The Use of Daunomycin-Antibody Immunoconjugates in Managing Soft Tissue Sarcomas: Nude Mouse Xenograft Model

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ABSTRACT

Analysis of human fibrosarcoma cells exposed to radiolabeled monoclonal antibody 19-24, which recognizes sarcoma-associated antigen p102, revealed that over 54% of the cell surface-bound radioactivity was internalized. No modulation of cell surface p102 antigen by monoclonal antibody 19-24 was observed in human fibrosarcoma cells. Monoclonal antibody 19-24 coupled to daunomycin via a dextran bridge was found to be most effective. In different preparations, the daunomycin:total protein molar ratio ranged from 1.9 to 6.1. In vitro cytotoxicity studies using human fibrosarcoma cells showed that, at 10 μg/ml concentration, this immunonjugate was 79.4% as efficient as free daunomycin and, at 1 μg/ml concentration, 36.8% as efficient. Control nonspecific murine monoclonal antibody P3 immunonjugates were relatively ineffective.

The distribution of 125I-Daunomycin, 125I-labeled monoclonal antibody 19-24, and 125I-labeled 19-24 immunonjugates was also evaluated over a 24-hour period in tumor and normal tissues of athymic mice bearing a human fibrosarcoma xenograft. Poor uptake of radiolabeled Adriamycin by the tumor tissue was observed. The level of 125I radioactivity in the tumor tissue never exceeded 1% of the total injected dose and was 24.8-fold lower than the radioactivity found in the spleen tissue. Tumor tissue uptake of radiolabeled monoclonal antibody 19-24 was characterized by the high tumor tissue: blood ratio of 1.62 ± 0.28 (SD). However, for monoclonal antibody 19-24 immunonjugates, this ratio decreased to 0.66 ± 0.05, which was still higher than normal liver (0.48 ± 0.02; lung, 0.48 ± 0.07; spleen, 0.28 ± 0.01) or nonspecific monoclonal antibody P3 immunonjugates. Thus, it appears that, compared to free daunomycin, monoclonal antibody 19-24 immunonjugates may be more efficient and less cytotoxic to normal tissues.

INTRODUCTION

MoAbs to human tumor-associated antigens have been intensely investigated for use as carriers of toxins (1, 2) or drugs (3-7). Previous studies have shown that immunonjugates prepared by different methods varied in usefulness in targeting antineoplastic drugs to tumor cells (8-10).

Antisarcoma highly specific MoAb 19-24 (11) was selected as a carrier of antineoplastic agents, based on our previous observations that this MoAb selectively localizes in tumors in vivo (12-16). Recently, we described a method in which ADR (doxorubicin) was coupled to MoAb 19-24 using a biotin-avidin-biotin bridge (17). In vitro cytotoxicity studies using human fibrosarcoma cells, this ADR-MoAb immunonojugate maintained 40% of the efficacy of free ADR. Immunonojugates using a biotin-avidin-biotin bridge may be less effective in vivo immunotherapy because egg white glycoprotein avidin M, 67,000 may induce an immune response after administration of immunonojugates into the bloodstream. Therefore, we decided to investigate other methods of coupling anthrapyrimidine drugs to MoAb.

Below, we report the in vitro cytotoxicity results of conjugation of DAU (daunorubicin) to MoAb 19-24 through a dextran bridge. In vivo distribution of MoAb 19-24 immunonojugates in fibrosarcoma-bearing athymic mice was also examined.

MATERIALS AND METHODS

Tissues and Tumor Cells

Human fibrosarcoma HT-1080 cells (18), purchased from the American Type Culture Collection (Rockville, MD) were grown in adherent monolayer culture with MEM-E, supplemented with L-glutamine and 15% heat-activated fetal bovine serum (GIBCO, Grand Island, NY).

Tumor Xenografts

HT-1080 cells (2 × 10⁶ cells/mouse) were injected s.c. into the right flanks of male athymic (nude) mice (NCI Frederick Cancer Research Facility, Frederick, MD) to initiate a tumor xenograft (12). When the tumors had grown to ~1 cm in diameter (after 10-14 days of growth), animals were entered into the study.

Monoclonal Antibodies

Antisarcoma mouse MoAbs 19-24 and 23-26 and nonspecific mouse myeloma MoAb P3 (all of isotype IgG1) were generated as described previously (11, 19). Purified MoAbs were radioiodinated using Na¹²⁵I (Amerham, Arlington Heights, IL) and chloramine-T (20).

