraperitoneally and 40×10^3, 60×10^3, and 80×10^3 IU intravenously 30 min before the intravenous administration of 25.9 MBq ^{99m}Tc/20 μg $F(ab')_2$. Mice in the control groups received ^{99m}Tc-$F(ab')_2$ but not the conjugate. Twenty-four hours later mice were killed and imaged, and tissues were removed for quantitative (percentage injected dose/g [% ID/g]) distribution of ^{99m}Tc. **Results:** In all conjugate-receiving mice, the tumor uptake was higher and the liver uptake was lower ($P < 0.01$) than that in the control mice with the exception of liver uptake, which was not significantly different in mice receiving 80×10^3 IU conjugate. The optimal results were apparent in mice pretreated with 40×10^3 IU conjugate in which tumor uptake was enhanced by a factor of 2.3 (4.8 ± 0.5 %ID/g versus 11 ± 0.7 %ID/g; $P < 0.01$). The renal uptake remained unchanged, and the tumor-to-muscle ratios increased from 11.5 ± 6.8 to 14.6 ± 3.9, and the tumor-to-blood ratios increased from 4.4 ± 1.8 to 8.3 ± 2.4. The liver uptake decreased from 9.5% ± 1% to 5% ± 1.6%. Results were attributed to enhanced tumor blood flow, increased antigenic expression, and blocking of hepatic nonspecific Fc receptors. **Conclusion:** A pretreatment with IFN–MAb conjugate is a worthwhile approach to consider in radioimmunoscintigraphy and RIT.

Key Words: immoconjugate; interferon–monoclonal antibody conjugate; improving RIS and RIT

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Oncologic applications of radiolabeled monoclonal antibodies (MAbs) in radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT) have continued to be exciting and yet challenging. The concept of receptor specificity mediated by the MAb has stimulated numerous investigators and invigorated the field of RIS and RIT. However, thus far, at least 2 fundamental problems have prevented radiolabeled MAbs from widespread applications in RIS and RIT. First, the low tumor uptake has deprived RIS of high sensitivity and has thwarted tumors from receiving a radiation dose high enough to eradicate malignant cells in RIT. The low tumor uptake may stem from a combination of several parameters, such as receptor heterogeneity, low expression of specific antigen density, poor tumor vascularity, interstitial hypertension, and relatively long transport distances to the interstitium after intravenous administration of radiolabeled MAbs (*1,2*). To circumvent these problems, antibody cocktails, tumor hyperthermia, external beam irradiation, and vasoactive conjugates have been used (*3–8*). However, none of these approaches has fully resolved the problem (*9,10*).

The second problem is the relatively high liver uptake. The high liver uptake has restricted the quantity of radioactivity that can be administered safely. Modifications in radiolabeling procedures, use of a variety of bifunctional chelating agents, and use of liposomally entrapped MAbs or the MAb fragments have not conclusively overcome the drawbacks (*11–15*).

In an attempt to minimize these weaknesses, we have evaluated the use of interferon (IFN), a biologic response modifier over the past few years. IFNs are naturally occurring, water-soluble proteins with molecular weights between 15 and 21 kDa. These cytokines are produced and secreted by cells in response to viral infections or to various synthetic and biologic inducers. Three major classes of these cytokines, α, β, and γ, have been identified. IFNs exert their cellular activities by binding to specific membrane receptors on the cell surface. One class of IFN, IFN-α-2b (19.2 kDa), is produced by a recombinant DNA technique and is known to possess high immunomodulating activity (*16*).

In addition to other biologic response modifiers, intravenous administration of IFN-α-2b has been shown to enhance blood flow and increase uptake of subsequently injected radiolabeled MAbs, not only in the tumor but also in other tissues, including the liver (*17–19*). This work has led us to

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hypothesize that with IFN–MAb immunoconjugate (IC) pretreatment, not only may the tumor uptake increase by preferential tumor targeting of IFN molecules but also the liver uptake of radiolabeled MAb may decrease by blockage of the nonspecific Fc receptors by MAb injected with IC. Indeed, the tumor uptake and the tumor-to-blood and tumor-to-muscle ratios have been shown to increase by more than 2-fold, and the liver uptake has been shown to decrease by a significant proportion (*20*).

