Phase I Study of Monoclonal Antibody-Ricin A Chain Immunotoxin XomaZyme-791 in Patients with Metastatic Colon Cancer

V. S. Byers, R. Rodvien, K. Grant, L. G. Durrani, K. H. Hudson, R. W. Baldwin, and P. J. Scannon


ABSTRACT

Monoclonal antibody 791T/36, recognizing a M, 72,000 antigen on the surface of colon carcinoma cells, has been used to construct an immunotoxin by conjugating to it the ribosomal inhibitor protein, ricin toxin A chain. The antibody 791T/36 has been shown to bind to membranes of freshly disaggregated tumor cells from human colon tumors, and to localize in tumors in vivo. Subcutaneous toxicity testing in rats receiving immunotoxin i.v. showed, at highest doses, weight loss, decreased serum albumin, and hepatocyte vacuolization without elevation in liver function tests.

A Phase I dose escalation study was carried out in which 17 patients with metastatic colorectal cancer were treated with doses of immunotoxin ranging from 0.02 to 0.2 mg/kg/day in 1-h i.v. infusions for a 5-day course. Side-effects included a composite of signs and symptoms thought to be generic to ricin A chain immunotoxins, including decreased serum albumin, mild fever, and flu-like symptoms, all being reversible. Two additional findings, reversible proteinuria and mental status changes, were also noted which may be characteristic of this immunotoxin.

By 10–20 days after therapy, most patients developed IgM and IgG antibodies against both the ricin toxin A chain and the immunoglobulin portion of the immunotoxin, which were asymptomatic. A strong anticomplementing site antibody response was seen. Biological activity manifest as mixed tumor regression was seen in five patients.

INTRODUCTION

MoAbs directed against human tumor-associated antigens have allowed drug targeting to be explored as a therapeutic modality in cancer (1–3). Cytotoxic moieties, when coupled to MoAbs, can be directed specifically to the relevant target cell. One such moiety, RTA, has been used to construct several immunotoxins (1, 4, 5). RTA is a ribosomal inhibiting protein which functions as an RNA N-glycosidase specific for the 28-S ribosomal subunit (6). Since RTA alone is poorly internalized, it is functionally inactive as a free agent. When coupled to monoclonal antibodies, however, it can be targeted to tumor cells, internalized, and is cytotoxic to the cells.

MoAb 791T/36 recognizes a M, 72,000 antigen present on tumor cells derived from ovarian, colorectal, and osteogenic sarcoma tissues (7–9). Flow cytometric analysis of tumor cells from colorectal and ovarian tumors, derived by enzymatic disaggregation of the tumors, demonstrated that 791T/36 MoAb binds to the majority of cells from over 80% of both types of tumors (8, 9), indicating the antigen is expressed on the cell surface. In vivo, this MoAb localizes in tumors, as demonstrated by studies in which the antibody, labeled with 111In or 111In, images primary and metastatic ovarian and colorectal cancers (9–11).

The MoAb has been used to construct an immunotoxin, XomaZyme-791, by conjugation with RTA chain (12, 13). In vitro studies indicate that the immunotoxin retains over 70% of its binding to cells carrying the M, 72,000 antigen, and when tested on 791T target cells using a [75Se]methionine incorporation assay, there was more than a 1000-fold difference between the molarity of immunotoxin and free RTA necessary to attain 50% inhibition of tumor cell growth in vitro (12). It specifically and effectively inhibits growth of human tumor xenografts (13). On the basis of these findings, animal toxicology studies were carried out, and XomaZyme-791 was then tested in a Phase I clinical trial for the treatment of metastatic colorectal cancer.

MATERIALS AND METHODS

Monoclonal Antibody. The generation of the hybridoma producing 791T/36 MoAb (IgG2a) has been previously described (12, 14). The MoAb used for these clinical trials was produced by XOMA Corporations from murine ascites and purified by affinity adsorption on Sephasose-protein A (13). The homogeneity of the IgG2a preparations, evaluated by SDS-PAGE, HPLC, and double immunodiffusion indicated preparations had purities of greater than 95%. Reactivity of the MoAb on normal tissues was assessed by immunoperoxidase staining on frozen sections of a range of normal adult and fetal tissue from five cadaveric donors using a modification of the ABC technique (15).