Preparation of Immunonojugates

**Method A.** Glutaraldehyde was used to cross-link the amino groups of DAU (Wyeth-Ayerst Laboratories, Inc., Philadelphia, PA) and the free amino groups of the MoAb (8, 21) as follows. To 1 ml of 0.1 M phosphate buffer (pH 6.8) containing 1 mg of MoAb and 0.17 mg of DAU, we added 0.05 ml 1% aqeous solution of glutaraldehyde and incubated it for 15 min at room temperature. The excess glutaraldehyde and DAU were removed by extensive dialysis against PBS (10 mg phosphate-0.15 w NaCl-I mg MgCl₂-0.02% Na₂HPO₄, pH 7.3).

**Method B.** This MoAb was covalently coupled to DAU with EDC using a conjugation kit supplied by Pierce Chemical Co. (Rockford, IL) (8, 22). Briefly, a mixture of 0.5 mg MoAb, 0.1 mg DAU, and 2.5 mg EDC, dissolved in conjugation buffer, was incubated for 1 h at room temperature in the dark. After Sephadex G-25 gel filtration, DAU-EDC-MoAb immunoconjugation fractions were pooled and analyzed.

**Method C.** Conjugation of DAU to MoAb or ¹²⁵I-labeled MoAb was effected through a dextran bridge (8, 23, 24). DEX (M, 40,000; Sigma Chemical Co., St. Louis, MO) was oxidized to polyaldehyde-dextran with sodium periodate. DEXoxid was then dialyzed against water, lyophilized, and stored at 4°C. The DEXoxid was incubated with DAU in PBS at drug:DEXoxid weight ratios varying from 1:7 to 1:1. The DAU-DEXoxid conjugate was separated from free DAU by dialysis against PBS. Finally, the DAU-DEXoxid conjugate was incubated with MoAb (19-24 or P3), and any remaining polyaldehyde-activating sites in the DAU-DEXoxid-MoAb immunonojugate were reduced by a 30-min incubation with sodium cyanoborohydride. Excess sodium cyanoborohydride was removed by dialysis against PBS.

The total protein and DAU concentrations in the immunonojugates were determined spectrophotometrically (24), and immunonojugates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (25).
In V'aro labeled DAU-Dextran-IgG. resolving gel; loading well; used in the immunoconjugate preparation with a molecular weight of --150,000; w,

plates with protein A-purified MoAbs 19-24 (10/xg/ml) or 23-26 (10/xg/ml) p102 epitope, direct radioimmunoassay was performed to determine the modu-

ment and other

with lzSI-labeled MoAb 23-26 (3.2  105 cpm/ml; specific activity, 5.2  107

measured by direct radioimmunoassay as follows. The cells were incubated

MEM-E media at 0, 1, 5, 24, or 72 h; the p102 antigen on the cell surface was

Decolorization of the samples was performed by treatment with hydrogen peroxide at room temperature. Because this assay reflects 14C-ADR and 14C-

labeled ADR metabolites, the data are expressed as cpm of 14C-ADR radio-

active equivalents/g of organ, weight (29). Recovery studies were per-

formed on set plasma and tissue samples containing known amounts of 14C-

ADR.

To investigate the penetration of MoAb or DAU-DEX-MoAb immunocon-

jugates into solid tumor tissue, fibrosarcoma-bearing athymic mice were given a single i.p. injection of 125I-labeled MoAb or DAU-DEX-MoAb (125I-labeled) immunoconjugate. At the stated times after injection, radioactivity was measured in blood, tumor, skin, muscle, brain, and the rest of the viscera. The results are expressed as cpm/ml(g) of blood (wet tissue) or in tissue:blood ratios.

RESULTS

Anti-p102 MoAb 19-24 conjugated to DAU using glutaraldehyde (Method A) or carbodiimide bridge (Method B) lost all binding ac-
tivity to p102 antigen; therefore, no additional studies were per-
formed.

Based on spectrophotometric analysis of DAU-DEX-MoAb 19-24 immunoconjugates, the calculated DAU:total protein molar ratio ranged from 1.9 to 6.1. The presence of high molecular weight immunoconjugates was clearly evident after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (Fig. 1, Lane 3). Protein G (Fig. 1, Lane 4) did not reorganize the immunoconju-
gates; therefore, we used protein G to remove untreated (free) anti-
body from the reaction mixture. Furthermore, there was no difference in binding between immunoconjugates diluted up to 1:5000 (60 ng

MoAb in immunoconjugate/ml) and free (unconjugated) MoAb 19-24 (60 ng/ml).

