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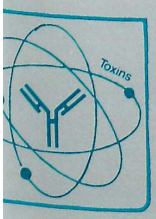

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ANTIBODY, IMMUNOCONJUGATES, AND
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Preclinical and Clinical Studies with a Variety of Immunoconjugates

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ABSTRACT

The use of monoclonal antibodies to target cytotoxic drugs to solid tumors is an attractive concept, but has yet to make a radical impact on the therapy of cancer. A number of variables that could influence the efficacy of drug-antibody conjugates were investigated - (a) Drugs - the covalent attachment of drug to monoclonal antibody was variable; anthracyclines such as adriamycin (AD) coupled poorly, yet analogs such as bromoidarubicin were coupled successfully; chlorambucil (CBL) and melphalan (MEL) were unique in that a greater number of drug molecules could be coupled to monoclonal antibody. The requirement for more cytotoxic drugs was clear when aminopterin (AMN), a more cytotoxic analog of methotrexate (MTX), was coupled to monoclonal antibody and found to be more effective than methotrexate conjugates. Of all the drugs used, idarubicin (Ida) was the most effective in vivo (Ida > MEL > AMN > MTX > CBL > AD). (b) Antibody - in several tumor growth models $F(ab')_2$ drug complexes were as effective as IgG, but not more so. (c) Access to tumor - the importance of tumor access was demonstrated when tumors growing subcutaneously were eradicated by the local injection of whole ricin-antibody conjugates and intraperitoneal (i.p.) tumors were also easily eradicated by i.p. treatment. By contrast, tumors growing in the subcutaneous site are less susceptible to therapy, however, vasoactive agents increased the in vivo efficacy of drug-antibody conjugates. (d) Tumor - the problem of tumor heterogeneity was addressed by using a cocktail of two drug-antibody conjugates for tumor therapy; the cocktails were clearly more effective than either conjugate used alone. On the basis of these results, phase I studies are in progress using MTX-anti-colon cancer monoclonal antibodies given intravenously and ricin anti-breast antibodies given into the tumors.

INTRODUCTION

In contrast to the treatment of lymphoma and leukemia where a proportion of patients obtain a complete remission, cytotoxic agents for the treatment of most solid tumors are clearly less effective (1) and there

is usually accompanying toxicity. Consequently there have been attempts to "target" cytotoxic agents to tumors. The advent of monoclonal antiodies (MoAbs) has been an important step in the development of drug targeting whereby the MoAbs can now be used to convey cytotoxic drugs to tumor cells (2-5). Drug-MoAb conjugates, upon binding to target cells, may be specifically internalized and degraded to free drug, which then acts on the target (6-9). This approach to drug targeting does however, present many problems both in vitro and in vivo. Firstly, it is difficult to couple cytotoxic drugs (mostly organic chemicals) to hydrophilic antibody molecules with retention of both drug and antibody activity; thus, despite retaining selective cytotoxicity for target cells, the conjugate is generally less cytotoxic than the free drug. As a result it is necessary to couple more cytotoxic drugs to MoAbs to obtain greater antitumor effects; our efforts have been directed towards conjugating MoAbs to more cytotoxic analogs of drugs. Other problems include the in vitro and in vivo stability of the conjugates, and the ability of immunoconjugates to penetrate throughout tumors. Here we present a review of our studies using different drug-antibody conjugates; including their potentiation with vasoactive agents and the use of cocktails of drug-antibody conjugates.

MATERIALS AND METHODS

Tumor Cells

E3, a clonal variant of the murine thymoma ITT(1)75NS (10) was maintained in vitro in Dulbecco's Modified Eagles Medium (DME) supplemented with 10% heat inactivated newborn calf serum (Flow Laboratories, Sydney, Australia), 2mM glutamine (Commonwealth Serum Laboratories (CSL), Melbourne, Australia), 100μg streptomycin (Glaxo, Melbourne, Australia) and 100IUml^{-1} penicillin (CSL). For in vivo experiments E3 cells were maintained by serial passage in ascites fluid in (C57BL/6 x BALB/c)F_1 ($B6CF_1$) mice. Human cell lines used were COLO 205 (11) a colon carcinoma, and CEM (12) a T-lymphocyte leukemia cell line; these were maintained in culture in RPMI-1640 medium (Flow Laboratories) with the same additives as above. Adherent cells were harvested with 0.125% trypsin (CSL), washed with RPMI-1640, and either used for the in vitro assays or injected subcutaneously into nude mice ($3-5x10^6$) and grown in vivo as xenografts on the abdominal wall of nude mice.

Mice

Nude mice (Swiss) were obtained from the Animal Resources Centre (Perth, Western Australia), and $B6CF_1$ mice were produced in our department.

Monoclonal Antibodies and Serology

Several monoclonal antibodies were used in this study (Table 1). A rosetting assay (18) was used to determine the antibody activity of the drug-antibody conjugates; as a control antibody that had undergone the same procedures used in the coupling methods (other than adding drug) was used.

Preparation of Drug-antibody Conjugates

Chlorambucil (19), N-acetyl melphalan (20, 21) and methotrexate (22) were coupled to MoAb as described previously using an active ester derivative of these drugs. Aminopterin was also coupled by a similar method but was dissolved in dimethyl sulphoxide rather than dimethylformamide (23). Idarubicin was coupled to MoAb via the

TABLE 1
Details of MoAbs used in Experimenal Studies.

Antibody	Reference	Purification Procedure	Target Cell	Immunoglobulin Subclass
Ly-2.1	13	Protein A	ITT(1) 75NS E3 thymoma	IgG2a
250-30.6	14	Affigel Blue	COLO205 (Ca colon)	IgG2b
anti-Transferrin Receptor (TFR)	15	Affigel Blue	CEM	IgG1
17.1	16	Affigel Blue	COLO205	IgG2a
Ly-1.1	17	Protein A	CBA thymus	IgG2a

bromoidarubicin (Br-Ida) derivative (24). Briefly, MoAb (1-2mg/ml in 0.05M Borate pH 8.0) were mixed with molar excesses (0-50) of 14-bromo-4-demethoxydaunomycin (Br-Ida) dissolved in N,N-dimethylformamide (DMF) at 10mg/ml. The reaction was maintained at room temperature for 4 hours, before centrifuging (400g x 5 minutes) to remove any precipitate; free Br-Ida and other unreacted materials were removed using a Sephadex G25 column (PD10) and the conjugates were then passed through a column of Porapak Q to remove non-specifically bound drug (25). The amount of idarubicin incorporated in drug-antibody conjugates was determined by absorbance spectrophotometry at 483nm (E_{483} = 3.4 x 10^3 m^{-1} cm^{-1}), and protein estimated (26). Adriamycin was coupled to MoAb using the iodoacetyl derivative (27); sulfydryl groups were exposed by treating antibody (1ml, 1mg/ml) with dithiothreitol (DTT) (75µl, 1M) for 45 mins; the mixture was desalted by gel filtration on a PD10 column equilibrated with deoxygenated 0.01M Tris-saline buffer pH 8.6, and the protein collected. Iodoacetyl adriamycin (0.4mg) in 100 l DMF was added to the reduced antibody (2.5ml), and allowed to stand for 2.5-3 hrs and the precipitate which formed was removed by centrifugation and the supernatant purified by gel filtration. The number of residues (N) of adriamycin molecules bound per immunoglobulin molecule was calculated to the formula:

$$N = 215{,}000 \times A_{280} / (11{,}600 \times A_{280}) - (8{,}600 \times A_{480})$$

where A_{480} and A_{280} are the absorbance of the conjugate at 480 and 280nm using the extinction coefficient of adriamycin at 480 and 280nm of 11,600 m^{-1} cm^{-1} and 8,600 m^{-1} cm^{-1} respectively; the extinction of immunoglobulin at 280nm is 215,000 m^{-1} cm^{-1}.

In vitro cell inhibition assays

Two types of assays were carried out to test for residual drug activity of the conjugates in comparison with free drug. (a) 24 hour assay: 100µl of cells (1-5 x 10^6/ml) was added to a 96-well flat bottom microtitre plate and incubated for 2-3 hours at 37°C; sterile antibody, free drug or conjugate was diluted in PBS and a 50µl aliquot was added to cells using duplicate wells per sample. Controls received 50µl of PBS and the cells were cultured at 37°C in 7% CO_2 for 24 hrs. (b) 30 minute assay: 200µl of cells (1-5 x 10^6/ml) was collected in sterile plastic tubes, resuspended in sterile antibody, free drug or conjugate and mixed for 30 minutes at 37°C. The cells were centrifuged (400g x 5 min) and resuspended in growth medium, then 100µl cells were added to microtitre wells in duplicate and incubated for 24 hrs. After the incubation period in both assays, 1µCi of [^{3}H]-thymidine (specific activity = 15Ci/mmol; Amersham Internation Ltd, Amersham, England) or [^{3}H]-deoxyuridne (specific activity = 15µCi/mmol in 50µl medium) was added and the plates incubated for a further 3-6 hours. Then the cells were harvested onto glass fibre filter paper, dried and the samples counted for radioactivity on a β counter. Incorporation of

radionucleotide was expressed as the percentage inhibition in incorporation of controls; the standard error for any given point did not exceed 5%.