The purpose of this investigation was to assess whether IFN modulates antigenic expression and to determine the most effective route of administration of the IC, the optimal duration of time to lapse between the administration of IC and the radiolabeled MAb, and the optimal quantity of the IC to be injected for best results.

MATERIALS AND METHODS

Preparation, Purification, and Quantification of IFN-α-2b MAb Conjugate

Intron-A (IFN-α-2b; 10×10^6 IU/mL; free of human serum albumin [HAS]) was a gift from Schering-Plough, Inc. (Kenilworth, NJ). To increase its concentration, the Intron-A solution was lyophilized and then taken up in 75 μL 0.1 mol/L phosphate buffer (pH 7.2). IFN-α-2b was then conjugated with anticarcinoembryonic antigen (CEA)-F6 MAb (IgG2a; Immunotech, Marseilles, France) using a method developed in this laboratory (*20*). The MAb is specific for human colorectal cancer.

Briefly, 12.5–25 mg 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate and *N*-hydroxysulfosuccinimide were each dissolved in 50 μL borate buffer (pH 9.2), and 500 μg to 1 mg MAb dissolved in 50 μL 0.1 mol/L phosphate buffer (pH 7.2) was added. The molar ratio was maintained at ~25:25:1. The mixture was stirred for 2–3 min at 4°C, and then 100–200 μg (2×10^7 to 4×10^7 IU) IFN-α-2b solution (75 μL) was added. The pH was adjusted to ~7, and the reaction mixture was stirred at 4°C overnight. The reaction was monitored by high-performance liquid chromatography (HPLC); the conjugated protein was separated by HPLC using a size-exclusion Protein Pak SW 300 column (Waters Corp., Milford, MA) and 0.05 mol/L phosphate buffer (pH 6.8) in 0.9% NaCl as a solvent at a flow rate of 0.5 mL/min. The conjugated protein fraction, as monitored by the optical density peak at 280 nm, was collected, and the protein was concentrated using a Centricon-30 (Amicon, Beverly, MA) molecular filtration device. The quantity of protein collected was determined spectrophotometrically using the equation $E^{1\%}_{280} = 1.46$, where $E^{1\%}_{280}$ is the extinction coefficient for a 1% solution at 280 nm. Conjugation efficiency and the number of IFN molecules bound to each MAb molecule were determined using ^{125}I-IFN-α-2b. The conjugate was stored at 4°C and monitored periodically for its stability by HPLC.

Preparation of ^{99m}Tc-F(ab′)$_2$

The ^{99m}Tc-MAb preparation was prepared as described (*21*). Briefly, 100 μg CEA-F6 F(ab′)$_2$ of the MAb (2 mg/mL) was incubated with 266 μg sodium ascorbate (pH 6.5) for 60 min at room temperature. ^{99m}Tc was then reduced with sodium dithionite (final concentration, 5 mg/mL) in bicarbonate solution (pH 11), added immediately to the reduced MAb, and incubated for another 30 min at 22°C. To eliminate any free ^{99m}Tc, the reaction mixture was centrifuged using an HSA-treated Centricon-30 molecular filtration device. The quality of the ^{99m}Tc-MAb preparation was examined by instant thin-layer chromatography (ITLC) (2 mol/L urea, ^{99m}Tc-MAb, $R_f = 0.0$; free ^{99m}Tc, $R_f = 1.0$; and a mixture of ethanol, NH_4OH, and H_2O (80:1:19); colloid, $R_f = 0.0$; ^{99m}Tc-MAb, $R_f = 1.0$) and HPLC using a size-exclusion Protein Pak SW 300 column and 0.05 mol/L phosphate buffer (pH 6.8) in 0.9% NaCl as the solvent at a flow rate of 0.5 mL/min (*21*).