To measure reactivity with hematopoietic progenitor cells, mature T-lymphocytes were removed from human bone marrow aspirates by first treating the cells with soybean lectin and removing the resultant agglutinates, then forming E rosettes and removing them. This technique produces a 4 log10 depletion of T-lymphocytes (16). Binding to the remaining cells was assessed by indirect immunofluorescence, measured by flow cytometry.

The purified MoAb is free of xenotropic and ecotropic viruses as well as the twelve murine viruses measured by the mouse antibody production test (a test in which contamination with 12 murine viruses is evaluated by injection of the test article into mice and determination of antibody production to the viruses of interest1).

Ricin Toxin A Chain. RTA was purified from castor beans by a series of column based separations, including immunooaffinity chromatography (17). The RTA was greater than 95% pure as judged by SDS-PAGE, and contained no detectable ricin, or ricin toxin B chain by any assay including immunoprecipitation. The IC50 level of the purified RTA as measured by a reticulocyte lysate assay (17) was less than 10 pm. This assay measures inhibition of protein synthesis in a cell free system. In a mouse toxicity assay, RTA injected into BALB/c mice at 10 mg/kg produced no deaths.

Immunotoxin. Immunotoxin XomaZyme-791 was prepared for clinical trials by conjugating purified ricin A chain to the murine monoclonal antibody 791T/36 by means of N-succinimidyl-3-(2-pyridyldithio)propionate reagent, forming a disulfide bond (4, 12). It was purified by gel filtration. Each lot was subjected to a series of tests prior to release. The free IgG level and amount of immunotoxin present in the immunotoxin preparation was determined by size exclusion HPLC. Binding of the conjugate to 791T target cells was compared to that of

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1 The abbreviations used are: MoAb, monoclonal antibody; RTA, ricin A chain; LDH, lactate dehydrogenase; SGOT, glutamic oxaloacetic acid transaminase; CT, computerized tomography; CEA, carcinoembryonic antigen; PBS, phosphate buffered saline; XomaZyme-791, immunotoxin made from 791T/36 MoAb (clinical material); CPK, creatinine phosphokinase; BUN, blood urea nitrogen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; EEG, electroencephalogram; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate.

2 Xoma Corporation, unpublished observations.

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the native antibody by flow cytometry analysis as previously described (18). The cytotoxicity of the immunotoxin against relevant and irrelevant target cells was determined using an in vitro assay in which target cell survival is determined by [3H]thymidine incorporation. Target cells (791T) or the erythro leukemia cell line, Molt-4 (ATCC Bethesda, MD) which does not carry the M, 72,000 antigen recognized by this MoAb, were plated at 4 x 10^4 cells/well in 100 ml of RPMI 1640 (GIBCO, Grand Island, NY) with 10% fetal calf serum (Hyclone Labs, Logan, UT). After incubation at 37°C for 3 h, various concentrations of immunotoxin ranging from 20 to 2000 ng/ml were added in 100-μl aliquots to triplicate wells. After 48 h, 1 μCi of [3H]thymidine was added to each well and 72 h later the wells were harvested and incorporated radioactivity determined. Results are expressed as amount of immunotoxin/ml producing 50% inhibition (IC50). These tests were repeated at monthly intervals after immunotoxin production with minimal change in reactivity. XomaZyme-791 was prepared for clinical use at a concentration of 1 mg/ml in phosphate buffered saline, pH 7.3. It was clear to visual inspection and was filtered through a low protein binding 0.22-μm filter into about 100 ml of normal saline just prior to infusion.