In vivo experiments

Several different approaches were used. (a) Survival: Tumor cells were injected intraperitoneally and mice received various treatments by the same route and the survival of the mice was calculated. (b) Tumor growth: Tumor cells were injected subcutaneously into the abdominal wall and allowed to develop into a palpable lump at which time treatment (i.p. or i.v.) was commenced. The size of the tumors was measured daily with a caliper square along the perpendicular axes of the tumor and the data was recorded as a mean tumor size (products of two diameters ± standard error). Experimental groups of 10-20 mice, all of the same sex and age, were used in each experiment. In other studies, tumors were injected directly.

Biodistribution

$B6CF_1$ mice bearing subcutaneous E3 tumors (0.5 - 1.0cm^2) were used to compare the distribution of ^{125}I-anti-Ly-2.1 in the presence or absence of therapeutic levels of different vasoactive agents. Groups of 4 mice were sacrificed at 24 hrs after the injection of labeled anti-Ly-2.1 and the biodistribution of ^{125}I-anti-Ly-2.1 was determined by counting the radioactivity in blood, heart, spleen, liver, kidneys and tumor from these mice. The distribution of isotope is reported as a localisation ratio ¼tissue (cpm/g) / blood (cpm/g) ¼. All mice received 500,000 cpm of ^{125}I (approx. 20 g protein) by tail vein injection, one hour after the intravenous or oral administration of the vasoactive agent.

Clinical Studies

In a study using MTX-MoAb conjugates, patients with metastatic, histologically confirmed, colorectal cancer were included if they had fulfilled the eligibility criteria of 3-month expected survival, Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, presence of measurable disease, no other therapy for at least 1 month prior to MoAb-MTX administration, and normal hepatic and renal funciton. Four patients have been treated, with informed consent obtained from each. The measured lesions were studied 1 and 3 months after treatment with the same technique as that used for the initial evaluation. Blood tests were done to assess potential hematological, renal or hepatic toxicity, and to detect human anti-mouse antibody (HAMA) formation (assayed by an ELISA based test). Serum levels of carcinoembryonic antigen (CEA) were determined using a radioimmunoassay for CEA (CEA-RIA, Abbott Laboratories, Chicago, Ill. USA).

Administration of Antibody Conjugates

Three patients each received a total dose of 100mg MoAb covalently bound to approximately 4mg of MTX. The second dose given to the first patient caused a mild allergic reaction, so the dose was not escalated until the lower dose was tolerated by the next patient. Dose escalation was according to the modified Fibonacci Sequence (28) up to a maximum dose of 500mg MoAb : 20mg MTX/metre^2 of body surface area. This is well below the usual therapeutic dose of MTX used in human without folinic acid rescue (25mg/m^2) compared with 1500mg/m^2 that can be given with folinic acid rescue. The antibody conjugate was diluted in 500mls of normal saline and administered over 6-8 hours whilst under strict medical observation with the patients being carefully monitored for change in pulse, blood pressure,

temperature and respiratory function for 24 hours. Complete physical examinations were made after completion of the treatment and on the following day. All patients received a 48 hour course of systemic corticosteroids (to diminish hypersensitivity phenomena). Human anti-mouse antibodies (HAMA) were measure, before and after treatment; no patient with pre-existing HAMA was found. Patients were examined for signs and symptoms of serum sickness for 1 month after the infusion.

Pharmacokinetics: To determine the pharmacokinetics of the MoAb-MTX conjugates, serial blood samples were obtained (at 12, 24, 48 and 72 hours) after infusion. Affinity-purified sheep anti-mouse IgG (SAMG; Amersham, UK) was diluted to 15ng/500µl in PBS, plated on 96 well PVC plates (Costar, Cambridge, MA) and incubated overnight at 4°C. the plates were then washed 6 times in PBS/0.05% Tween 20 (Sigma Chemical Co., St Louis, MO, USA) and serum samples were diluted 1:32 in PBS/Tween diluent. Diluted serum (50µl) was then added with 10 counts per minutes (cpm) of ^{125}I-MoAb labeled competitor (in 50µl of the same diluent) to the SAMG coated wells. After overnight incubation at 4°C, the plates were washed in PBS/Tween and dried at 37°C and the wells were counted in a gamma counter to determine the amount of radioactivity bound per well. Each time the assay was performed a standard curve was generated using dilutions of purified unlabeled MoAbs of the isotype being assayed. The standard curve was generated by plotting the percentage of bound radioactivity/well (minus background) versus the log concentration of unlabeled competitor MoAb. The amount of MoAb in the serum samples was then calculated by relating the average cpm bound/well to the concentration of unlabeled MoAb producing an equivalent level of bound radioactivity.

Response Criteria

Response to treatment was assessed using standard criteria as suggested by Miller et al (29). **Complete Response:** (CR) is the disappearance of all clinical evidence of tumor for at least 4 weeks. **Partial Response:** (PR) is a reduction of at least 50% in the sum of the products of all diameters of measured lesions, lasting for at least 4 weeks. There must be no objective progression of any existing lesion and no new lesions may appear. There must be a significant reduction in the size of an evaluable lesion. **Stable Disease:** (SD) is an objective regression of measurable disease less than required to meet the criteria for partial response or less than a 25% decrease in a measurable lesion over 4 weeks. **Progressive Disease:** (PD) is an increase in the sum of the product of the two greatest perpendicular diameters of any measurable lesion by 25% or more, or obvious increase in an evaluable lesion. Appearance of new areas of malignant disease signifies progressive disease. **Duration of Response:** Duration of response was measured from the achievement of maximal response to the first sign of disease progression.

RESULTS

Conjugation of Antineoplastic Drugs to Monoclonal Antibodies

We have examined a number of different drugs in an attempt to obtain the most potent drug/antibody conjugate. The conjugation procedures are detailed here and the activity discussed in the following sections.

Adriamycin: Despite the side effects associated with the use of adriamycin, it is widely used; and although the exact nature of its cytotoxicity is not clear, intercalation of DNA is important. To reduce systemic toxic effects, several groups have coupled adriamycin to monoclonal antibodies with varying success (30). However, adriamycin is coupled to antibody with some difficulty, so a number of analogs to facilitate coupling were prepared (Fig. 1A-D). Of these, only the

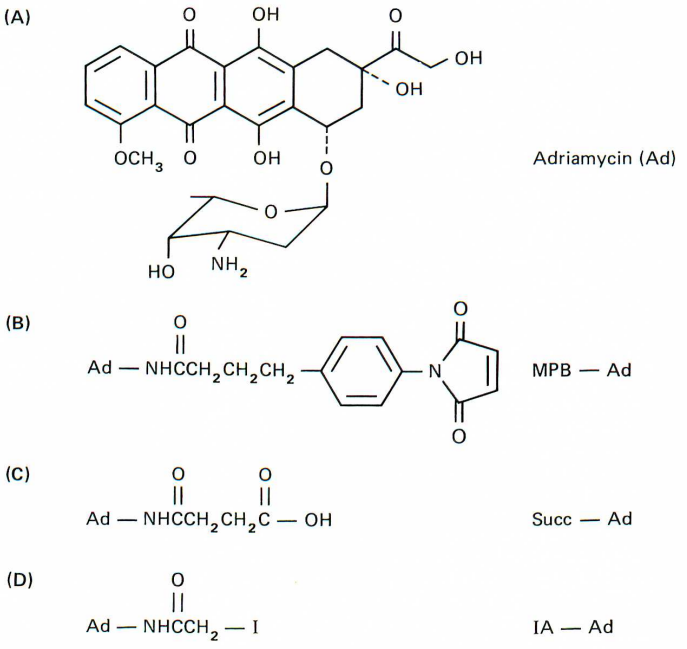


FIGURE 1

Chemical structures of Adriamycin (A) and derivatives. B, Maleimidophenyl-butyryl adriamycin (MPB-Ad); C, Succinyl adriamycin (Succ-Ad) and D, Iodoacetyl adriamycin (IA-Ad). Reprinted with permission from Targeted Diagnosis and Therapy (27).

iodoacetyl adriamycin analog resulted in an active conjugate (Fig. 2) where up to 8 molecules of adriamycin could be coupled to antibody with good protein recovery and antibody activity (Table 2). In these studies adriamycin non-covalently bound to MoAb was removed using a porapak Q column (25).

TABLE 2
Conjugation of Drugs and MoAbs.

