Phase I Evaluation of an Anti-Breast Carcinoma Monoclonal Antibody 260F9-Recombinant Ricin A Chain Immunoconjugate

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ABSTRACT

Four women with metastatic breast cancer were treated with monoclonal antibody 260F9-ricin A chain immunotoxin (IC) which targets a, 55,000 antigen expressed by human mammary carcinoma. Patients were treated by daily, 1-h i.v. injections for 6 to 8 consecutive days. Two patients were treated with 10 µg/kg daily and the two others were treated with 50 µg/kg daily. The trial was suspended after four patients had been treated because patients treated with a continuous infusion schedule with this IC had developed significant neurological toxicity at doses similar to those used in this study. The half-life of the IC showed a t1/2 of approximately 1.8 h, a t1/2 of approximately 8.3 h, and a peak concentration of about 200 ng/ml at the lower dose level, and showed a t1/2 of approximately 2.5 h, t1/2 of about 10.4 h, and a peak concentration of 500 and 850 ng/ml for the two patients at the higher dose level. All four patients developed evidence of a human anticonjugate antibody response within 16 days of the onset of therapy. The treatment was associated with significant toxicity, manifest by a syndrome consisting of weight gain, edema, hypoalbuminemia, and dyspnea. Similar symptoms were observed in patients treated by continuous infusions of the IC. This clinical syndrome, seen at doses of IC which were insufficient to saturate antigen-expressing malignant tumor deposits in this trial, has been seen in other IC therapy trials and in clinical trials using the cytokine interleukin 2. To investigate a possible mechanism responsible for this toxicity, human monocytes were incubated with varying concentrations of IC. There was detectable binding of IC to human monocytes at IC concentrations which were achieved clinically in this trial. Furthermore, the binding appeared to be abrogated by preincubation of the monocytes with pooled human immunoglobulin, thus suggesting that binding occurs via Fcγ receptor-mediated mechanisms. Binding was not affected if different linkers between recombinant A chain and the antibody were used or if a different antibody moiety was used in the IC preparation. Chemically linked dimers of MOPC-21 bound to human monocytes at least as well as the IC; this binding was not abrogated by preincubation with pooled human immunoglobulin. Since the IC preparations used in this clinical trial contained small percentages of dimers and/or multimers, the clinical toxicity syndrome which we observed may be related to this series of observations. A more complete understanding of the relationship of this previously unreported mechanism of IC binding to human cells expressing Fcγ receptors with the clinical manifestations of the capillary leak syndrome will await production and testing of F(ab')2 ICs or highly purified whole antibody IC preparations which contain only monomers. Further investigations into the mechanisms by which IC binding to Fcγ receptor-bearing cells may lead to disruption of endothelial cell integrity may provide important clues to the pathogenesis of the capillary leak syndrome seen with a variety of biological therapies.

INTRODUCTION

The availability of monoclonal antibody technology has made it possible to develop antibody-toxin conjugates that can deliver the toxin moiety to malignant cells in a highly specific manner. A variety of toxins have been tested; among the most potent of these is ricin. This toxin catalytically inhibits protein synthesis by binding to and cleaving the N-glycosidic bond of position A4342 in 28S RNA of 60S subunits of mammalian ribosomes (1-3). The native toxin is composed of two chains, A and B, linked by a disulfide bond. The B chain is responsible for binding to cell surfaces, while the A chain contains the enzymatic activity. ICs containing purified A chains have been tested extensively in preclinical models and clinical trials (4-14).

The IgG1 murine monoclonal antibody 260F9 is specific for a M, 55,000 antigen expressed by approximately 50% of breast cancer cells (15). This antibody has been conjugated to recombinant ricin A chain to form the immunotoxin 260F9 MAb-rRA (16). Following extensive preclinical testing which confirmed the selectivity, biological activity, and acceptable toxicity profiles of the IC, Phase I evaluations of this compound were begun using two different administration schedules. This communication describes our observations of a daily injection schedule and focuses on the capillary leak syndrome seen with this IC. We show that this and other ICs bind to human cells that do not express the antigen identified by 260F9, via uptake by cellular Fcγ receptors.