Determination of Modulation of CEA Expression by IFN in LS174T Human Colorectal Cells In Vitro

We examined whether IFN modulates CEA expression on LS174T human colorectal cancer cells (American Type Culture Collection, Rockville, MD) as indicated by ^{99m}Tc-F(ab′)$_2$ binding to the cells. Cells were grown in Dulbecco's modified Eagle's medium (DMEM), and 2×10^6 confluent cells were incubated with 10^3 IU IFN in a 24-well plate for predetermined periods of up to 24 h. The DMEM was then removed, and cells were resuspended and incubated with ^{99m}Tc-F(ab′)$_2$ (74 kBq/0.2 μg) at 37°C for 30 min. Cells were then washed free of radioactivity and dissolved in 0.5 mol/L NaOH; radioactivity associated with the cells was then determined. Data were plotted as the percentage of radioactivity associated with cells as a function of time for which cells were incubated with IFN. Experiments were performed in triplicate.

Animal Studies

Establishment of LS174T Human Colon Cancer Xenografts. Animal tissue distribution studies were performed in athymic NCr nude mice bearing LS174T human colorectal tumors in the right thigh. Cells were grown in culture, and $\sim 5 \times 10^6$ viable cells were implanted intramuscularly in the right thigh of each mouse, which weighed 18–25 g. Tumors were allowed to grow to no more than 1 cm in size. Five animals were used per group. Studies were repeated at least once.

Determination of Influence of Route of Administration. The purpose of this part of the study was to determine the best route of administration for the IFN–MAb conjugate. In this study, tumor-bearing mice received 20×10^3 IU of the conjugate intravenously, intraperitoneally, or intramuscularly and then 30 min later were given the ^{99m}Tc-labeled F(ab′)$_2$ (25.9 MBq/20 μg) intravenously in 200 μL 0.9% NaCl. All animals were euthanized in a halothane gas chamber 24 h after administration of the radiolabeled MAb. The animals were imaged with a γ camera (Starcam 300; General Electric Medical Systems, Milwaukee, WI) in posterior position, and tissues were then harvested for quantitative determination of radioactivity distributed in the tissues. The institutional animal care and use committee approved the procedure (protocol no. 118L). Tissues were rinsed free of blood, blotted, and weighed, and radioactivity associated with the tissues was determined in an energy-calibrated γ counter (5000 series; Packard Instrument Co., Downers Grove, IL). Standard radioactive solutions were prepared at the time of injection. The exact radioactivity received by each mouse was determined by measuring the radioactivity in the syringe before and after injection.

Determination of Optimal Time at Which ^{99m}Tc-F(ab′)$_2$ Should Be Injected After Treating Tumor-Bearing Mice with IC. Groups of 5 mice each were injected intravenously with 20×10^3 IU IFN–MAb conjugate, and then approximately 25.9 MBq/20 μg F(ab′)$_2$ was injected intravenously at 30 min and 1.5, 3, 6, and 24 h after IFN–MAb IC injection. Twenty-four hours later, animals were killed and imaged, and tissue was dissected. Quantitative tissue distribution studies were performed as described.

Dose–Response of IC. The response of IC dose on tumor uptake and tissue distribution was examined in nude mice bearing

intramuscular LS174T human colorectal tumors. IC containing 20×10^3, 40×10^3, 60×10^3, and 80×10^3 IU IFN, each in 200 μL 0.9% NaCl, was administered through a lateral tail vein. a group of 5 mice was used for each concentration of the IC. Thirty minutes later, each mouse was injected intravenously with 25.9 MBq ^{99m}Tc labeled to 20 μg F(ab')$_2$ of the MAb. Twenty-four hours later, the mice were killed and imaged, tissues were harvested, and radioactivity associated with the tissues was counted. The percentage injected dose/gram (%ID/g) tissue was determined.

Statistical evaluation was performed using the Student *t* test. Results were considered to be significantly different when $P \leq 0.05$.

