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 immunoconjugate was 79.4% as efficient as free daunomycin and, at 1 μg/ml concentration, 36.8% as efficient. Control nonspecific murine monoclonal antibody P3 immunoconjugates were relatively ineffective. The distribution of 14C-Daunomycin, 125I-labeled monoclonal antibody 19-24, and 125I-labeled 19-24 immunoconjugate was also evaluated over a 24-h 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 14C 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 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 immunoconjugates, this ratio decreased to 0.66 ± 0.05, which was still higher than normal (liver; 0.48 ± 0.82; lung, 0.48 ± 0.07; spleen, 0.25 ± 0.01) or nonspecific monoclonal antibody P3 immunoconjugates. Thus, it appears that, compared to free daunomycin, monoclonal antibody 19-24 immunoconjugates may be more efficient and less cytotoxic to normal tissues.

INTRODUCTION

MoAbs1 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 immunoconjugates prepared by different methods varied in usefulness in targeting antineoplastic drug 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 covalently coupled to MoAb 19-24 using a birutinin-biotin-biotin bridge (17). In vitro cytotoxicity studies using human fibrosarcoma cells, this ADR-MoAb immunoconjugate maintained 40% of the efficacy of free ADR. Immunoconjugates using a birutinin-biotin-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 immunoconjugates into the bloodstream. Therefore, we decided to investigate other methods of coupling anthracycline 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 immunoconjugates in fibrosarcoma-bearing athymic mice was also examined.

METHODS AND MATERIALS

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).

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 radiiodinated using Na125I (Amersham, Arlington Heights, IL) and chloramine-T (20).

Preparation of Immunoconjugates

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% aqueous solution of glutaraldehyde and incubated it for 15 min at room temperature in the dark. The excess glutaraldehyde and DAU were removed by extensive dialysis against PBS (10 mM phosphate-0.15 M NaCl-1 mM MgCl2-0.02% NaN3, pH 7.3).

Method B. A MoAb was covalently coupled to DAU with EDC using a conjugation kit supplied by Pierce Chemical Co. (Rockford, IL) (8, 22). Briefly, a mix 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 immunoconjugate 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 topolyaldehyde-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-active sites in the DAU-DEXoxid-MoAb immunoconjugate 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 immunoconjugates were determined spectrophotometrically (24), and immunoconjugates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (25).

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2 To whom requests for reprints should be addressed, at Specialized Cancer Center (M/C820), 840 South Wood Street, Chicago, IL 60612.
3 The abbreviations used are: MoAb, monoclonal antibody; ADR, Adriamycin; DAU, daunomycin (daunorubicin); PBS, phosphate buffered NaCl solution; EDC, ethyldimethyl amino propyl carbodiimide; DEX, dextran; DEXoxid, oxidized dextran; DEXred, reduced dextran; MEM-E, Eagle’s minimal essential medium; %ID, percentage of injected dose.

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In vitro Experiments

Using two anti-p102 MoAbs (19-24 and 23-26), each recognizing a different p102 epitope, direct radioimmunoassay was performed to determine the modulation of the cell surface sarcoma-associated antigen p102 (11, 19). Fibrosarcoma HT-1080 cells (10⁴ 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 radioimmunoassay as follows. The cells were incubated with 125I-labeled MoAb at 37°C or 4°C for 1 h. The cells were then washed and harvested, and 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-epitope, direct radioimmunoassay and autoradiography (Fig. 1, Lane 3) or carbodiimide bridge (Method B) lost all binding activity to p102 antigen; therefore, no additional studies were performed.

In a different experiment, ~6 × 10⁶ fibrosarcoma cells were grown in a flask and incubated with 125I-labeled MoAb 19-24 at 37°C in 5% CO₂ for 24 h. After incubation, the cells were washed or incubated in fresh media for 24 h and 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 immun conjugates was determined by incubating HT-1080 cells with the immun conjugates. The cells (4 × 10⁴ 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 visera were analyzed for the presence of 14C-ADR and its radioactive metabolites. Briefly, plasma (200 μl) or weighed tissue samples (~100 μg) were transformed to scintillation vials and solubilized in 0.5 ml 1 N KOH (28). Determination of the sample 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 radioactivity equivalents/g of organ, wet weight (29). Recovery studies were performed on set plasma and tissue samples containing known amounts of 14C-ADR.

To investigate the penetration of MoAb or DAU-DEX-MoAb immun conjugates 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) immun conjugate. At the stated times after injection, radioactivity was measured in blood, tumor, skin, muscle, brain, and the rest of the visera. 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 activity to p102 antigen; therefore, no additional studies were performed.

Based on spectrophotometric analysis of DAU-DEX-MoAb 19-24 immun conjugates, the calculated DAU:total protein molar ratio ranged from 1.9 to 6.1. The presence of high molecular weight immun conjugates 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 immun conjugates; therefore, we used protein G to remove untreated (free) antibody from the reaction mixture. Furthermore, there was no difference in binding between immun conjugates diluted up to 1:5000 (60 ng MoAb in immun conjugate/ml) and free (unconjugated) MoAb 19-24 (60 ng/ml).