MATERIALS AND METHODS

Clinical Study Design. Four patients with metastatic breast cancer were treated with this immunotoxin between May and August 1987. Their clinical characteristics are summarized in Table 1. Patients 1 and 2 were assigned to receive 10 µg/kg daily of immunotoxin, administered by a 1-h infusion for 8 consecutive days. Patient 3 was scheduled to receive 50 µg/kg daily by the same route for 8 consecutive days, while patient 4 was assigned to receive 50 µg/kg daily for 6 days based upon pharmacokinetic studies in the first three patients. The patients were hospitalized to receive all treatments in the clinical study unit at Fox Chase Cancer Center.

The patients were evaluated for toxicity on a daily basis while receiving treatment and at approximately weekly intervals thereafter. Unless specified otherwise, all toxicity is graded according to the recently developed National Cancer Institute common toxicity criteria.

IC Preparation. 260F9 MAb-rRA IC was prepared by the Cetus Corporation. Briefly, purified 260F9 MAb was linked to rRA through a linker containing a disulfide bond. Ricin A chain was produced in Escherichia coli K12 from the gene sequence of ricin A chain cloned in 28S RNA of 60S subunits of mammalian ribosomes (4-14). The IgG1 murine monoclonal antibody 260F9 IC was prepared by the Cetus Corporation. Briefly, purified 260F9 MAb was linked to rRA through a linker containing a disulfide bond. Ricin A chain was produced in Escherichia coli K12 from the gene sequence of ricin A chain cloned in the complementary DNA of the castor bean. The final IC was purified free of unconjugated rRA and conjugates containing multimers of MAb and rRA; however, a small fraction of contaminating MAb-rRA persisted in the final product. Clinically used IC preparations contained a small concentration of dimers and multimers, less than 10% as assessed by nonreducing gel electrophoresis.

ICs 260F9 MAb-L1-rRA and 260F9-L2-rRA were prepared at Cetus using two thioether linkers, L1 and L2, to aid in determining the role...
than 95% of the cells in the monocyte gates expressed Leu-M3 (data not shown). Results are expressed as percentage of cells with more fluorescent intensity than 95–98% of the negative control cell population. Statistics were performed using paired t test analysis, as described (19).

RESULTS

Clinical Course. The courses of treatment are summarized in Table 2. Patients 1 and 2 received their planned therapy at full doses for all 8 days. Patient 3 required a 1-day treatment break and 50% dosage reductions thereafter, so that she received therapy for 7 full days, with 62.5% of her planned dose actually administered. Patient 4 received full doses of a 6-day planned treatment course. The shorter treatment course was undertaken because of the demonstration of ant conjugate antibody responses observed in the previous patients (see below).

One patient, #3, had a clinical response, with the disappearance of her sole site of disease, a 1-cm³ lung nodule identified by chest computer-assisted tomographic scan. However, she had other lung abnormalities, also seen on computer-assisted tomographic scan, of uncertain disease significance, which did not change with therapy. She had recurrence of her tumor at a chest wall/skin site 3.5 months after the initiation of her therapy.

A number of toxicities were observed with this therapy, even at doses which were too low to yield detectable binding of the immunoconjugate to the target antigen of 260F9 (Table 3). Malaise and other constitutional symptoms such as fever or myalgias were commonly seen but were not dose limiting. While all patients experienced performance status deterioration, only patient 3 declined significantly. Peripheral blood monocyte counts did not change with therapy. Anemia was universal and could not be totally accounted for by multiple phlebotomies. Weight gain averaging 4.2 kg was observed as was edema, hypoalbuminemia, hypoproteinemia, and eosinophilia. Patient 3 experienced a number of toxicities, including fever, myalgias, weight gain averaging 4.2 kg, and edema (Table 3).

Patient dose (mg/kg) dose (nig) Planned Actual received (nig)

<table>
<thead>
<tr>
<th>Patient</th>
<th>assigned daily dose (mg)</th>
<th>planned total dose (mg)</th>
<th>Duration of therapy (days)</th>
<th>total dose received (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6.8</td>
<td>8</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5.5</td>
<td>8</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>20.7</td>
<td>8</td>
<td>13.0</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>17.7</td>
<td>6</td>
<td>17.7</td>
</tr>
</tbody>
</table>

*This patient received full doses for 3 days. Treatment was held for 1 day and then resumed at 50% doses for the remaining 4 days.