RESULTS

As shown previously (*20*), the IFN–MAb conjugation yield was >70%, and 1 IFN molecule was bound to each molecule of the MAb. The labeling yield of ^{99m}Tc-F(ab')$_2$ was >85%, and the colloid formation as determined by HSA-impregnated ITLC was <5%. It was shown previously that the antibody antiCEA-F6 ^{99m}Tc-F(ab')$_2$ has high affinity (binding constant, 5.9×10^{-9} mol/L) for LS174T cells (*20*).

Although it has been reported that IFN enhances tumor blood flow (*18,19*), its influence on cell-associated antigen modulation was not examined. Figure 1 shows that in the presence of IFN, the CEA expression (as indicated by increased ^{99m}Tc-F(ab')$_2$ binding) continued to enhance for up to 20 h and then declined, perhaps because of antigen internalization. This finding is consistent with the observation of Murray et al. (*22*), who reported that such decreased antigenic expression occurs when cells are incubated with IFN for 24 h and attributed this decline to antigen internalization. Table 1 and Figure 2 present the tissue distribution of

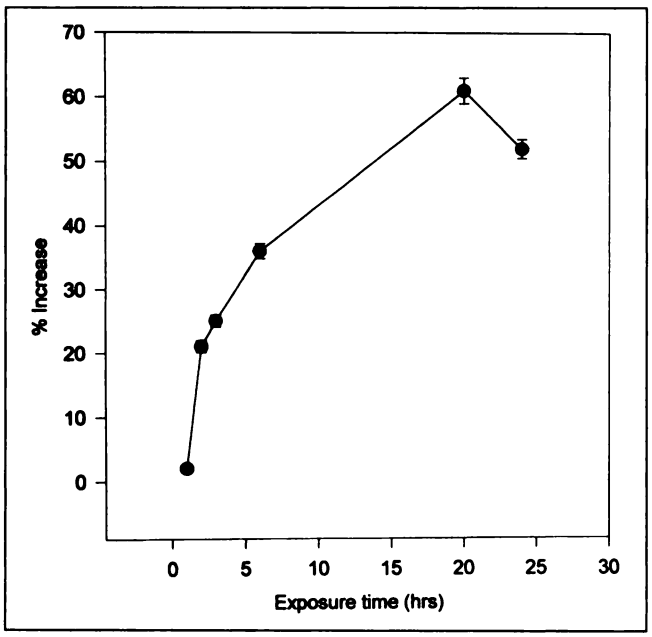

FIGURE 1. Percentage increase in ^{99m}Tc-F(ab')$_2$ binding on LS174T human colorectal cancer cells as function of IFN exposure time in vitro.

TABLE 1
Twenty-Four–Hour Tissue Distribution of ^{99m}Tc–CEA-F6 F(ab')$_2$ in Nude Mice Bearing LS174T Human Colorectal Cancer

Tissue	Intravenous*†	Intramuscular*	Intraperitoneal†
Muscle	0.84 ± 0.21	0.77 ± 0.11	0.74 ± 0.07
Intestine	0.81 ± 0.08	0.79 ± 0.23	1.21 ± 0.58
Heart	1.05 ± 0.14	0.92 ± 0.19	1.25 ± 0.21
Lungs	1.44 ± 0.07	1.39 ± 0.30	1.47 ± 0.24
Blood	1.96 ± 0.24	1.94 ± 0.43	2.15 ± 0.47
Spleen	3.31 ± 0.61	4.54 ± 1.06	5.52 ± 1.89
Kidneys	26.61 ± 2.12	25.25 ± 4.40	30.42 ± 3.13
Liver	6.58 ± 0.91	9.48 ± 1.30	11.34 ± 2.53
Tumor	8.72 ± 1.00	8.45 ± 2.57	8.67 ± 1.60
Tumor-to-muscle ratio	11.08 ± 3.84	11.49 ± 4.89	13.64 ± 2.09
Tumor-to-blood ratio	4.50 ± 0.76	4.38 ± 1.07	4.03 ± 0.24

*P for intravenous and intramuscular injections of labeled MAb ≥ 0.01 except in tumor ($P < 0.01$).