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

Fig. 2. Uptake of anti-p102 MoAb 19-24 by fibrosarcoma cells. Cells (10⁴ cells/ml) were incubated with 125I-labeled MoAb at 4°C (•) or 37°C (○) in MEM-E. At the times indicated, cells were washed and harvested, and radioactivity was measured. Points, mean of 3 determinants.
After 36 h, the cells were harvested, resuspended in trypan blue, and counted. Cytotoxicity of radiolabeled MoAb 23-26. The mean was similar (2.5 ± 0.3% ID) 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 ± 0.01 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 Table 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 immunoconjugate than nonspecific immunoconjugate 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 DEXoxi (~96%), or DAU linked to DEXred (~93%). DAU in the immunoconjugate 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 immunoconjugate revealed no cytotoxicity to fibrosarcoma cells. The cytotoxicity of DEXoxi 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 receiving i.p. injections of 14C-ADR. Radioactivity of 14C was measured in blood, tumor, skin, muscle, brain, and the rest of the viscera (Table 2). The mean total tumor tissue weight in six mice used in this experiment was 1.51 ± 0.27 (SD) g. The levels of ADR 14C-radioactive equivalents in blood and brain tissue were low, corresponding to only 0.3% of the injected radioactivity. The radioactivity of the tumor tissue was slightly higher than that of the blood; however, it never exceeded 1% 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 visera (liver, spleen, kidney, and lung) radioactivity was significantly less than 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 immunoconjugate. 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 immunoconjugate with nonspecific 125I-labeled MoAb P3 was much lower (0.22 ± 0.03).

**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 mannos containing a glycoprotein with a molecular weight 102,000.

**Table 1. Inhibition of fibrosarcoma cells growth**

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>10.0</th>
<th>1.0</th>
<th>0.1</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAU</td>
<td>97.8</td>
<td>0.5</td>
<td>9.8</td>
<td>4.6</td>
</tr>
<tr>
<td>DAU-DEXred</td>
<td>92.6</td>
<td>0.9</td>
<td>6.8</td>
<td>2.6</td>
</tr>
<tr>
<td>DAU-DEX-MoAb 19-24</td>
<td>77.7</td>
<td>1.6</td>
<td>5.2</td>
<td>1.1</td>
</tr>
<tr>
<td>DAU-DEX-MoAb P3</td>
<td>14.1</td>
<td>1.8</td>
<td>2.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

| DAU-DEX-MoAb 19-24 (10/~g of DAU/ml) killed 77.7% (compared to untreated cells) of the fibrosarcoma cells, and the nonspecific 125I-labeled MoAb P3 was much lower (0.22 ± 0.03). |  

**Table 2. Tissue uptake of 14C-daunorubicin in fibrosarcoma-bearing athymic mice**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>3.7*</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Liver</td>
<td>140.5</td>
<td>247.5</td>
<td>60.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>66.5</td>
<td>155.3</td>
<td>42.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>123.2</td>
<td>249.7</td>
<td>151.5</td>
</tr>
<tr>
<td>Lung</td>
<td>33.6</td>
<td>70.8</td>
<td>27.9</td>
</tr>
<tr>
<td>Heart</td>
<td>37.3</td>
<td>38.6-48.9</td>
<td>24.4-49.8</td>
</tr>
<tr>
<td>Muscle</td>
<td>16.2</td>
<td>20.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Skin</td>
<td>24.9</td>
<td>15.7-16.7</td>
<td>12.9-27.3</td>
</tr>
<tr>
<td>Brain</td>
<td>4.2</td>
<td>14.5-17.3</td>
<td>10.1-39.6</td>
</tr>
<tr>
<td>Tumor</td>
<td>15.3</td>
<td>8.9-10.6</td>
<td>9.8-16.8</td>
</tr>
</tbody>
</table>

*Mean value from two animals.

Numbers in parentheses, range.
Table 3: Tissue: blood ratio of 125I-labeled MoAb 19-24 and Daunomycin-dextran-MoAb (125I-labeled MoAb 19-24) in fibrosarcoma-bearing athymic mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MoAb 19-24</th>
<th>Daunomycin-dextran-MoAb 19-24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Liver</td>
<td>0.57 ± 0.03</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.57 ± 0.09</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.51 ± 0.08</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Lung</td>
<td>0.52 ± 0.09</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>Heart</td>
<td>0.18 ± 0.02</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.24 ± 0.07</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Skin</td>
<td>0.10 ± 0.04</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Brain</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.36 ± 0.21</td>
<td>0.64 ± 0.08</td>
</tr>
</tbody>
</table>

*Time after i.p. injection.  
*Mean ± SD from three animals.

(p102) with pl 4.7 (11, 25). 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) (31). In our studies, p102 modulation was not found in more than 5 x 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. 4

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 possible p102 modulation. Internalization of 125I-labeled MoAb 19-24 by fibrosarcoma HT-1080 cells was examined by the method described by Uadia et al. (26) and by the direct method, in which radioactivity was measured in the cytoplasm and cell membranes. Data obtained by both methods indicate that MoAb 19-24 is internalized by fibrosarcoma cells. 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 glutaraldehyde bridge produced immun conjugates with moderate activity, whereas using carbodiimide yielded inactive conjugates (8). In our attempts to couple MoAb 19-24 and DAU, both methods produced immun conjugates 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 T101 bound in ADR-DEX-MoAb T101 immun conjugate proved to be within 20% of free MoAb T101 (33). We cannot explain why immun conjugates prepared by similar methods differed in binding activity. On the basis of previous studies (35), we suggest that the reduced immunoreactivity of MoAb T101 in the immun conjugate 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 immun conjugates 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 immun conjugates 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 immun conjugate with control antibody (Table 1). In addition, DAU-DEX-MoAb 19-24 immun conjugates 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 immun conjugates 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 immun conjugates 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 immun conjugate 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 immun conjugates.

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. 4 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 radiiodine, ~1-18 %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 immun conjugate by tumor tissue is higher than by normal tissues, supporting our suggestion that MoAb 19-24 immun conjugates may play an important role in managing soft tissue sarcomas.

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