**In Vitro Experiments**

Using two anti-p102 MoAbs (19-24 and 23-36), each recognizing a different p102 epitope, direct radiolmmunoassay was performed to determine the modu-

lation of the cell surface sarcoma-associated antigen p102 (11, 19). Fibrosar-

coma HT-1080 cells (10^6 cells/well) were incubated in sterile 96-well culture plates with protein A-purified MoAbs 19-24 (10 /µg/ml) or 23-26 (10 /µg/ml) at 37°C in 5% CO₂ for 24 h. The cells were washed and incubated in fresh

MEM-E media at 0, 1, 5, 24, or 72 h; the p102 antigen on the cell surface was

measured by direct radiolmmunoassay as follows. The cells were incubated

with 125I-labeled MoAb 23-26 (3.2 x 10^5 cpm/ml; specific activity, 5.2 x 10^7

cpm/µg) for 1 h. The supernatant was removed, and washed cells were lysed

in 0.05 ml of 1% sodium dodecyl sulfate. A cotton swab was used to wipe out

the lysed cells, and the radioactivity was measured. All assays for this experiment and other *in vitro* studies reported here were performed in triplicate.

The internalization of anti-p102 MoAb 19-24 was examined using p102-
positive fibrosarcoma HT-1080 cells incubated with 125I-labeled anti-p102 MoAb at 37°C or 4°C for 1 h. The cells were then washed and incubated for

1, 6, or 24 h at 37°C or 4°C. Finally, radioactivity was measured in cells

harvested with 0.05% trypsin-EDTA. The cells were homogenized (4°C,

and the radioactivity was measured for cytoplasm and crude plasma membrane

fraction (27).

The cytotoxicity of DAU-DEX-MoAb immunoconjugates was determined by incubating HT-1080 cells with the immunoconjugates. The cells (4 x 10^4

cells/well) were grown in sterile 96-well culture plates at 37°C in 5% CO₂ for

24 h, then washed with culture and MEM-E medium, and incubated with DAU,

DEXoxid, DEXred, DAU-DEXoxid, DAU-DEXred, DAU-DEXoxid-MoAb,

or DAU-DEXred-MoAb diluted in culture medium for 24 h. After incubation,
cells were washed and harvested with 0.05% trypsin-EDTA. The cells were

resuspended in 0.4% trypan blue (GIBCO), and live cells were counted.

**Tissue Distribution Studies**

14C-ADR (Amersham; specific activity, 96 /µCi/µg) was injected i.p. at a
dose of 0.02 /µCi/µg into a group of six fibrosarcoma-bearing athymic mice. At

1, 6, and 24 h after the ADR administration, 2 mice from each of the 3 time

intervals were sacrificed; blood, tumor, skin, muscle, brain, and the rest of the

viscera were analyzed for the presence of 14C-ADR and its radioactive me-

tabolites. Briefly, plasma (200 µl) or weighed tissue samples (~100 µg) were

transformed to scintillation vials and solubilized in 0.5 ml of KOH (28).

Decolorization of the samples was performed by treatment with hydrogen peroxide at room temperature. Because this assay reflects 14C-ADR and 14C-

labeled ADR metabolites, the data are expressed as cpm of 14C-ADR radio-

active equivalents/g of organ, weight (29). Recovery studies were per-

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![Fig. 1. Polyacrylamide gel electrophoresis (7% under nonreducing conditions) of 125I-labeled DAU-Dextran-IgG. Lane 3, molecular weight of over 200,000 at the top of the resolving gel; Lane 4, supernatant after incubation with agarose-bound protein G; Lane 1, 125I-labeled albumin with a molecular weight of 66,000; Lane 2, 125I-labeled mouse IgG used in the immunoconjugate preparation with a molecular weight of ~150,000; w, loading well; t, top of resolving gel.](image1.png)

**RESULTS**

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tivity to p102 antigen; therefore, no additional studies were per-
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gates; therefore, we used protein G to remove untreated (free) anti-
body from the reaction mixture. Furthermore, there was no difference in binding between immunoconjugates diluted up to 1:5000 (60 ng

MoAb in immunoconjugate/ml) and free (unconjugated) MoAb 19-24 (60 ng/ml).