Animal Toxicology Studies. The LD50 level was assessed on BALB/c mice; subacute toxicity studies were performed in Sprague-Dawley rats, both from Charles River laboratories. Mice were injected i.v. through the tail vein and observed for 5 days. After at least 7 days quarantine, three groups of rats, 12 per group, were dosed i.v. with either saline, or 1 mg/kg or 5 mg/kg of 791T/36-RTA (RTA:MoAb ratio of 4:3:1; 3.7% free RTA) through the tail vein daily for 10 days (study days 1–10). Each group of animals was weighed daily, and three in each group were bled, sacrificed, and necropsied on days 6, 11, or 17. Other Sprague-Dawley rats (three rats per group, Simonsen Labs) were given i.v. injections of 0.2, 1.0, or 5 mg/kg of RTA alone or of saline (2 ml/kg) for 5 sequential days. Animals were bled and necropsied on day 5. Serum chemistries included SGOT, serum glutamic pyruvate transaminase, bilirubin, BUN, creatinine, total protein, albumin, CPK, uric acid, LDH, glucose, and electrolytes. Hematology included indices and platelets were estimated.

Patient Population and Treatment Plan. Seventeen patients were entered in the trial. All had at least one measurable lesion. No patient had received a murine monoclonal antibody prior to this therapy. No patient studied had significant organ dysfunction; i.e., neurological, cardiological, and pulmonary functions were within normal ranges. Signed informed consent was obtained from all patients prior to entry into the study which was conducted under a U. S. FDA investigational new drug exemption notice. All patients signed informed consent.

XomaZyme-791 was given as 1-h daily i.v. infusions for 5 days, with the ability to postpone doses for up to 3 days if suspected side effects intervened. Immunotoxin doses, from 0.02 to 0.2 mg/kg/day, were infused over 1 h. Most patients were skin tested prior to the first dose with 100 μg of unconjugated antibody; some received an i.v. challenge of the equivalent amount of diluted immunotoxin, with infusions proceeding 15 min later if no reaction was seen. No adverse reactions to the test dose were noted. Physical exams and laboratory evaluation, including hematological and serum chemistry panel and urinalyses, were done daily through study day 6, and then at study days 15, 28, and 60. Prothrombin time, partial thromboplastin time, complement levels and electrocardiograms were carried out on study days 0 and 6. Where indicated, EEG and CT examinations of the head were performed. Patients were evaluated by sequential chest X-rays or CT scans of the abdomen, CEA levels, blood chemistries, and urinalyses for up to 6 months after completion of therapy. In most patients proteinuria was quantitated by dipstick where 1 = 30 mg/dl and 4 = 2000 mg/dl. One patient with 4+ proteinuria had quantitation of the urinary densities of each well were read at 405 nm and serum titers determined as the serum dilution producing 50% of the maximum ELISA value (19). Anti-combining site antibodies were detected using a flow cytometry assay in which the capacity of patient’s serum to block binding of FITC conjugated 791T/36 (791T/36-FITC) to target cells was determined (20). This was expressed as titer of serum which produced 50% inhibition of the maximum 791T/36-FITC binding to target cells.

RESULTS

Reactivity of MoAb and Immunotoxin

Apart from tumor cells, reactivity of the MoAb assessed by immunoperoxidase staining is primarily with stromal (noncellular) tissue, although there is cytoplasmic staining in the region of the juxtaglomerular apparatus and occasional reactivity with pulmonary epithelium and isolated kidney glomeruli in some sections. There is no detectable binding to progenitor cells by flow cytometry. Other studies have found weak antigen binding mitogen stimulated (but not resting) lymphocytes (21). Two preparations of immunotoxin were used for clinical trials (Table 1). Analysis by SDS-PAGE indicated several species of immunonconjugate were present with antibody:RTA ratio of 1:1 to 1:5. Less than 10% aggregates were present by weight. The intrinsic variation of the binding assay is 15% and that of the cytotoxicity assay is 50%; monthly analysis during the time the lots were in clinical use indicated all variations were within this range. No increase in free antibody or change MoAb:RTA ratio was noted.

While both lots fell within the accepted ranges, the first had a higher MoAb:RTA ratio than the other and correspondingly less free antibody. The binding to target cells was decreased as compared to the lot with lower conjugation ratios but the in vitro cytotoxicity was similar.