Drug	Number of drug molecules per antibody molecule	Protein Recovery (%)	MoAb	MoAb Activity Before conjugation	MoAb Activity After conjugation
Adriamycin	8	70	Ly-2.1	1/128,000	1/32,000
Chlorambucil	25	70	Ly-2.1	1/50,000	1/15,000
Melphalan	25	65	Ly-2.1	1/75,000	1/20,000
"	25	55	Ly-2.1 (IgG1) $F(ab')^2$	1/32	1/32
Methotrexate	13	80	Ly-2.1		
Aminopterin	6	97	Ly-2.1		
Idarubicin	5	50	Ly-2.1	1/80,000	1/56,000
"	4	60	250-30.6	1/33,000	1/11,000

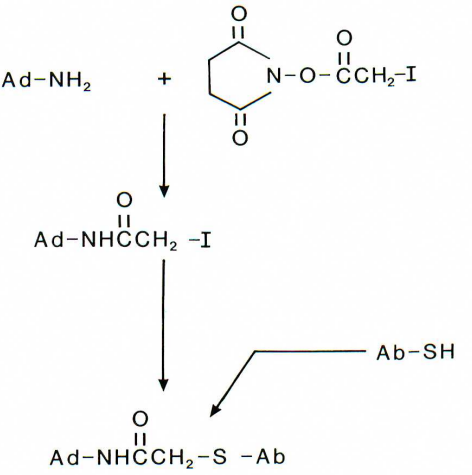
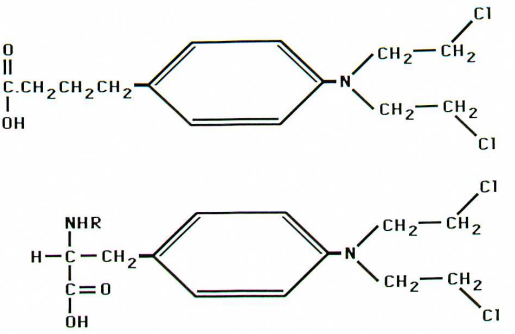


FIGURE 2

Coupling of adriamycin to monoclonal antibody. Reprinted with permission from Targeted Diagnosis and Therapy (27).

Chlorambucil: This alkylating agent is used for the treatment of various leukemias, lymphomas and for breast and ovarian carcinoma, although marrow suppression is one of the side effects. Chlorambucil (Fig. 3A) was one of the first drugs used for coupling to polyclonal antibodies and indicated that complexes with CBL may be formed at low or high pH, although the exact nature of the bond was unknown.

CHLORAMBUCIL



R = H MELPHALAN
R = CH_3CO N-ACETYL MELPHALAN

FIGURE 3

Chemical structures of chlorambucil (CBL), melphalan (MEL) and N-acetyl melphalan (N-AcMEL).

An active ester derivative of CBL was coupled to monoclonal antibodies (Fig. 4); this is a mild procedure and 20-30 CBL molecules could be coupled to an antibody molecule with good protein recovery and antibody activity (Table 2); 80% of CBL was covalently linked and 85% of the alkylating activity was preserved.

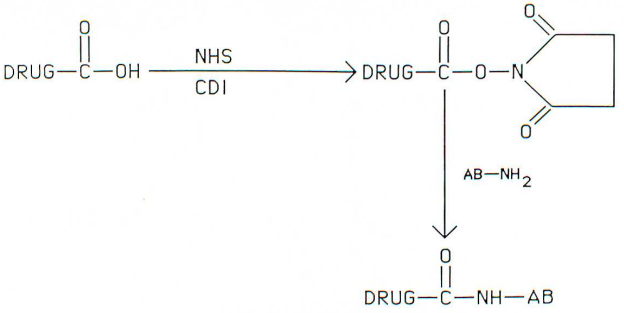


FIGURE 4
Coupling of Chlorambucil (CBL), N-acetyl melphalan (N-AcMEL), methotrexate (MTX) and aminopterin (AMN) to monoclonal antiobdies using the active ester method.

Methotrexate (MTX) and Aminopterin (AMN): MTX was coupled to antibody using an active ester (Fig. 4) as the γ-glutamyl carboxyl group of MTX (Fig. 5A) can be modified without effect on the dihydrofolate reductase binding ability. Conjugates with a drug:antibody ratio of 13 were formed with good yield and activity (Table 2). The more toxic folic acid antagonist, aminopterin (AMN) (Fig. 5B), was coupled to antibody in a similar way to MTX, but due to its low water solubility, the incorporation of AMN was less than MTX, with 6 molecules of aminopterin per antibody molecule bound (Table 2).

Melphalan: This is a more potent alkylating agent than chlorambucil and was coupled to antibody using a new approach. Melphalan enters cells via the amino acid transport system and its multifunctional nature makes it

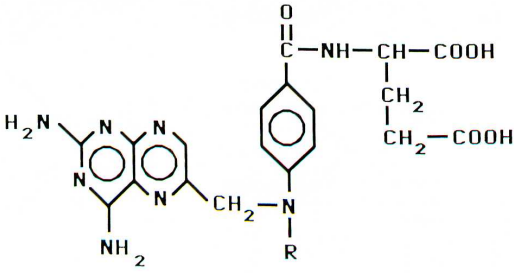
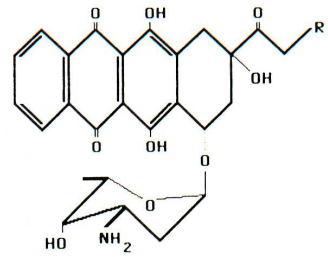


FIGURE 5
Chemical structures of methotrexate (MTX) and aminopterin (AMN).

difficult to couple to antibodies; the amino group of melphalan, necessary for cell uptake but not cytotoxicity, was blocked with an acetyl group and the resulting N-AcMEL (Fig. 3C) was coupled to antibody via an active ester (Fig. 4). The procedure removed the ability of the Melphalan to enter cells by active transport, however the MoAb provided the alternative route of cell entry. Similarly to CBL, 20-30 residues of MEL could be coupled to MoAb (Table 2).

Idarubicin: Idarubicin (Fig. 6A) is an anthracycline analog and is 10 times more cytotoxic than adriamycin. The 14-bromo analog of idarubicin (Fig. 6B) was coupled to monoclonal antibodies using a reaction which could give rise to two types of linkage; by testing the stability to base, it was concluded that 50% of the drug was ester linked (Fig. 6D) and 3-5 residues of drug could be coupled with good recovery of antibody activity and protein (Table 2).



A IDARUBICIN R = H

B BROMOIDARUBICIN R = Br

C AMINE LINK R = NH-MoAb

D ESTER LINK R = O-C(=O)-MoAb

FIGURE 6
Chemical structures of idarubicin (Ida) and possible linkages to antibody.

In Vitro Activity of the Drug-Antibody Conjugates

The pharmacologic activity of the free drug and MoAb bound drugs were tested in vitro on cell lines by measuring the inhibition of DNA and RNA synthesis previously found to correlate well with cell death. The in vitro cytotoxicity data for the various drug antibody conjugates is summarized (Tables 2 and 3) and there are several interesting points to note. Of all the drug-antibody conjugates, only Chlorambucil-antibody conjugates were more toxic than free drug (10 fold). Examination of the I.D. for methotrexate showed a 40 fold decrease in drug activity when bound to antibody, and aminopterin a 20 fold decrease in activity. However, the aminopterin conjugate was nearly as toxic as free methotrexate, emphasising the improvement in cytotoxicity with more potent analogs. In addition, the coupling of adriamycin to antibody using iodoacetyl adriamycin caused a 40 fold decrease in cytotoxicity; however by using idarubicin, where the conjugate was coupled via the C-14 carbon, the resulting conjugate had similar cytotoxicity to that of the free drug. Melphalan, when modified to N-AcMEL was 25 times less active, however when this was coupled to antibody there was a 10 fold increase in cytotoxicity.

TABLE 3
In Vitro Cytotoxicity of Drug-MoAb conjugates

Drug	Number of drug molecules per Ab molecule	Free drug ID_{50} (M)[1]	Drug-MoAb immunoconjugate ID_{50} (M)
Adriamycin	not done	$2.3x10^{-1}$	not done
Iodoacetyladriamycin	8	$8.0x10^{-1}$	$2.6x10^{-1}$ (anti-Ly-2.1)
Idarubicin	4	$2.2x10^{-1}$	$3.0x10^{-1}$ (anti-TFR)
Methotrexate	8	$8.2x10^{-1}$	$1.8x10^{-1}$ (250-30.6)
Aminopterin	8	$4.2x10^{-1}$	$8.4x10^{-1}$ (anti-Ly-2.1)
Chlorambucil	25-30	$1.7x10^{-1}$	$1.6x10^{-1}$ (anti-Ly-2.1)
Melphalan	not done	$3.1x10^{-1}$	not done
N-acetyl melphalan	25-30	$7.5x10^{-1}$	$7.5x10^{-1}$ (anti-Ly-2.1)

[1] Concentration at which 50% inhibition of the incorporation of ^{3}H-thymidine or ^{3}H-uridine uptake relative to the control occurs.

It was of interest that with certain compounds, e.g. MTX and CBL (where the former was approximately 400 times more potent than CBL as free drug) the potency was similar in the immunoconjugate. The advantage of the alkylating agents is clearly the ability to bind large amounts of these to antibody with a resulting increase in potency. The drugs examined were mostly those in common usage, however our feeling is that other drugs, discarded because of their toxicity, would be worth investigating, as the toxicity is usually decreased when they are coupled to antibody to form an immunoconjugate.