![Fig. 2. Uptake of anti-pl02 MoAb 19-24 by fibrosarcoma cells. Cells (10^6 cells/ml) were incubated with 125I-labeled MoAb at 4°C (O) or 37°C (•) in MEM-E. At the times indicated, cells were washed and harvested, and radioactivity was measured. Points, mean of 3 determinants.](image2.png)
After 36 h, the cells were harvested, resuspended in trypan blue, and counted. Cytotoxicity binding of radiolabeled MoAb 23-26. The mean was similar (2.5 ± SD) of cells exposed for 24 h to unlabeled MoAb 23-26 and then grown in MEM-E) and cells exposed to MoAb 19-24. However, when is expressed in percentage of dead cells SD as compared to untreated cells.

MoAb 23-26 was significantly lower (due to competition) with respective values for times 0, 1, 5, 24, and 72 h of 0.3, 0.72, 1.22, 1.93, and 1.91 ± 10^4 cpm.

Binding of 125I-labeled anti-p102 MoAb to human fibrosarcoma HT-1080 cells was examined at 37°C and 4°C. The data are shown in Fig. 2. The amount of cell-bound radioactivity after 24 h of incubation at 37°C was 3.6-fold higher than at 4°C. A progressive increase was observed in the uptake of MoAb during the 24-h period: 0.05, 0.09, and 0.21 ng at 1, 6, and 24 h, respectively.

In order to examine how much MoAb was internalized by human fibrosarcoma cells, the radioactivity of plasma cell membranes and cytoplasm was measured. Of the total radioactivity, 54% was located in the plasma cell membranes and 41% in the cytoplasm. However, when fibrosarcoma cells were analyzed immediately after incubation with radiolabeled MoAb, 94.6% of radioactivity was located in the cell membranes and only 3.9% in the cytoplasm.

Cytotoxicity assays revealed significantly higher specificity of the DAU-DEX-MoAb 19-24 immunonjugate than nonspecific immunonjugate DAU-DEX-MoAb P3; also, it showed a greater ability to reduce the viability of in vitro fibrosarcoma cells [expressed as a percentage of dead cells (Table 1)]. The highest concentration of DAU-DEX-MoAb 19-24 (10 μg of DAU/ml) killed 77.7% (compared to untreated cells) of the fibrosarcoma cells, and the nonspecific control DAU-DEX-MoAb P3 killed 14.1%. At concentrations of 10 μg/ml, a high level of cytotoxicity was observed with free DAU (~98% of cells were killed), DAU linked to DEXoxid (~96%), or DAU linked to DEXred (~93%). DAU in the immunonjugate with MoAb 19-24, at a concentration of 1 μg/ml, was able to kill 25.4% of fibrosarcoma cells, which was about 36.8% of the efficacy of free DAU. While free DAU at a concentration of 0.1 μg/ml was able to kill 56.8% of the fibrosarcoma cells, DAU at the same concentration in the immunonjugate revealed no cytotoxicity to fibrosarcoma cells. The cytotoxicity of DEXoxid and DEXred was tested at concentrations up to 230 μg/ml; neither showed any cytotoxic effect on the growth of fibrosarcoma cells.

To examine the time course of ADR uptake into the fibrosarcoma xenograft, sarcoma-bearing mice received injections of 14C-ADR. Two mice were sacrificed at 1, 6, and 24 h after radiolabeled Adriamycin was administered i.p. (0.02 μg/g) into fibrosarcoma-bearing athymic mice; it is expressed as ADR 14C radioactive equivalents in cpm/ml blood or g wet tissue.

| Table 1 Inhibition of fibrosarcoma cells growth |
|-----------------|-------------|-----------------|-----------------|-----------------|-----------------|
| Cytotoxicity     | 10.0 μg/ml  | 1.0 μg/ml       | 0.1 μg/ml       | 0.001 μg/ml     |
| Daunomycin       | 97.8 ± 0.5  | 68.9 ± 1.7      | 56.8 ± 2.5      | 25.4 ± 4.6      |
| DAU-DEXoxid      | 96.1 ± 1.2  | 58.8 ± 5.7      | 35.8 ± 4.7      | 4.4 ± 7.6       |
| DAU-DEXred       | 92.6 ± 0.9  | 64.8 ± 1.0      | 43.8 ± 2.2      | 8.2 ± 9.4       |
| DAU-DEX-MoAb 19-24 | 77.7 ± 1.6  | 25.4 ± 5.2      | 0.0 ± 0.0       | 0.0 ± 0.0       |
| DAU-DEX-MoAb P3  | 14.1 ± 4.8  | 3.2 ± 2.1       | 0.0 ± 0.0       | 0.0 ± 0.0       |