Patient Characteristics. The characteristics and sites of disease of the 17 cancer patients (10 females and 7 males) evaluated in the immunotoxin study are summarized in Table 2. The age range was 30–70 years. Sixteen patients had colorectal cancer; one patient (Patient 14) had the diagnosis of colorectal cancer later revised to ovarian cancer after laparotomy. Sixteen had liver metastases documented by CT scan; one patient (Patient 4) did not. Ten patients also had pulmonary metastases. All measurable lesions were less than 12 cm in size. Most patients had the primary tumor removed. Some had received other therapy such as 5-fluorouracil chemotherapy of IL-2-LAK cell immunotherapy no less than one month prior to immunotoxin

<table>
<thead>
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<th>Table 1 Characteristics of clinical lots of XomaZyme-791</th>
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<td>Lot</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>791T Molt 4</td>
</tr>
<tr>
<td>62</td>
</tr>
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</table>

* Determined by SDS-PAGE.
Relative to antibody 791T/36: determined by competitive inhibition of binding of MoAb 791T/36-FITC to 791T cells.

Determined by in vitro cytotoxicity of immunotoxin for cultured target cells. Cell survival determined by [3H]thymidine incorporation. Values expressed in terms of amount of immunotoxin/ml necessary to produce 50% inhibition.
A gain of 5% or greater was noted in seven of 17 patients and was either stabilized or began to increase by day 15 (Fig. 1a). Weight There was no obvious correlation between dose of immunotoxin infusion. Asymptomatic proteinuria not associ
manifest as peripheral edema. Pulmonary edema was not seen. One patient developed fever of 39°C during or within 6 h of
immunotoxin therapy are summarized in Table 3. Patients
received a full course of five doses of immunotoxin; doses were
taken at 2-wk intervals. One patient (Patient 6) received only one infusion
derived from colon (or in one case ovarian) carcinoma. Fifteen patients
received a full course of five doses of immunotoxin; doses were
temporarily postponed in two patients (Patients 9 and 13) because of neurological events (increased unilateral tremor and transient mental status change) thought possibly due to drug
Table 3. One patient (Patient 6) received only one infusion
because of an anaphylactoid reaction consisting of periorbital edema. His skin test was negative prior to immunotoxin treat
ment. Karnofsky scores were greater than 70; all had normal levels of blood urea nitrogen and serum creatinine; and no more than 1+ proteinuria by dipstick. Complement and coagulation parameters were within normal limits as were hemoglobin and white blood cell counts. Of the 17 patients, five had normal liver function tests, 10 patients had mildly elevated LDH levels, and seven had elevation of SGOT. Most patients had values less than twice normal for each test; one had values less than three times normal, and two had mild bilirubin elevations less than 30% above normal. Serum albumin was normal in all. None had other serious diseases or tumors apart from colon (or in one case ovarian) carcinoma. Fifteen patients received a full course of five doses of immunotoxin; doses were temporarily postponed in two patients (Patients 9 and 13) because of neurological events (increased unilateral tremor and transient mental status change) thought possibly due to drug
(Table 3). One patient (Patient 6) received only one infusion
because of an anaphylactoid reaction consisting of periorbital edema. His skin test was negative prior to immunotoxin treat
ment. Another patient (Patient 17) received only four doses
because of mental status change which required more than 3
days to resolve.

Clinical Observations. Clinical observations associated with immunotoxin therapy are summarized in Table 3. Patients
generally tolerated the immunotoxin well. Decrease in serum albumin levels was noted in all patients, beginning during the
5 days of infusion. This occurred with all doses of immunotoxin, and serum albumin levels fell to 12-48% of the starting level.
There was no obvious correlation between dose of immunotoxin and degree of albumin drop. In all cases the albumin levels
either stabilized or began to increase by day 15 (Fig. 1a). Weight gain of 5% or greater was noted in seven of 17 patients and was
manifest as peripheral edema. Pulmonary edema was not seen.