In Vivo Efficacy of Drug-Antibody Conjugates

In vivo models: Several different models were used. In the first the murine thymoma ITT(75)NS was grown in the Ly-2 congenic strain either subcutaneously or in the peritoneum (B6.PL(75NS)). This tumor is Ly-2.1^{-} but grows progressively, without rejection in C57BL/6 mice which are Ly-2.2^{+}. Thus, the monoclonal anti-Ly-2.1 antibodies are effectively "tumor specific" in that the antibody reacts only with the tumor and with no other normal tissues. In a second model human colon carcinoma cell lines were grown subcutaneously in nude mice and in a third, recently established model, fresh samples of colon carcinoma were growing subcutaneously in nude mice. All three models were used for preclinical studies and can also be used to examine the influence of the route of injection and the site of the tumor.

Adriamycin-MoAb Conjugates: The in vivo efficacy of Ad-anti-Ly-2.1 conjugates was tested in mice bearing ITT(1)75NS thymomas growing in the peritoneum. Groups of mice bearing established tumors were treated on days 1, 3, 5 and 7 with a total of 16μg of Ad in the conjugate. It was found that this increased the lifespan of 30% of mice by >200 days (Table 4);

TABLE 4
Efficacy of a variety of Drug-anti-Ly-2.1 conjugates on the survival of mice bearing the thymoma ITT(1)75NS E3.

Drug conjugated	Treatment Schedule[1] Total Dose administered (g) Drug	MoAb	Days after tumor inoculation	Days of survival (% survived) PBS	Drug	MoAb	Drug+ MoAb	Drug-MoAb conjugate
CBL	6x4	6x40	0,1,2,3,7,13	20	22	48	>200/80%	>200/80%
MEL	2x15	2x150	0,1	25	30	35	35	>200/90%
AD	4x4	4x50	1,3,5,7	28			32	>200/30%
Ricin	0.05	0.22	1	55			52	>200/90%

[1] All treatments were administered i.p.

when used with subcutaneously growing tumors, the immunoconjugates had little effect (Fig. 7).

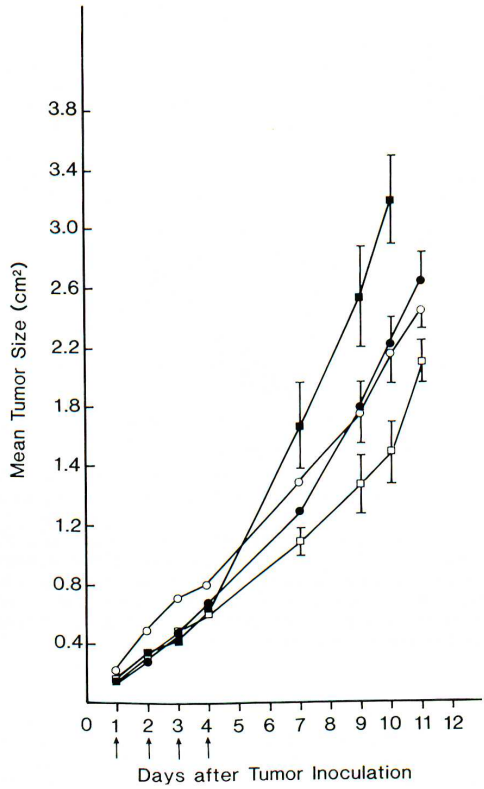


FIGURE 7

Growth of the Ly-2.1$^+$ thymoma ITT(1)75NS in B6CF$_1$ mice injected subcutaneously with 3 x 10^6 cells. Groups of 10 mice were given treatments i.p. (↓); PBS (■), adraimycin (○), non conjugated mixture of adriamycin and anti-Ly-2.1 (●) and Ad-anti-Ly-2.1 conjugate (□). Error bars represent ± standard error of the mean tumor size. Reprinted with permission from Targeted Diagnosis and Therapy (27).

Chlorambucil-anti-Ly-2.1 conjugates: Mice with intraperitoneal tumors treated with 24>g of CBL conjugate survived indefinitely (Table 4), although a non-covalent mixture of CBL and MoAb also increased the lifespan of 80% by >200 days. This is clearly a synergistic effect as similar doses of free drug and antibody did not have any effect when administered alone. In mice with subcutaneously growing tumors, those receiving CBL-MoAb conjugates had tumors significantly smaller than mice receiving a mixture of CBL and MoAb or MoAb alone (Fig. 8).

Melphalan-MoAb conjugates: Melphalan coupled to anti-Ly-2.1 using the N-AcMEL derivative was tested in mice with the thymoma growing in the peritoneal cavity (Table 4); 90% of the mice receiving the conjugate survived tumor free >200 days whereas all other mice died with tumor by day

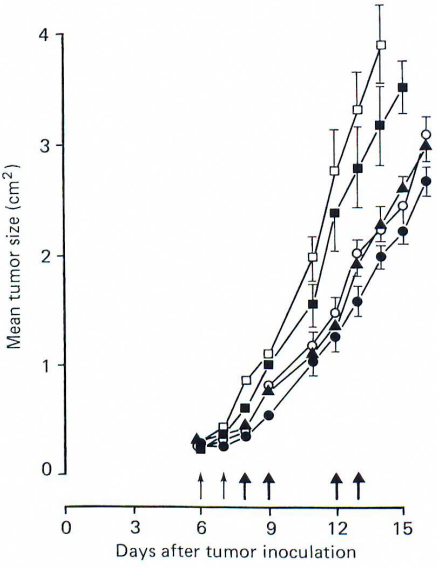


FIGURE 8

Growth of the thymoma ITT(1)75NS E3 in $B6CF_1$ mice given a s.c. injection of 2 x 10^6 cells. Groups of 10 mice were given treatment i.p. denoted (↓); PBS (□), free CBL (■), CBL-anti-Ly-2.1 conjguate (●), non-covalently conjugated CBL-anti-Ly-2.1 (○) and anti-Ly-2.1 (▲). Error bars represent ± standard error of the mean.

35. The effect on solid tumors was tested in nude mice bearing COLO 205 and using N-AcMEL-30.6 conjugates (60μg) in 10% of the mice, tumors were eradicated (Fig 9), and on day 28 the mean size of the tumors of the mice treated with conjugate was 50% that of mice in the control groups. However, while effective, the conjugates were limited by their cytotoxicity and consequently more toxic analogs were examined.

Aminopterin and Methotrexate-MoAb conjugates: In vitro experiments demonstrated AMN to be 10 times more cytotoxic than MTX and AMN-MoAb to be 20 times more cytotoxic than MTX-MoAb conjugates. Both conjugates were tested in vivo in nude mice bearing the COLO205 tumor, the total dose of AMN, either free or conjugated, was 35μg and of MTX, 75μg. On day 19 the AMN conjugate treated tumors were 60% smaller than the MTX tumors although the dose was only half that of MTX (Fig. 10). The more toxic AMN analog is clearly more potent in vivo.

Idarubicin-antibody conjugates: Colon carcinoma xenografts in nude mice were treated with a total of 275μg of Ida given i.v. either as conjugate or free drug. It was noted that many mice receiving unconjugated Ida died due to toxic effects (Fig 11): 80% of those receiving Ida alone, and 100% of those receiving the mixture of Ida and 30.6 antibody. Of the group that received Ida-MoAb conjugate, 20% tumors were eradicated and the mean tumor size of this group was about 20% of the PBS treated mice on day 20.

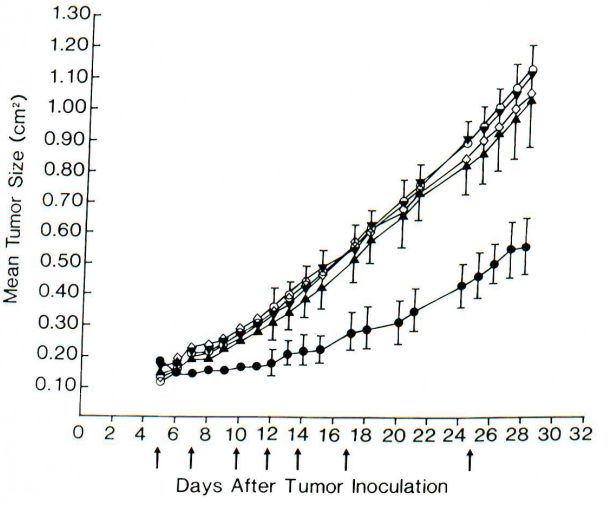


FIGURE 9

Tumor growth of COLO 205 xenograft in nude mice. Groups of 10 mice bearing preexisting tumors were given one of the following treatments i.v. denoted (↓); PBS (○), a mixture of N-AcMEL and 250-30.6 (◇), free N-AcMEL (▼), 250-30.6 (▲) and N-AcMEL-250-30.6 conjugate (●). Error bars represent ± standard error of the mean.