†P for intravenous and intraperitoneal injections of labeled MAb > 0.01 except in tumor ($P < 0.01$).

Mice were injected with 20×10^3 IU of IC 30 min before injection of MAb (each group, n = 5). Control mice did not receive IC.

^{99m}Tc-F(ab')$_2$ of the MAb, which was administered 30 min after injection of 20×10^3 IU IFN conjugate given intramuscularly, intraperitoneally, or intravenously. With the administration of the IC, the tumor uptake was consistently and significantly greater ($P < 0.01$) than was the tumor uptake in the control animals, which had not been treated with the IC. The enhanced tumor uptake was independent of the route of administration, but the liver uptake was the lowest in mice that had received the IC intravenously ($P < 0.01$). None of the routes of administration of IC reduced the blood uptake, which was the lowest with the intravenous injection of IC. To best serve our aim of increasing the tumor uptake and decreasing the liver uptake, the intravenous route of IC administration was preferable.

Figure 3 indicates that the tumor uptake in mice was twice as high as that in the control animals when mice were injected intravenously with ^{99m}Tc-F(ab')$_2$ at 30 min after injection of the conjugate. The tumor uptake remained almost unchanged in the mice that were injected at 1.5 and 3 h after administration of the conjugate but then decreased in the mice that received ^{99m}Tc-F(ab')$_2$ at 6 and 24 h after injection of IC. At these time points, the liver uptake was also significantly ($P < 0.01$) less than that in the control animals. It was apparent, however, that the combination of high tumor uptake and low liver uptake, as well as low spleen uptake, was best achieved by injecting the ^{99m}Tc-F(ab')$_2$ 30 min after administration of IC. At this time point, the kidney uptake, which was the highest of all organs, with or without the administration of IC, was not affected significantly.

Table 2 and Figure 4 present the results of the dose–

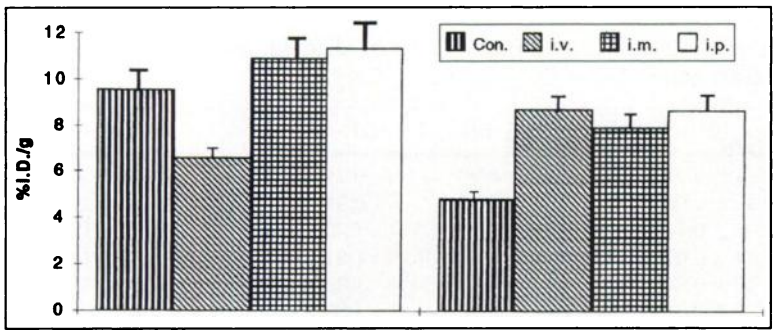

FIGURE 2. Influence on ^{99m}Tc–CEA-F6 F(ab')$_2$ uptake in liver and tumor when nude mice (n = 5) bearing LS174T human colorectal tumors were injected intravenously (i.v.), intramuscularly (i.m.), or intraperitoneally (i.p.) with 20 × 10^3 IU IFN–MAb conjugate 30 min before administration of ^{99m}Tc-F(ab')$_2$. Control (Con.) mice (n = 5) did not receive conjugate.

response on tissue distribution. The tumor uptake at 40 × 10^3 IU IFN conjugate was 2.3-fold greater than that in the control animals and was significantly higher than that in the mice that received 20 × 10^3 IU IFN conjugate. The liver uptake was also low when 40 × 10^3 IU were administered 30 min before injection of the labeled F(ab')$_2$. The tumor-to-blood ratio was also optimal with this concentration (Fig. 4). At 60 × 10^3 IU IFN conjugate, the tumor uptake remained approximately the same, and the tumor-to-muscle ratio increased from 14.6 ± 3.9 to 17.8 ± 8.1. However, the liver uptake increased from 5.0 ± 1.6 %ID/g to 8.8 ±1.2 %ID/g, and the tumor-to-blood ratio decreased from 8.3 ± 2.4 at 40 × 10^3 IU IFN IC to 5.7 ± 0.9.