One patient developed fever of 39°C during or within 6 h of immunotoxin infusion. Asymptomatic proteinuria not associ
ated with other renal abnormalities was first noted on days 5-10 of study and increased through day 15 (Fig. 1b). Decreased serum albumin and weight gain occurred before onset of this delayed proteinuria. In all cases but one, the proteinuria resolved to 1+ or less after 30-45 days. The one patient (Patient 3) in whom proteinuria persisted had an accompanying urinary tract infection. This delayed proteinuria was noted in 11/11 patients treated with doses at or greater than 0.1 mg/kg/day, and in three of five patients treated with 0.05 mg/kg/day or less. Protein was quantitated in one of two patients with 4+ proteinuria (receiving a dose of 0.1 mg/kg/day) and totalled 2 g protein in a 24-h period. Urine protein electrophoresis demonstrated that the protein was primarily albumin. Urine sediment in all patients was unremarkable. In no case was there any decrease in serum complement levels (C3, C4, or CH50).

No patient’s serum albumin level decreased after the onset of proteinuria (Fig. 1). No increase in serum creatinine or BUN was seen in any patient. In most patients there was a slight decrease in platelet counts within the first 6 days of an average of 79,000 which was not related to dose. The lowest count reached was 146,000 in one patient. Counts returned to baseline or above within 15-20 days in all patients. There was no decrease in white blood cells or red cells and no change in prothrombin time, partial thromboplastin time, or fibrinogen levels.

On study day 6, only one patient (Patient 12) had a significant increase in any liver function test; this was a twofold increase in the total bilirubin value which had returned to base line by day 15 occurring in a patient with liver metastases Over the initial 28-day study period, one patient had SGOT and bilirubin values increasing by at least twofold.

In one patient, there was worsening of a preexistent tremor of the left hand and four of 17 patients had reversible mental status changes; all had been treated at a dose of 0.1 mg/kg or higher (Table 3). In three patients, mild fatigue, slurred speech,
**Clinical observations in patients treated with XomaZyme-791**

<table>
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<tr>
<th>Patient number</th>
<th>Dose (mg/kg)</th>
<th>Total dose (mg)</th>
<th>Maximum drop in serum albumin (%)</th>
<th>Weight gain % (peak day)</th>
<th>Neurologicala events</th>
<th>Feversb</th>
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<tr>
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<td>11.0</td>
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<td>3 (6)</td>
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<tr>
<td>5</td>
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<td>48</td>
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<td>+</td>
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</table>

a Headache, dementia, or tremor.
b During therapy.
c RTA: MoAb ratio of 2.0:1; other patients treated with lots with RTA: MoAb ratio of 3.5:1.

* Received only one dose.

Neurological events Fevers during therapy.

Biological Activity. Although this was a Phase I dose escalation study, observations concerning antitumor activity were made. Of 16 patients with hepatic metastases (Table 4), two (Patients 10 and 16) had objective evidence by abdominal CT scans of decreasing size in large metastases and disappearance of smaller lesions. These changes were seen at 3 months without additional intervening treatment. In another case (Patient 7), new calcification of the hepatic metastases, with stabilization of growth, was noted at 2 months, but there was increased size at 6 months. Three patients (Patients 8, 11 and 14) had fixed supraclavicular nodes which decreased in size or disappeared by study day 30. Three also had liver metastases which increased in size or number over the 2-month follow-up period. One patient (Patient 6) had both liver and pulmonary metastases; the liver metastases increased in size but the lung metastases decreased in size 5 months after therapy. These patients received no additional chemotherapy after the immunotoxin therapy.

Although there were transient decreases in the CEA values of some patients (Table 4), these could not be correlated with decreased tumor size. Of the three patients (Patients 7, 10, and 14) who had calcification or decrease in size of the hepatic metastases, one had CEA values that decreased during the period of observation.

Immunological Studies. Thirteen patients were skin tested with the unmodified antibody prior to initiation of therapy and all tests were negative. Between days 10 and 15, onset of erythema and induration at the skin test site was noted in 12 of 13 patients. The exception was one patient (Patient 4), treated at 0.05 mg/kg/day. In all cases the reaction lasted 3–5 days and then resolved.