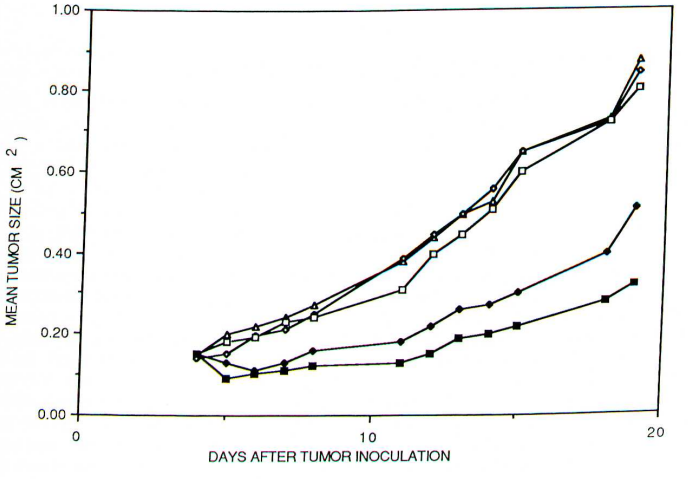


FIGURE 10

Tumor growth of COLO 205 xenograft in nude mice. Groups of 10 mice bearing preexistent tumors were given one of the following treatments i.v. on days 4, 5, 8, 11 and 13, PBS (△), free AMN (□), free MTX (◇), MTX-250-30.6 conjugate (◆) and AMN-250-30.6 conjugate (■). Standard error for each point was no more than ±0.03 cm^2.

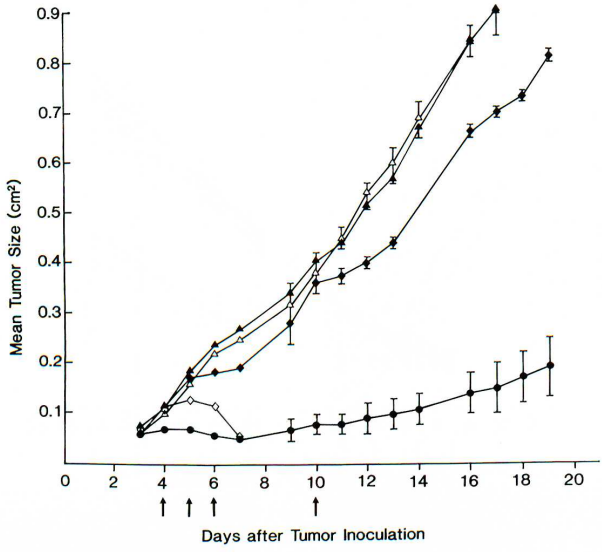


FIGURE 11

Tumor growth of COLO 205 xenograft in nude mice. Groups of 10 mice bearing established tumors were given one of the following treatments i.v. (↑); PBS (Δ), free Ida (◆), 250-30.6 (▲), a mixture of Ida and 250-30.6 (◇) and Ida-250-30.6 conjugate (●). Error bars represent ± standard error of the mean. Reprinted with permission from Targeted Diagnosis and Therapy (27).

In vitro and in vivo efficacy of ricin-MoAb conjugates

In order to explore the potential of using extremely cytotoxic agents, ricin was coupled to monoclonal antibody (31) to form a conjugate devoid of galactose binding activity. Conjugates were tested for their ability to inhibit [^{3}H]-leucine incorporation into target cell lines (Fig 12A); in the absence of lactose the ricin-anti-Ly-2.1 conjugate had an $I.D._{50}$ of 45ng/ml, equal to that of native ricin, while the control anti-Ly-1.1-Ricin conjugate was not toxic; demonstrating that neither the specificity of the anti-Ly-2.1 conjugate activity nor the toxin acivity was lost on coupling. When the assay was carried out in the presence of 100mM lactose, the cytotoxicity of ricin was decreased while the cytotoxicity of the immunoconjugate was not affected (Fig 12B).

The conjugate was tested in a study using intraperitoneal tumors (Table 4) where 90% of the mice receiving a dose of 0.05 g ricin coupled to MoAb survived >200 days. The efficacy of the conjugate on the growth of s.c. tumors (Fig 13) was also examined: a dose of 0.1μg of ricin conjugate given on days 9 and 11 resulted in a 70% reduction in tumor size while no other group demonstrated a significant decrease in tumor size. Mice receiving this conjugate had extensive liver and kidney damage. Thus the most potent material known - ricin - was only moderately effective against tumors growing in the s.c. site, although it can eradicate tumors growing i.p. where better access can be obtained. Clearly access of antibodies to the tumor site is of major importance.

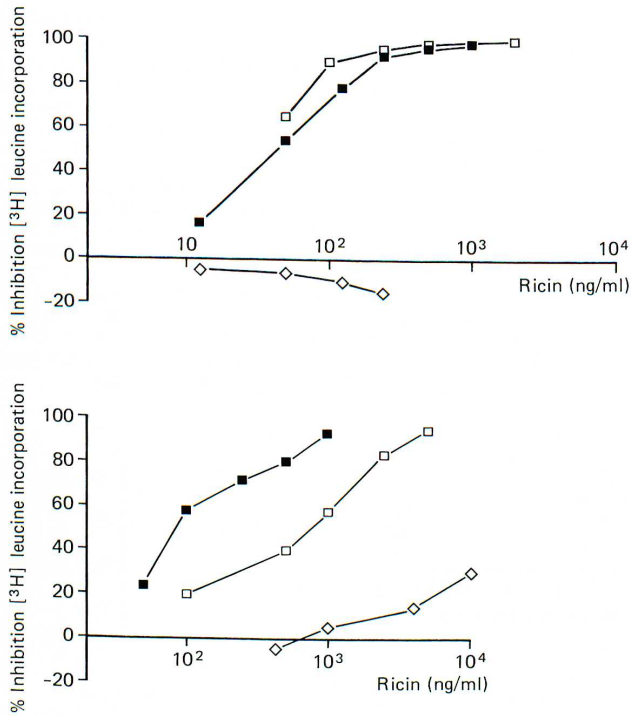


FIGURE 12

Inhibition of protein synthesis by ricin (□), ricin-anti-Ly-2.1 (■) and ricin-anti-Ly-1.1 (◇) in the absence (a) and presence (b) of 100mM lactose. Reprinted with permission from Membrane Mediated Cytotoxicity (31).

Studies to Improve Antitumor Efficacy of Drug-MoAb Conjugates

Intratumor treatment: Ricin-MoAb conjugates, when used intravenously, caused some liver and kidney damage due to the clearance by the reticuloendothelial system and the efficacy of these conjugates on solid tumors may have been reduced because their size impeded their ability to penetrate into tumors. However, by injecting the immunoconjugates directly into tumors the conjugates were not subject to the same physiological and anatomical barriers. Mice with subcutaneous tumors 0.5cm received injection into the tumor with either an irrelevant immunoconjugate, PBS, anti-Ly-2.1-ricin immunoconjugate or antibody alone. Within 48 hours, using a dose of ricin 0.22 g, 40% of the ricin-anti-Ly-2.1. treated tumors were completely eradicated. Some tumors that regressed, reappeared and regrew from a surviving peripheral rim of tumor. By improving the injection technique 100% of the tumors could be eradicated using the ricin immunoconjugate. The non-specific conjugate was not effective (Fig 14).

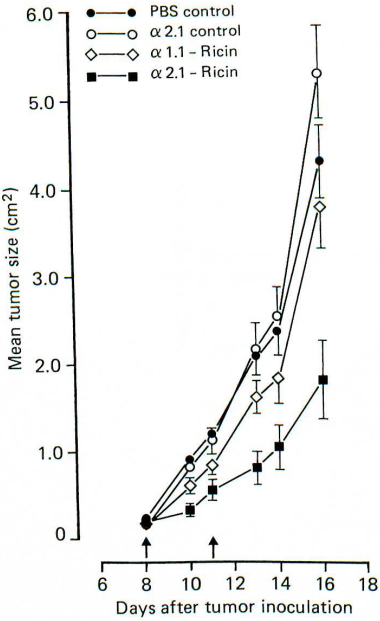


FIGURE 13

Growth of the thymoma ITT(1)75NS E3 in $B6CF_1$ mice given s.c. injection of 1 x 10^5 cells. Groups of 10 mice were given one of the following treatments i.v. (↑); PBS (●), free anti-Ly-2.1 (○), ricin-anti-Ly-1.1 (◇) and ricin-anti-Ly-2.1 (■). Error bars represent ± standard error of the mean. Reprinted with permission from Membrane Mediated Cytotoxicity (31).