It was reasonable to conclude from these data that the intravenous administration of 40 × 10^3 IU IFN conjugate 30 min before the injection of ^{99m}Tc-F(ab')$_2$ not only increased the tumor uptake appreciably but also decreased ($P < 0.01$) the liver uptake significantly.

DISCUSSION

Our interest in IFN-α-2b stemmed from its broad spectrum of biologic activities that exert a complex sequence of intracellular responses, such as the induction of certain enzymes, inhibition of viral replication in virus-infected cells, suppression of malignant cell proliferation, enhancement of phagocytic function, and modulations in cell-associated antigenic expression (*23–33*). Our previous work has shown repeatedly that pretreatment with IFN–MAb conjugate followed by the administration of ^{99m}Tc-F(ab')$_2$ of the same MAb significantly enhanced the tumor uptake and diminished the liver uptake (*17–20*). These results were attributed to the enhanced tumor blood flow that resulted from the selective accumulation of IFN in the tumor and to the blockage of nonspecific Fc receptors in the liver by the MAb molecules injected with the conjugate (*17–20*). This study was designed to further illustrate the influence of other parameters, such as the upregulation of the antigen density, the quantity of the conjugate injected, the route of its administration, and the time lapse between the 2 injections.

Figure 1 indicates that IFN exerts its effects on tumor cells and modulates its antigen density much sooner than shown previously. Rowlinson et al. (*34*) treated mice with 2 × 10^5 IU IFN daily for 4–11 d before injecting them with ^{131}I-MAb. Tumor uptake increased by 110% (1.1-fold) in these animals. As seen in Figure 1 and as reported by Murray et al. (*22*), the cell-surface antigen density diminishes at prolonged treatment with IFN, presumably by internalization. This phenomenon supports the results of Rowlinson et al. (*34*) and the results of this study (Fig. 3), which show low tumor uptake in mice in which ^{99m}Tc-F(ab')$_2$ was injected 24 h after the injection of IC. The 30-min duration between the 2 injections not only provides better results but also is convenient in the clinical practice of this pretargeting technique.

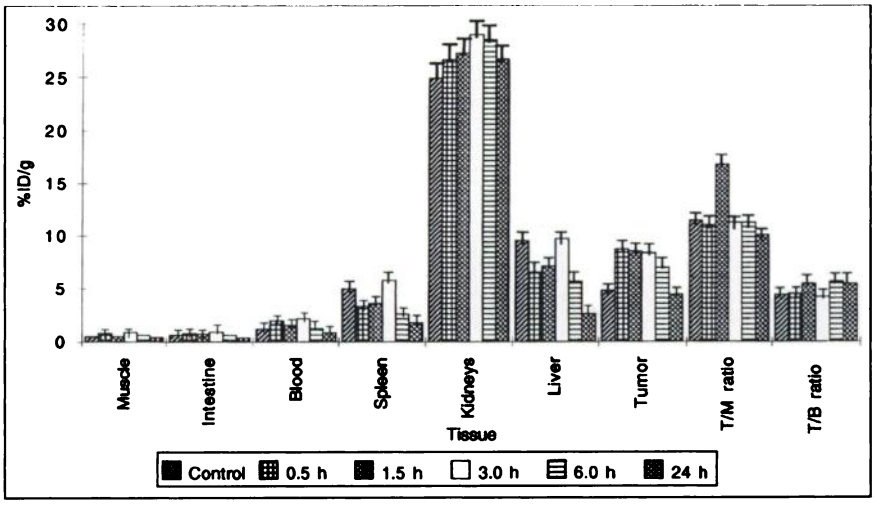

FIGURE 3. Tissue distribution of ^{99m}Tc–CEA-F6 F(ab')$_2$ in nude mice bearing LS174T human colorectal tumors. Mice were killed 24 h after administration of ^{99m}Tc-MAb. Before administration of ^{99m}Tc–CEA-F6 F(ab')$_2$, mice in each group (n = 5) were injected intravenously with 20 × 10^3 IU IFN–MAb conjugate either 30 min or 1.5, 3, 6, or 24 h previously. Mice in control group (n = 5) did not receive IC. T/M ratio = tumor-to-muscle ratio; T/B ratio = tumor-to-blood ratio.