Humoral antibody responses to murine 791T/36 immunoglobulin and RTA components of the immunotoxin were observed in all but one patient, including Patient 6 who received a single injection of immunotoxin (Table 5). Most patients produced IgM and IgG responses to 791T/36 immunoglobulin; the one patient who did not (Patient 15) was only tested as late

[Fig. 1. a, serum albumin levels in colorectal cancer patients treated with immunotoxin XomaZyme-791 (dose range, 0.02–0.2 mg/kg/day). Study days are plotted on a log scale to allow comparison between serum albumin and proteinuria, and patients are identified by dose in mg/kg: 0.02 (●), 0.05 (□), 0.15 (▲), and 0.2 (○). b, proteinuria assayed by dipstick, scale 1–4 in colorectal patients treated with XomaZyme-791.]

irritability, or expressive aphasia were noted. These events usually began about study day 4 and were largely resolved within 2 days although complete resolution could take seven days. One patient treated at the highest dose (0.2 mg/kg) became frankly demented; the patient received steroids and this condition reversed after 3 days. EEGs done on the four patients with mental status changes after therapy revealed diffuse slowing and/or paroxysmal bursts, and both the clinical examination and EEG were most compatible with a mild toxic encephalopathy. The patient who developed dementia had a normal head CT scan and no other etiology for the dementia. Nausea with vomiting was noted in four other patients during therapy; headaches were seen in two.
as study day 19. The IgM response was first detected between days 5 and 20 of study, with maximum responses around day 30. The overall pattern was that of a rapid rise in the IgM antibody response and then a fall in antibody levels up to day 80 of study. Peak IgM responses against the MoAb portion of the immunotoxin ranged in titer from 0 to 1:500.

The IgG response to 79IT/36 immunoglobulin was markedly greater than the IgM response, with peak antibody titers of 1:7000 or greater observed in four patients. In comparison, the maximum IgM antibody titer was 1/500 (Patient 12). The pattern of the IgG response was different from the IgM response with IgG antibodies continuing to rise from approximately days 10 to 30 and remaining elevated for up to 80 days of study. (Fig. 2).

Sera were screened for antibodies recognizing 79IT/36 immunoglobulin (IgG\textsubscript{2b}) in comparison with nonspecific mouse IgG\textsubscript{2a} and IgG\textsubscript{2b} immunoglobulins to determine if the predominant response was anti-mouse IgG, anti-subclass, or anti-variable region (Table 5 and Fig. 2). The predominant response was specific for the 79IT/36 immunoglobulin, where peak serum titers greater than 1:1000 were obtained in samples from 11 patients. In 13 of 17 patients the anti-79IT/36 response was at least twice as high as the response to mouse myeloma IgG\textsubscript{2a} or IgG\textsubscript{2b}.

The pronounced antibody response to 79IT/36 immunoglobulin (IgG\textsubscript{2b}) compared to normal mouse IgG\textsubscript{2a} and IgG\textsubscript{2b} suggested that the patients generated antibody to the monoclonal antibody 79IT/36 combining site. This was confirmed using a flow cytometry assay which measured the capacity of patients' serum to block binding of fluorescein labeled 79IT/36 (FITC-79IT/36) with target antigen on tumor 79IT cells (18, 20). Titers were determined as the dilution of patients' serum which produces a 50% inhibition of FITC 79IT/36 binding to target 79IT cells. Anti-combining site antibodies were detected in sera from 14 of 17 patients (Table 5 and Fig. 3). Peak serum titers were variable ranging from up to 1:5 in two patients to 1:100 or greater in 12 patients. The kinetics of this response in patients is illustrated in Fig. 3 showing that the response begins about days 10 to 30. In most instances, antibody levels remained elevated up to day 70. In three patients there was a pronounced fall of the anti-combining site antibody titer. Antibody responses to RTA were detected in sera from 15 patients (Table 5 and Fig. 4). One patient (Patient 15) receiving 0.15 mg/kg/day of immunotoxin (total dose 45 mg) did not produce antibody to either RTA or 79IT/36 immunoglobulin. A second patient (Patient 16) treated at the 0.15 mg/kg dose (total dose 61.4 mg) also produced only minimal responses to RTA (and also 79IT/36). Significant titers of IgM antibodies to RTA were detected in sera from 12 patients, titer range 1/50 to 1/16,600 (Table 5). IgG anti-RTA antibodies were detected in 13 patients with titers ranging from 1/50 to 1/316,000. The kinetics of the anti-RTA IgG response (Fig. 4) indicates a rapid rise in antibody titer between study day 10 to 20, this being 5 to 15 days after completion of immunotoxin treatment. In one of the patients (Patient 9) who generated a very pronounced response, the anti-RTA antibody levels decreased over a period of 70 days. In most patients, however, the IgG antibody level remained elevated throughout the 80-day period of investigation.