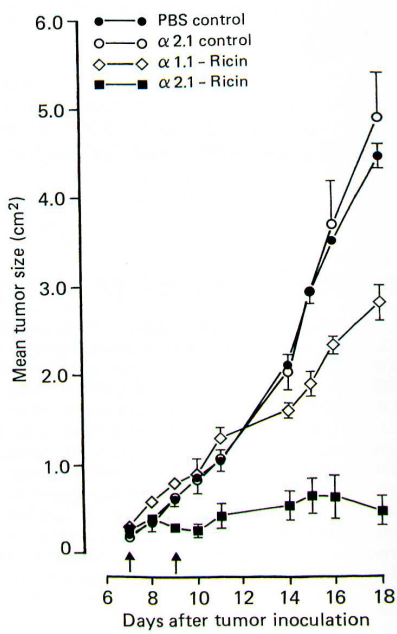


FIGURE 14

Growth of the thymoma ITT(1)75NS E3 in $B6CF_1$ mice given a s.c. injection of 3 x 10^6 cells. Groups consisting of 10 mice were given one of the following treatments directly into the tumor (↑); PBS (●), anti-Ly-2.1 (○), ricin-anti-Ly-1.1 (◇) and ricin-anti-Ly-2.1 (■). Error bars represent ± standard error of the mean. Reprinted with permission from Membrane Mediated Cytotoxicity (31).

F(ab')$_2$ -Conjugates: As an alternative means of improving tumor localization and access, F(ab')$_2$ fragment-drug conjugates were used. These conjugates, being only 70% of the size of the intact IgG conjugate, should, theoretically permeate the tumor more effectively. N-AcMEL was coupled to an F(ab')$_2$ fragment of the anti-Ly-2.1 MoAb and its effects were compared in vivo with intact IgG conjugate (Fig 15). Mice were treated with either PBS, free MEL, F(ab')$_2$ conjugate, IgG conjugate and F(ab')$_2$ at a total dose of 30μg N-AcMEL. Of the mice that received intact MoAb conjugate 3/10 tumors were eradicated while 4/10 tumors were eradicated in the group receiving F(ab') conjugate, i.e. there was no difference between these groups.

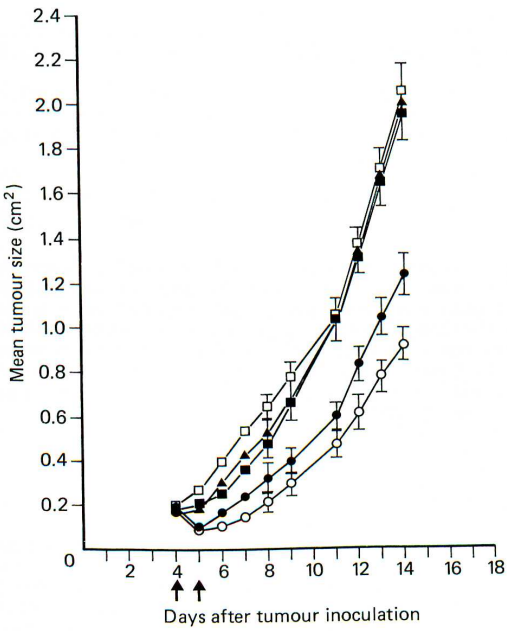


FIGURE 15
Growth of the thymoma ITT(1)75NS E3 in B6CF mice injected s.c. with 3 x 10^6 cells. Groups of 10 mice were treated i.v. denoted (↑); PBS (□), free N-AcMEL (■), N-AcMEL-anti-Ly-2.1 conjugate (●), N-AcMEL-F(ab')$_2$ conjugate (○) and anti-Ly-2.1-F(ab')$_2$ (▲). Error bars represent ± standard error of the mean tumor size. Reprinted by permission from British Journal of Cancer (20).

Vasoactive Agents: To determine the therapeutic effect of changing the blood flow in and around a tumor mass, several vasoactive agents were tested for their ability to alter the blood flow to tumors. Their effect was detected by measuring the tumor:blood ratio of mice receiving radiolabeled specific antibody in the presence or absence of the vasoactive agent (32). Table 5 demonstrates that several -adrenergic blockers are capable of increasing the tumor:blood ratio threefold, but when -blockers were used the tumor:blood ratios were unchanged (data not shown).

The therapeutic effect of these vasoactive agents in combination with Ida-MoAb was examined using the E3 murine thymoma model (Fig 16 a, b, c).

TABLE 5
Effect of β-Adenoreceptor Blocking Agents on ^{125}I-anti-Ly-2.1 Uptake in Mouse ITT(1)75NS E3 Thymoma.

^{125}I-anti-Ly-2.1 accumulation as mean % injected dose/g tissue ± SE.

Item	Control	Propanolol 10mg/kg	Control	Pindolol 0.6mg/kg	Control	Oxprenolol
Blood	14.00±1.63	7.52±1.36	12.05±1.29	5.29±1.21	11.51±0.92	6.72±0.75
Tumor	10.56±1.64	16.03±1.80	9.02±1.00	14.83±1.69	15.32±1.82	22.83±0.89
Spleen	5.10±1.11	4.41±0.59	4.06±0.58	1.52±0.71	8.01±1.00	6.48±0.41
Liver	2.74±0.20	1.35±0.15	2.31±0.20	1.02±0.05	2.89±0.18	1.62±0.18
Kidneys	6.05±0.79	3.32±0.31	4.77±0.35	2.89±0.35	5.48±0.18	4.04±0.03
Heart	4.22±0.68	2.28±0.61	3.09±0.82	1.96±0.06	4.46±0.07	3.03±0.06
Tumor/ Blood	0.75±0.11	2.13±0.40	0.75±0.13	2.80±0.57	1.33±0.26	3.36±0.43
Tumor/ Liver	3.85±0.55	11.87±1.62	3.90±0.79	14.53±1.68	5.30±1.05	14.09±1.50
Plasma $t_{\frac{1}{2}}$(hrs)	3-5		3-4		1-2	

Several points are evident; there was no difference in tumor growth between the groups of mice receiving free Ida or PBS with or without the blocking agent. Groups of mice that received either pindolol or propranolol in combination with the Ida-MoAb conjugate had more tumors eradicated (60%) and a smaller mean tumor size than the groups that only received conjugate (20%). Oxprenolol, however, was less effective, possibly due to its shorter half life and lower potency as a blocking agent compared with propranolol and pindolol (Table 5).

Use of Antibody Cocktails

On the basis that two MoAbs with differing specificities may improve tumor localisation of coupled drug, two different drug-MoAb conjugates to colon cancer were used in Swiss nude mice bearing COLO205 xenografts. The conjugates were given alone or in total combination such that the amount of Ida administered was identical in each group (Fig. 17). Although only approximately half as much of each MoAb was used when the two conjugates were administered together as a cocktail, the two conjugates together were more effective than either used alone - presumably by the delivery of more drug to the tumor site.

Preliminary Clinical Results Using MTX-MoAb Conjugates

Immunohistochemical testing on biopsy specimens

The results of immunoperoxidase staining, performed on sections of biopsy specimens, using a panel of MoAbs, are summarised in Table 6. There was a wide range of staining grades with different MoAbs (Table 6) although all MoAbs demonstrated some degree of staining. To overcome the problem of tumor cell heterogeneity and to ensure maximal immunoreactivity, a combination of two MoAbs was used - the combined use of 30.6 and A3C6 gave maximal staining of the colonic sections.

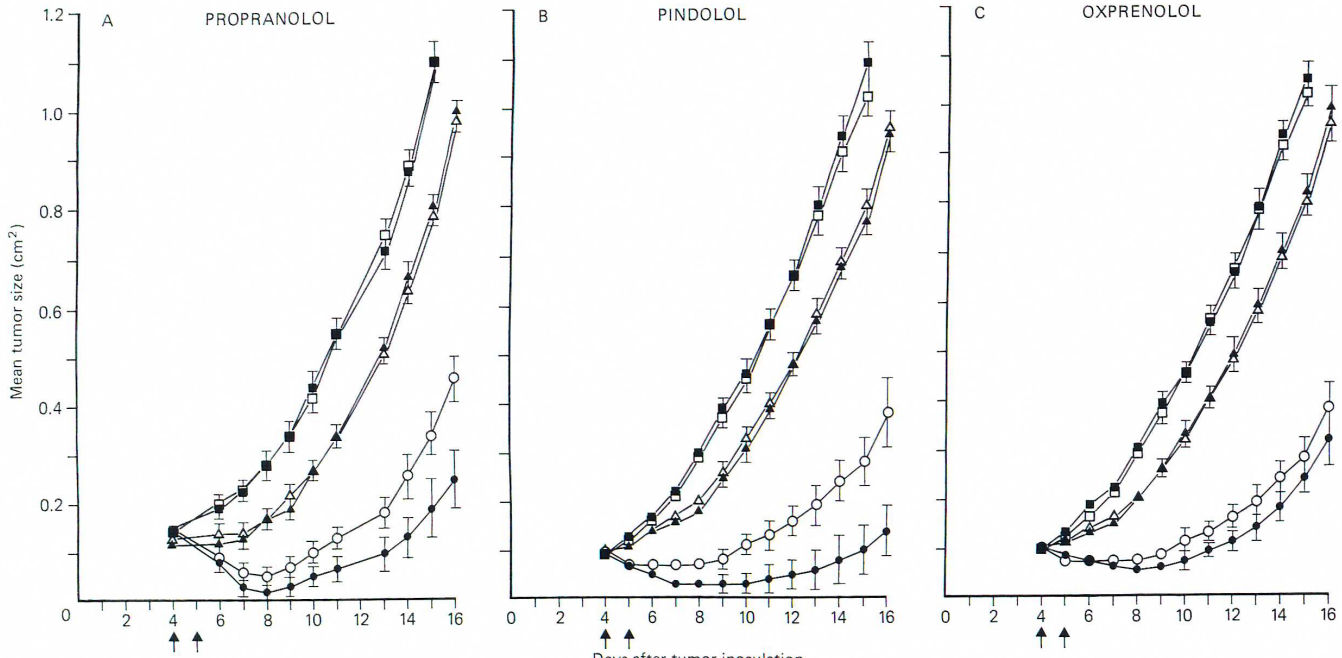


FIGURE 16

Growth of the thymoma ITT(1)75NS E3 in B6CF$_1$ mice injected s.c. with 2 x 10^6 cells. Groups of 10 mice were given the following treatments i.v. denoted (↑); PBS (□), PBS and vasoactive (■), Ida (△), Ida and vasoactive agent (▲), Ida-anti-Ly-2.1 conjugate (○) and Ida-anti-ly-2.1 conjugate and vasoactive agent (●). Error bars represent ± standard error of the mean tumor size.