DISCUSSION

This paper reports on our continuing effort to use MoAbs to human sarcoma-associated antigens as a tool in diagnosing and treating soft tissue sarcoma (11-17). To date, we reported the production of several MoAbs against sarcoma-associated antigens p102 (11), p160, and p200 (29). Previous studies have shown that MoAb 19-24 generated against a malignant fibrous histiocytoma reacted with a cell surface mannose containing a glycoprotein with a molecular weight 102,000 of the total injected radioactivity (Table 2). At 6 h, radioactivity was 19-fold higher in the liver and spleen and 12-fold higher in the kidney than the tumor xenograft. Similarly, all other normal tissues (lung, heart, muscle, and skin) showed higher levels of radioactivity than the tumor.

In order to examine the uptake of anti-p102 MoAb by tumor tissue, fibrosarcoma-bearing athymic mice received injections of 125I-labeled MoAb 19-24. The %ID/ml of blood was markedly higher at 6 h (15.89 ± 0.39) than at 1 h (9.13 ± 0.39) or 24 h (5.16 ± 0.34).

The viscerum (liver, spleen, kidney, and lung) blood ratios of 125I-labeled MoAb 19-24 at 1 h ranged from 0.51 to 0.57, and, except for the lung, the organ:blood ratios were lower at 6 and 24 h. The tumor tissue: blood ratio was 0.36 ± 0.21 (0.95 %ID) at 1 h and 0.61 ± 0.08 (9.47 %ID) at 6 h. At 24 h, the tumor tissue: blood ratio reached the highest value, 1.62 ± 0.28 (7.86 %ID), which was 3.7-fold higher than the uptake by the liver and about 5.6-fold higher than the uptake by the spleen. The uptake of nonspecific MoAb P3, measured at 24 h after i.p. injection of 125I-labeled MoAb P3, showed a tissue: blood ratio similar to that of normal tissue tested for 125I-labeled MoAb 19-24. However, the tumor tissue: blood ratio was lower (0.34 ± 0.01).

The uptake of 125I-labeled DAU-DEX-MoAb by tumor and normal tissues of fibrosarcoma-bearing athymic mice at 24 h after i.p. injection was examined in the same fashion as described above. Data listed in Table 3 clearly indicate similar blood ratios in normal tissue and in a group of mice that received free 125I-labeled MoAb 19-24 or radiolabeled MoAb linked to the immunonjugate. A decrease in the tumor tissue: blood ratio from 1.62 ± 0.28 to 0.66 ± 0.05 was observed in the group of mice after injection of 125I-labeled DAU-DEX-MoAb 19-24. However, the uptake of the immunonjugate with nonspecific 125I-labeled MoAb P3 was much lower (0.22 ± 0.03).

| Table 2 Tissue uptake of 14C-adriamycin in fibrosarcoma-bearing athymic mice |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Tissue          | Cpm x 10^3     | 1 h              | 6 h              | 24 h             |
| Blood           | 3.7² (3.6-3.9)³ | 4.4              | 3.2              |
| Liver           | 140.5           | 247.5            | 60.6             |
| Kidney          | 66.5            | 155.3            | 42.2             |
| Spleen          | 123.2           | 240.7            | 151.5            |
| Lung            | 33.6            | 70.8             | 27.9             |
| Heart           | 37.3            | 37.3             | 14.9             |
| Muscle          | 16.2            | 20.1             | 9.1              |
| Skin            | 28.3            | 27.3             | 8.4              |
| Brain           | 14.5-17.3       | 10.1-39.6        | 14.3-29.5        |
| Tumor           | 13.9            | 13.9             | 6.1              |

a Mean value from two animals.

b Numbers in parentheses, range.
Radiolabeled MoAb 19-24 was shown to localize specifically to tumor when injected into human sarcoma xenograft-bearing athymic mice (12, 14, 15) and sarcoma-bearing humans, with negligible binding to normal tissue (13, 16). MoAb 19-24 has also been shown to have a high affinity to target antigen (8.1 × 10^9 M^-1) found in more than 5 × 10^5 sites/cultured human fibrosarcoma HT-1080 cell (25).