TABLE 2
Dose–Response Effect on 24-Hour Tissue Distribution of ^{99m}Tc–CEA-F6 F(ab')$_2$ in Nude Mice Bearing LS174T Human Colorectal Cancer

Tissue	Control*†‡§	20 × 10^3 IU*	40 × 10^3 IU†	60 × 10^3 IU‡	80 × 10^3 IU§
Muscle	0.49 ± 0.19	0.84 ± 0.21	0.80 ± 0.20	0.71 ± 0.26	1.12 ± 0.52
Intestine	0.64 ± 0.14	0.81 ± 0.08	0.58 ± 0.08	0.91 ± 0.14	1.31 ± 0.07
Heart	1.67 ± 0.11	1.05 ± 0.14	0.74 ± 0.10	1.20 ± 0.22	1.51 ± 0.02
Lungs	1.04 ± 0.16	1.44 ± 0.07	1.19 ± 0.20	1.31 ± 0.11	1.56 ± 0.06
Blood	1.24 ± 0.55	1.96 ± 0.24	1.40 ± 0.32	1.98 ± 0.15	2.21 ± 0.40
Spleen	4.98 ± 0.59	3.31 ± 0.61	2.74 ± 0.78	4.69 ± 0.46	5.68 ± 0.09
Kidneys	24.87 ± 6.14	26.61 ± 2.12	28.94 ± 3.83	28.99 ± 1.05	32.89 ± 1.30
Liver	9.55 ± 1.00	6.58 ± 0.91	5.04 ± 1.58	8.83 ± 1.21	10.78 ± 0.56
Tumor	4.81 ± 0.53	8.72 ± 1.00	11.04 ± 0.75	11.26 ± 1.36	10.33 ± 4.19
Tumor-to-muscle ratio	11.50 ± 6.83	11.08 ± 3.84	14.59 ± 3.92	17.80 ± 8.16	9.54 ± 0.05
Tumor-to-blood ratio	4.42 ± 1.80	4.50 ± 0.76	8.31 ± 2.42	5.72 ± 0.93	4.85 ± 2.65

*P for control and MAb–IFN conjugate injected (20 × 10^3 IU) before labeled MAb < 0.05 except in kidney (P = 0.53) and tumor-to-blood ratio (P = 0.93).

†P for control and MAb–IFN conjugate injected (40 × 10^3 IU) before labeled MAb < 0.05 except in spleen (P = 0.01), liver (P = 0.01), and tumor ($P \leq 0.01$).

‡P for control and MAb–IFN conjugate injected (60 × 10^3 IU) before labeled MAb < 0.05 except in intestine (P = 0.01), heart (P = 0.01), blood (P = 0.01), and tumor (P = 0.01).

§P for control and MAb–IFN conjugate injected (80 × 10^3 IU) before labeled MAb < 0.05 except in intestine (P = 0.16) and tumor-to-blood ratio (P = 0.52).

Mice in each group (n = 5) were injected intravenously with IC 30 min before administration of MAb. Control mice did not receive IC.

Although the route of administration of the IC did not make a significant difference in the enhancement of tumor uptake, the liver uptake was significantly low with the intravenous injection. The reasons for this are unclear. We believe that 2 intravenous injections 30 min apart would not contribute to any inconvenience or any burden in executing the procedure.

Data presented in Table 2 and Figure 4 support for the use of 40 × 10^3 IU IFN conjugate. If this quantity is translated proportionally in a 25-g mouse, then a 70-kg man or woman would require 112 × 10^6 IU IFN conjugate. This with a 1:1 IFN–MAb conjugate will result in the use of ~4.3 mg MAb. Although this mathematic translation from mouse to man or woman is seldom applicable in practice, the use of 4.3 mg MAb may not be prohibitive because such quantities are not uncommon in RIT.