### Animal Toxicology

The LD\textsubscript{50} level of the immunotoxin assessed in BALB/c mice receiving a single i.p. injection of doses from 10 to 100 mg/kg, given in a volume of 0.5 ml, was calculated to be 81 mg/kg. In the subacute toxicology studies, rats receiving the highest dose of immunotoxin had a significant decrease in body weight from...
Table 5 Antibody responses to immunotoxin XomaZyme-791 in colorectal cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sera tested thru study day</th>
<th>Skin testa</th>
<th>Immunotoxin dose per kg/total</th>
<th>XMMCO-791 IgM</th>
<th>XMMCO-791 IgG</th>
<th>Murine IgGm</th>
<th>Murine IgGc</th>
<th>Murine IgGp</th>
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a Late erythema and induration appearing at site of skin test. Patients 5, 12, 13, 14 received i.v. challenge instead of skin test.

b Expressed as reciprocal of the serum dilution.

c NT, not tested.

Fig. 2. Kinetics of the IgG and IgM antibody responses to 79IT/36 immunoglobulin (●), mouse myeloma IgG2b (○), and mouse myeloma IgG2a (△) in two patients; nos. 17 (a, b) and 7 (c, d). Serum titers determined as serum dilution producing 50% of maximum reaction in ELISA. IgG responses (a, c); IgM responses (b, d).

day 3 through day 10 of the study followed by a weight gain from day 10 through the end of the study. They also had a significant increase in absolute neutrophil counts to seven times higher than that seen in the control animals; this had returned to baseline by day 11 (data not shown). Serum albumin was significantly decreased in the high dose group on day 6 and 11 of study but had returned to baseline by day 17 (Fig. 5). There were no changes in other serum chemistries including liver function tests, CPK, LDH, or renal function tests.

Histopathologically, there was mild vacuolization in hepatocytes in groups receiving either 1 or 5 mg/kg/day of immunotoxin although this effect was not seen until day 11. This persisted through day 17 of the study; i.e., 7 days after the last injection. Mild renal tubular necrosis was seen at day 6 in the group receiving the highest dose; this was reversing by day 11.

DISCUSSION

This Phase I study on the administration of XomaZyme-791 immunotoxin in patients with metastatic colorectal cancer has established the side-effects and clinical responses in patients receiving doses ranging from 0.02 mg/kg/day for 5 days to...
commonly seen in animals receiving higher doses, was not seen in humans receiving this immunotoxin although decreased ap-

through 0.2 mg/kg/day for up to 5 days.

Animal toxicology studies predicted the decreased albumin which has been seen in animals treated with RTA alone, and in animals and humans treated with other immunotoxins (4, 22). Clinically this results in a mild weight gain, and is not dose limiting. Hepatocyte vacuolization was seen in animals treated with immunotoxin, but there was no increase in liver function tests, nor was there evidence of hepatic dysfunction in the patients, even though most had liver metastases and abnormal liver function tests prior to immunotoxin therapy. Weight loss, commonly seen in animals receiving higher doses, was not seen in humans receiving this immunotoxin although decreased ap-