In the first phase of our studies, the potent and broad-spectrum antineoplastic agent ADR and its conjugate with MoAb 19-24 were tested for in vitro cytotoxicity using human fibrosarcoma cells (19). Analysis of DAU cytotoxicity to the fibrosarcoma cells revealed that DAU was, on average, 2.3 times more effective than ADR. Therefore, in this study we investigated the specificity and ability of MoAb 19-24 to deliver DAU to human fibrosarcoma cells. Two important issues needed to be addressed: internalization of MoAb 19-24; and the modulation of cell surface antigen was found to be a major obstacle in tumor cell destruction by antibody-mediated cytotoxicity (31). In our studies, p102 modulation was not observed in fibrosarcoma HT-1080 cells exposed to MoAb 19-24. The internalization and modulation data clearly indicate that fibrosarcoma cells are suitable for in vitro cytotoxicity studies.

Several methods of coupling anthracycline antineoplastic drugs to antibodies have been described (8–10). Conjugating DAU and antibody via a glutaraldehye bridge produced immunocomjugates with moderate activity, whereas using carbodiimide yielded inactive conjugates (8). In our attempts to couple MoAb 19-24 and DAU, both methods produced immunocomjugates with no binding activity to p102 antigen. Several investigators have extensively examined conjugates of DAU and ADR covalently coupled through dextran bridge to antibodies (3, 23, 32–34). Although, in the present study, the binding activity was not changed for MoAb 19-24 linked to DAU via a dextran bridge to a solid-phase p102 antigen, the immunoreactivity of anti-human T-cell MoAb T 101 bound in ADR-DEX-MoAb T 101 immunocomjugate proved to be within 20% of free MoAb T 101 (33). We cannot explain why immunocomjugates prepared by similar methods differed in binding activity. On the basis of previous studies (35), we suggest that the reduced immunoreactivity of MoAb T 101 in the immunocomjugate is not caused by the difference in molecular structure between ADR and DAU. In vitro experiments, based on inhibition of thymidine incorporation, confirmed that ADR-DEX-MoAb immunocomjugates retained their cytotoxicity, which was similar to the inhibition pattern of ADR (33, 34). However, the loss of in vitro cytotoxicity of MoAb linked to ADR (33) and similar cytotoxicity patterns of immunocomjugates with test and control antibody (34), were evident. In our study, the in vitro cytotoxicity of DAU-DEX-MoAb 19-24 was found to be lower than the efficacy of free DAU, but it was much higher than the cytotoxicity of immunocomjugate with control antibody (Table 1). In addition, DAU-DEX-MoAb 19-24 immunocomjugates retain in vivo specificity to tumor tissue (Table 3). The molar ratios of DAU to MoAb 19-24 (1.6 to 6.1) were similar to those reported previously (33, 34).

A number of in vivo experiments have been performed to investigate the ability of immunocomjugates to kill tumor cells (3–5, 7, 23). Most of these studies based in vivo cytotoxicity evaluation on survival time of experimental animals and retardation of tumor growth under variable conditions: single or multiple doses; and administration of free drug mixed with immunocomjugates and/or free MoAb. Thus, it is difficult to compare data obtained by different investigators. We assume that the therapeutic or toxic effect of a drug or immunocomjugate depends mostly on its tissue concentration and its prolonged action time in a given tumor. Therefore, we decided first to examine the tissue uptake and clearance of an antineoplastic drug and of MoAb 19-24 immunocomjugates.

There was low uptake of i.p.-injected ADR by tumor tissue [<1.1 %ID Table 2]. Normal tissue uptake of ADR by athymic mice differed from that described previously for rats (29) and CD1 mice (36). The normal liver:heart tissue ratio in athymic mice was 6.7:1, in CD1 mice it was 1:7:1, and in rats it was ~0.3:1.

Our previous studies investigated dehalogenation of 125I-labeled MoAb 19-24. The labeled material mixed with mouse serum was stable to storage, yielding over 87% trichloroacetic acid-precipitable radioactivity after 4 days at 37°C. In vivo dehalogenation with rapid clearance of the tracer from tumor and normal tissues is a major problem when using antibodies labeled with iodine isotopes and when expressing data in %ID (15). Based on excretion of free radiodiode, ~18 %ID is dehalogenated over the first 24 h (3).

In summary, the results of our studies show that the uptake of the MoAb 19-24 immunocomjugate by tumor tissue is higher than by normal tissues, supporting our suggestion that MoAb 19-24 immunocomjugates may play an important role in managing soft tissue sarcomas.

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