Although intravenous doses of 95 × 10^6 IU IFN (50 × 10^6/m^2) have been given daily for 5 d every other week to human subjects, to our knowledge, a single dose of 110 × 10^6 IU has not been used (*16*) and its toxicity has not been documented. No acute toxic effects were noted in mice during this study. However, further studies may be necessary to determine the quantity of IC that should be injected into humans for optimal results and to evaluate its toxicity, if any.

Contrary to our hypothesis that the preinjection of the MAb injected with the IC may chelate any circulating antigen and thereby help reduce the blood background activity, no reduction in %ID/g of blood was noted in mice. However, the presence or absence of circulating antigens in mouse blood was not determined.

Furthermore, the technique did not decrease the renal uptake, which, in RIT applications, may result in a high radiation dose to the kidneys. Preadministration of amino acid has been shown to minimize renal uptake of some

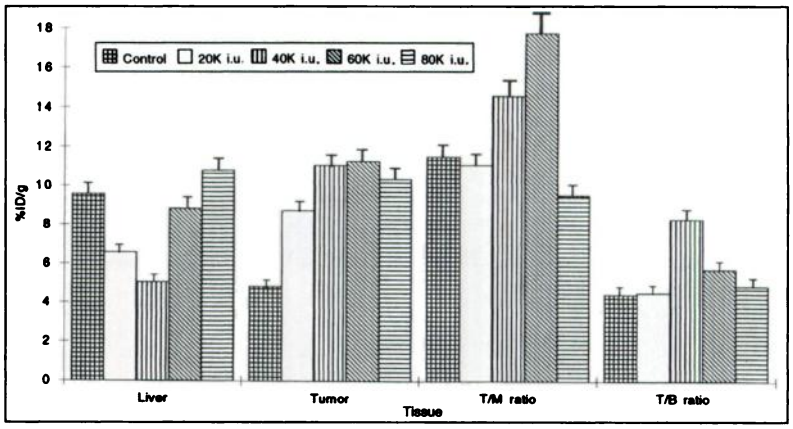

FIGURE 4. Influence of quantity of IC injected on tissue distribution of ^{99m}Tc–CEA-F6 F(ab')$_2$ in mice (n = 5) bearing LS174T human colorectal tumors. IC was injected intravenously 30 min before injection of ^{99m}Tc-MAb (K i.u. = 1000 IU), and mice were killed 24 h after administration of labeled MAb. Control mice did not receive IC. T/M ratio = tumor-to-muscle ratio; T/B ratio = tumor-to-blood ratio.

compounds in rodents (*34,35*). However, whether this approach would be successful in humans is unknown.

Langmuir et al. (*33*) suggested that even 2-fold enhancement in tumor uptake not only will increase the sensitivity in RIS but also will promote the MAb use in RIT. Furthermore, Langmuir et al. have postulated that with such enhancement of tumor uptake, radiolabeled MAbs can also serve as adjuvant therapeutic agents because they can reduce the external beam dose by 10%–20% and alleviate complications of radiotherapy.

The data of this study and previous studies (*17–20*), which showed that the use of this IC enhances the tumor uptake and decreases the liver uptake of radiolabeled MAbs, have also been found in studies of nude mice bearing not only human colorectal tumors but also ME 31.3 human melanoma tumors (*17–20*). The results have been consistent and lead us to believe that these preclinical data are promising and warrant studies in humans.

CONCLUSION

Our data provide evidence that intravenous administration of IFN-α-2b–antibody conjugate enhances blood flow and upregulates cell-surface antigenic expression, which lead to increased tumor uptake of radiolabeled MAbs by a factor of more than 2. As a result of the preadministration of the IC, the liver uptake also decreases significantly. These preclinical data are promising and suggest that the preadministration of IC may be useful in diagnostic and therapeutic applications of MAbs labeled with a radionuclide of appropriate characteristic.

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