petite has been noted with other immunotoxins (4, 23). Delayed asymptomatic proteinuria was also a common finding associated with therapy with XomaZyme-791 in these patients. This generally began on study days 10–15 and reversed by days 30–45. It began in the presence of a serum albumin level which was either stable or increasing, and thus could not be implicated as a cause of the early drop in serum albumin. 791T/36 MoAb is known to bind to stromal (noncellular) elements of normal tissues, and it is possible that the antibody portion of the immunotoxin has a longer dwell time in organs such as the skin and kidneys because of its stromal binding. The temporal association between skin test flare, onset of proteinuria, and humoral immune response suggests that the onset of proteinuria could be related to the onset of the immune response causing a mild and reversible antigen-antibody complex disease. There was poor correlation between antibody titers and proteinuria in individual patients, and two patients (Patients 1 and 4) with antibodies did not develop proteinuria. However, the phenomena has been reproduced in a rat model, showing that the onset of proteinuria occurs at the time that antibody responses to immunotoxin components are detected, and is accelerated by passive transfer of antibodies to the 791T/36 MoAb.3

Other observations were more subtle. A decreased platelet count was noted in most patients, but this was not clinically significant since the platelet count remained above 100,000. There was no indication in the preclinical studies that this antibody bound to human platelets or progenitor cells, and the effect was not seen in the animal toxicology studies. The neurological abnormalities noted with XomaZyme-791 have not been seen with other immunotoxins, but may have been overlooked due to the mildness of the abnormality.

Neither the decreased serum albumin, constitutional changes, or protein urea were dose limiting. One patient treated at the highest dose developed a marked mental status change and as a result only received 4 doses of drug. This patient was also the oldest subject treated and had several documented episodes of atrial fibrillation during the 3 days of observation after termination of therapy. However, he had no prior evidence of mental status change and no evidence by computerized tomography of the head of any abnormalities and it was possible that this was a true effect of drug. Additional patients were thus accrued at lower doses but although subtle mental status changes were noted the finding was not repeated. Thus a dose range of 0.1–0.15 mg/kg/d for 5 days has been established as safe, and it is felt doses above this should be monitored carefully.

All but one of the patients produced IgG and IgM responses to the immunoglobulin and ricin A chain components of the immunotoxin, the anticombining site response being a predominant feature of the anti-immunoglobulin response. Both the antiisotypic and anticombining site antibodies would be expected to alter the biodistribution and pharmacokinetics of the immunotoxin. The influence of antiisotypic antibodies on the pharmacokinetics of XM11CO-791 antibody has been demonstrated in rats (24). That study showed that the blood survival of 125I-791T/36 as well as normal mouse IgG2b was markedly reduced with an associated liver uptake in rats producing anti-791T/36 antibody. The influence of antibodies reacting with the combining site of 791T/36 immunoglobulin has also been demonstrated using BALB/c mice immunized with 791T/36-RTA. In this case the biodistribution of 791T/36 immunoglobulin (but not normal mouse IgG2b) was modified in immunized

V. S. Byers et al., unpublished observations.
mice (25). It is expected that anti-ricin A chain antibodies will also influence immunotoxin biodistribution and pharmacokinetics. This issue is currently being investigated and it has been shown that murine monoclonal anti-RTA antibodies increase the hepatic uptake of 125I-RTA.

Evidence of biological activity was seen in the three patients with hepatic metastases, who had initial calcification of lesions followed by resolution of the smaller lesions, and fading of the larger lesions. One of the patients with pulmonary metastases had a decrease in size of the lesions; another with a supravacualicular node had resolution of that lesion but concomitant growth of others.

Pharmacokinetic studies have demonstrated that ricin A chain immunotoxins are rapidly taken up by the liver, since the circulation time and tumor localization. Methods to control the systemic availability will allow retreatment. Both these measures should lead to prolong blood circulation and tumor localization. Methods to control the immune response to immunotoxins are under development, and will allow retention. Both these measures should lead to increased efficacy of the immunotoxin.

REFERENCES


Phase I Study of Monoclonal Antibody-Ricin A Chain Immunotoxin XomaZyme-791 in Patients with Metastatic Colon Cancer

V. S. Byers, R. Rodvien, K. Grant, et al.


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