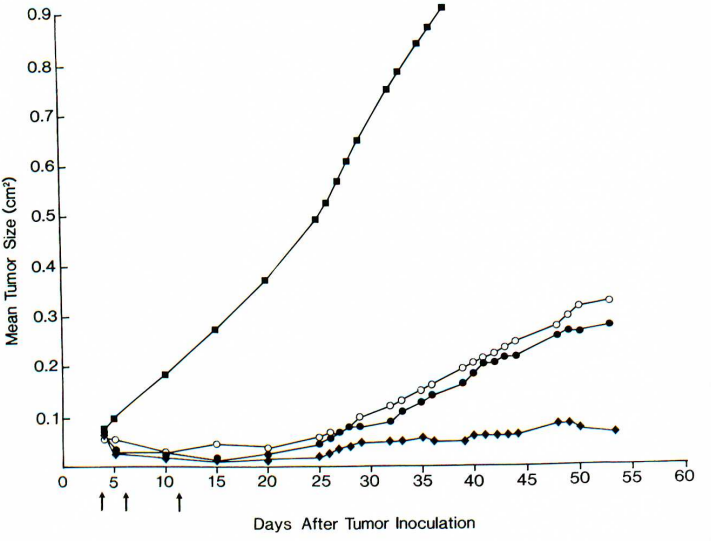


FIGURE 17

Tumor growth of COLO205 xenograft in nude mice. Groups of 10 mice bearing preexisting tumors were given one of the following treatments i.v. (↑); PBS (■), Ida-250-30.6 conjugate (●), Ida-17.1 conjugate (○) and a mixture of Ida-250-30.6 conjugate and Ida-17.1 conjugate (◆). Standard error for each point was not more than 0.03cm .

TABLE 6
Immunoperoxidase Staining of Primary or Metastatic Tissue with Different Monoclonal Antibodies

Patient	Tissue Tested	Staining grade 30.6 CaColon	A3C6 TFR	17C4 CEA	5C1 CaColon	30.6+A3C6
AD	Primary	+++	+++	+++	++	++++
DZ	Metastasis	++	++	+	+++	++++
HM	Metastasis	+++	+++	++	++	++++
MC	Metastasis	++++	+++	+++	+	++++

Toxicity

Table 7 summarizes the results of the patients treated with intravenous MTX-MoAb conjugates. In general, the slow intravenous infusions were well tolerated with no significant disturbance in hematological or biochemical parameters. Patient AD experienced moderate nausea, vomiting and fever up to 38.8°C, commencing 1 hour after the second infusion of conjugate had been completed. He had mounted a positive HAMA response (Titer 1/256 of negative control) to his first treatment, assayed two days prior to the second, and was thus given 4 mg of dexamethasone 1 hour before infusion. Three months later he had a strongly positive HAMA assay (Titer 1/2048). In view of this reaction, patient DZ was not given an escalated dose and

had no toxicity. Patient HM received a higher dose (95mg MoAb) without any untoward side effects. Patient MC was given two doses of MTX-MoAb conjugate three days apart (due to a shortage of MoAb supply) with no toxicity apart from mild nausea after the second infusion. All patients developed HAMA 3 months after treatment (Table 7). No patients had delayed symptoms that could be associated with serum sickness.

TABLE 7
Results of MTX-MoAb Conjugate Infusion

Patient	Day of infusion	Dose(mg) MTX:MoAb	CEA level Pre-infusion	CEA level Post-infusion	HAMA 0mo[1]	HAMA 3mo[2]	Status (treatment time)
AD	Day 0	1.7:50	20.5	19.1	-		
	Day 16	1.9:50	22.7	-	+	++	AWPD(10mo)
DZ	Day 0	2.1:50	1.7	1.6	-	+	AWPD(09mo)
HM	Day 0	3.9:95	30.8	31	-	+	AWPD(07mo)
MC	Day 0	1.8:50	152	160	-	+	
	Day 3	1.6:46		-	N.T.	N.T.	AWSD(03mo)

[1] Initial screening for human anti-mouse antibodies.
[2] Screening for human anti-mouse antibodies 3 months after treatment.

Response

There were no objective responses to treatment, although all are alive at the time of writing, AD had stable disease for four weeks as evidenced by a plateau in his steadily rising CEA level (data not shown) and no change in the size of a 2.5cm solitary hepatic metastasis on CT scanning over a two month period. However, obstructive renal failure necessitated radiotherapy one month later. DZ and HM are both alive with slowly progressive disease, starting within two months of immunotherapy. MC had a thoracic CT scan repeated two months after the initial scan, which showed some insignificant regression of a large paraspinal tumor deposit, but no change in the size of multiple pulmonary secondaries.

Pharmacokinetics

No detectable antibody could be found in the serum of MC at 24 hours; peak concentration at 1 hour after infusion was 20μg/ml, which fell to 10μg/ml by 12 hours. Serial samples were not obtained immediately after infusion in the other patients.

DISCUSSION

Ehrlich's concept of using molecules with an affinity for target tissue as carriers of cytotoxic drugs had not been fully examined until the advent of monoclonal antibodies (33). However, this concept was tested in the mid 1950s with methotrexate bound to polyclonal antibodies against L1210 cells in mice and encouraging results obtained (34). Davies and colleagues used Chlorambucil antibody preparations, resulting in the use of non-covalently bound preparations in humans (35) and since that time several antineoplastic drugs have been coupled to antibodies using a variety of coupling procedures (36). Most of the studies showed in vitro efficacy, but extensive characterization for in vivo efficacy was lacking. The most common model was the growth of tumor in the peritoneum, where treatment was

also administered intraperitoneally. Such a model is a mere extension of an in vitro test where tumor cells and drug-conjugates are freely accessible to each other and with no consideration to normal anatomical and physiological barriers seen with solid tumors. From these results, and ours presented here (Table 4), it appears that drug-antibody conjugates are highly efficacious against tumors growing in the peritoneum. Such a therapeutic approach could be used for the treatment of ovarian cancer and bladder cancer where local treatment is possible. This approach is currently used with ^{125}I-antibody conjugates for the treatment of ovarian cancer (37).

Most of the tests done with drug-antibody conjugates for efficacy on solid tumors, frequently used tumors that were not fully established, thus not ideally representing the clinical situation. In this study we used established murine thymomas or human tumor xenografts. However, quantitative studies carried out using radiolabeled antibodies indicate only a fraction of conjugate binds to the tumor (38) and this is a limitation of the use of monoclonal antibody conjugates for the treatment of solid tumors. A number of approaches can be used to increase the dose of drug carried to the tumor such as using conventional drugs bound to antibodies via an inert carrier molecule such as human serum albumin or to use highly toxic drugs or toxins. Both these solutions will still be limited by accessibility to tumor. The effect of delivering a highly toxic dose to the tumor is seen when ricin-antibody conjugates are administered directly into the tumor result in the eradication of tumors within 48 hrs (Fig 14).

The major problem with the use of highly cytotoxic proteins such as ricin and drugs bound to antibody using inert carriers is their size, and the approach taken was to couple toxic analogs of the conventional drugs to monoclonal antibodies. Idarubicin, an analog more active than adriamycin when coupled to antibody, resulted in conjugates which showed a similar effect although coupling adriamycin via the amino group led to the loss of 40 fold activity while coupling via the C-14 led to no loss in activity. In vivo, idarubicin was more efficacious that adriamycin, leading to the cure of established tumors (Fig 7, Fig 11). A similar trend was seen for aminopterin and methotrexate conjugates (Fig 10). Chlorambucil, when coupled to antibody was more active than when free, and also gave rise to high drug/antibody ratios. The more active alkylating agent melphalan behaved similarly and cured a proportion of nude mice bearing colon carcinoma xenografts.

Ricin antibody conjugates devoid of non-specific binding when tested on subcutaneously growing tumors caused a reduction of tumor growth although there were no complete cures (Fig 13). The lack of a strong inhibitory response by the ricin-antibody conjugates on solid tumors could be due to either the clearance by the RES due to the size of the conjugate with poor penetration into the tumor. Alternatively the ricin, although devoid of galactose binding activity, could also bind (non-specifically) by the presence of mannose residues.

Several possibilities were tested to improve tumor access. The smaller size of $F(ab')_2$ fragments should diffuse more freely into tumors than intact immunoglobulin and also the lack of Fc portions may prevent uptake by the Fc receptors on cells, however comparison of N-AcMEL-IgG conjugates with N-AcMEL-$F(ab')_2$ conjugates showed similar effects on solid tumors, probably because the shorter half life of the $F(ab')_2$ fragments balanced the greater diffusion (Fig 15). An alternative approach to increase access was to alter the blood supply to the tumor. The tumor vasculature lack smooth muscle and therefore are unresponsive to vasodilation and vasoconstriction by vasoactive agents (39). Administration of β-adrenergic blocking agents, together with drug-antibody conjugates, yielded 3 times higher tumor to blood ratios and decreased tumor:liver ratios (Table 6) and an increased cure rate was seen when pindolol and propranolol were used (Fig 16). The increased efficacy was probably due to the vasoconstriction of most blood vessels to organs and muscle tissue, resulting in an

increased flow through the tumor vessels and therefore the exposure of more conjugate to the tumor.

Another point addressed in this study is tumor heterogeneity. The use of a mixture of two conjugates, 17.1 and 30.6, against two different antigens, resulted in a greater efficacy than either alone in nude mice bearing colon xenografts.

On the basis of these and other reults, a number of principles can be summarized for the use of drug-antibody immunoconjugates.

1. Preservation of drug activity is important (e.g. Ida vs Ad).
2. Maximal amounts of coupled drug should be sought, e.g. CBL and MEL vs other drugs.
3. More potent analogs of the drugs are usually more effective, e.g. AMN vs MTX or Ida vs Ad or MEL vs CBL.
4. Immunoconjugates with $F(ab')_2$ fragments will not necessarily be more potent.
5. There is a major problem with tumor access but vasoactive agents may direct immunoconjugates towards the tumor.
6. In spite of these problems immunoconjugates can be delivered to the tumor site and can effectively cure tumors, e.g. intraperitoneal tumors and intratumor injection of immunoconjugates.
7. Cocktails of antibodies are more effective than single antibodies - presumably due to the fact that more drug enters each tumor cell or alternatively the heterogeneity of cells in the population leads to a more extensive eradication with multiple antibody.

After our studies with methotrexate a phase I clinical trial was commenced and 4 patients were treated (another 3 patients were also treated but the data collection was incomplete and not included). The doses of antibody used were small but it was clear that none of the patients had major side effects due to the antibody or the drug. In one patient (data not shown) over 500mg of antibody conjugated to methotrexate was given without ill effect. However, there was little noticeable effect on the tumors measured, although there was some levelling out in the CEA for some weeks in several patients. However, on the basis of subsequent studies where more potent analogs were available and improved results were seen with other drugs, we have abandoned this trial and we are currently performing a phase I study with melphalan-MoAb conjugates and we are soon to begin similar studies with idarubicin and ricin conjugates.

REFERENCES

1. Devita, V. Jr, Hellman, S. and Rosenberg, S.A. In "Cancer Principles and Practice", J.B. Lippincott, Philadelphia, 1984.

2. Embleton, M.J., Rowland, G.F., Simmonds, R.G., Jacobs, E., Marsden, C.H. and Baldwin, R.W. Br. J. Cancer 47:43 (1983).

3. Kato, Y., Tsukada, Y., Hara, T. and Hirai, H. J. Appl. Biochem. 5:313 (1983).

4. Gallego, J., Price, M.R. and Baldwin, R.W. Int. J. Cancer 33:737 (1984).

5. Deguchi, T., Chu, T.M., Leong, S.S., Horoszewicz, J.S. and Lee, C-L. Cancer Res. 46:3751 (1986).

6. Uadia, P., Blair, A.H., Ghose, T. and Ferrone, S. J. Natl. Cancer Inst. 74:29 (1985).

7. Smyth, M.J., Pietersz, G.A. and McKenzie, I.F.C. Immunol. Cell Biol. 65(Pt 2):189 (1987).

8. Shen, W-C., Ballou, B., Ryser, H.J-P and Hakala, T.R. Cancer Res. 46:3912 (1986).

9. Smyth, M.J., Pietersz, G.A. and McKenzie, I.F.C. Immunol. Cell Biol. 65:315 (1987).

10. Hogarth, P.M., Henning, M.M. and McKenzie, I.F.C. J. Natl. Cancer Inst. 69:619 (1982).

11. Semple, T.U., Quinn, L.A., Woods, L.F. and Moore, G.E. Cancer Res. 38:1345 (1978).

12. Foley, G.E., Lazarus, H., Farber, S., Geren Uzman, B., Boone, B.A. and McCarthy, R.E. Cancer 18:522 (1965).

13. Hogarth, P.M., Edwards, J., McKenzie, I.F.C., Goding, J.W. and Liew, F.Y. Immunology 46:135 (1982).

14. Thompson, C.H., Jones, S.L., Pihl, E. and McKenzie, I.F.C. Br. J. Cancer 47:595 (1983).

15. Panaccio, M., Zalcberg, J.R., Thompson, C.H., Leyden, M.J., Sullivan, J.R., Lichtenstein, M., and McKenzie, I.F.C. Immunol. Cell Biol. (submitted) (1986).

16. Thompson, C.H., Whitehead, R.H. and McKenzie, I.F.C. J. Natl. Cancer Inst. 70:490 (1983).

17. Hogarth, P.M., Potter, T.A., Cornell, F.N., McLaughlin, R. and McKenzie, I.F.C. J. Immunol. 125:1618 (1980).

18. Parish, C.R. and McKenzie, I.F.C. J. Immunol. Methods 20:173 (1978).

19. Smyth, M.J., Pietersz, G.A., Classon, B.J. and McKenzie, I.F.C. J. Natl. Cancer Inst. 76:503 (1986).

20. Smyth, M.J., Pietersz, G.A. and McKenzie, I.F.C. Br. J. Cancer 55:7 (1987).

21. Smyth, M.J., Pietersz, G.A. and McKenzie, I.F.C. Cancer Res. 47:62 (1987).

22. Kanellos, J., Pietersz, G.A. and McKenzie, I.F.C. J. Natl. Cancer Inst. 75:319 (1985).

23. Kanellos, J., Pietersz, G.A., Cunningham, Z. and McKenzie, I.F.C. Immunol. Cell Biol (in press) (1987).

24. Pietersz, G.A., Smyth, M.J. and McKenzie, I.F.C. Cancer Res. (Submitted) (1987).

25. Niederwieser, A. J. Chromatog. 54:215 (1971).

26. Bradford, M.M. Anal. Biochem. 72:248 (1976).

27. Pietersz, G.A., Smyth, M.J. and McKenzie, I.F.C. In: Targeted Diagnosis and Therapy. J.D. Rodwell (Ed), Marcell Dekker, Vol. 1., 1987.

28. Working Party on Clinical Use of Antibody, prepared by a Joint Committe of the Cancer Research Campaign, National Institute for Biological Standards and Control. Br. J. Cancer. 54:557 (1986).

29. Miller, A.B. Hoogstarten, B., Staquet, M. and Winkler, A. Cancer 47:207 (1981).

30. Hurwitz, E., Levy, R., Maron, R., Wilchek, M., Arnon, R. and Sela, M. Cancer Res. 35:1175 (1975).

31. Pietersz, G.A., Kanellos, J. and McKenzie, I.F.C. In "Membrane-Mediated Cytotoxicity", UCLA Symposia on Molecular and Cellular Biology, New Series, Volume 45, (Eds) B. Bonavida and R.J. Collier. Alan R. Liss, New York, 1986, p213.

32. Smyth, M.J., Pietersz, G.A. and McKenzie, I.F.C. J. Natl. Cancer Inst. (in press) (1987).

33. Kohler, G. and Milstein, C. Nature 256:495 (1975).

34. Mathe, G., Loc, T.B. and Bernard, J. C.R. Acad. Sci. Paris 246:1626 (1958).

35. Newman, C.E., Ford, C.H.F., Davies, D.A.L. and O'Neill, G.J. Lancet 2:163 (1977).

36. Pietersz, G.A., Kanellos, J., Smyth, M.J., Zalcberg, J. and McKenzie, I.F.C. Immunol. Cell Biol. 65(2):111 (1987).

37. Epenetos, A.A. Cancer Drug Delivery 2:233 (1985).

38. Epenetos, A.A., Snook, D., Durbin, H., Johnson, P.M. and Taylor-Papadimitriou, J. Cancer Res. 46:3188 (1986).

39. Chan, R.D., Babbs, C.F., Vetter, R.J. and Lamar, C.H. J. Natl. Cancer Inst. 72:145 (1984).

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