Adverse events during therapy

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Karnofsky performance status drop (%)</td>
<td>10 10 10 10</td>
</tr>
<tr>
<td>Highest fever (grade)</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Most wi gain (kg)</td>
<td>5 5 5.2 1.7</td>
</tr>
<tr>
<td>Hypotension (grade)</td>
<td>0 0 3 0</td>
</tr>
<tr>
<td>Tachycardia (grade)</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Edema (grade)</td>
<td>0 1 2 2</td>
</tr>
<tr>
<td>Largest hemoglobin drop (g/100 ml)</td>
<td>3.1 2.4 1.6 1.7</td>
</tr>
<tr>
<td>Eosinophilia (%)</td>
<td>4 10 8 3</td>
</tr>
<tr>
<td>Serum albumin (% of baseline)</td>
<td>69 77 60 79</td>
</tr>
<tr>
<td>Serum total protein (% of baseline)</td>
<td>71 83 69 86</td>
</tr>
<tr>
<td>Rash (grade)</td>
<td>0 0 1 0</td>
</tr>
<tr>
<td>Dyeusras (grade)</td>
<td>0 2 0 1</td>
</tr>
<tr>
<td>Neurological (grade)</td>
<td>0 0 1 1</td>
</tr>
<tr>
<td>Nausea, vomiting (grade)</td>
<td>0 0 2 1</td>
</tr>
<tr>
<td>Flu-like symptoms (grade)</td>
<td>0 0 1 1</td>
</tr>
</tbody>
</table>

* Delayed onset of dyspepsia in absence of pulmonary tumor, edema, or further weight gain.
* Delayed onset of tinnitus. Sensation of Tumor in brachial plexus found on magnetic resonance imaging scan.
3 experienced grade 3 orthostatic hypotension which was associated with weight gain, edema, hypoalbuminemia, and hypoproteinemia. Her clinical picture closely matched that of the capillary leak syndrome which has been observed in patients receiving high dose interleukin 2 therapy (20, 21). In addition, this patient experienced a grade 2 nonpruritic rash on her trunk and extremities following the last dose of immunoconjugate.

Three of the four patients experienced delayed symptoms which were possibly attributable to the immunoconjugate therapy. Patient 2 experienced grade 2 dyspnea 6 days after the conclusion of therapy in the absence of any demonstrated pulmonary tumor, edema, or weight gain. She was thought to have congestive heart failure by her local treating physicians, although this was never documented by noninvasive or invasive studies. Patient 3 developed the delayed onset of fingertip paresthesias in the arm contralateral to her prior mastectomy incision. These symptoms resolved completely within 6 months. She has since developed evidence of local skin recurrence but has had no evidence of axillary or brachial plexus involvement. Finally, patient 4 experienced progressive left arm weakness and diminished sensation in that arm following therapy. However, extensive tumor was found in the left brachial plexus upon magnetic resonance imaging scanning.

Pharmacokinetics and Anti-Drug Antibodies. Pharmacokinetics are displayed in Table 4A. The two patients treated with 10 μg/kg/day developed peak serum concentrations of approximately 200 ng/ml. τα was approximately 1.8 h and τβ was approximately 8.3 h for these two patients. For the two patients treated with doses of 50 μg/kg/day, τα was approximately 2.5 h, τβ was approximately 10.4 h and the peak serum levels were approximately 500 and 850 ng/ml, respectively. Trough levels of IC were noted to decline in patient 1 during the last 2 days of therapy (from 25 to 10 ng/ml). The role of anti-drug antibodies in mediating this decrease is unclear. Peak serum levels did not change in this patient from days 1 to 8 (264 to 248 ng/ml).

As shown in Table 4B, all four patients developed anticonjugate antibody titers, which in all cases exceeded the titers to (h)Amplitude (h)<0 compartment (ml/kg) * « either the antibody or ricin A chain components of the conjugate. Patient 1, this appeared to be largely an antiidiotype response since the human anti-mouse antibody response against the IgG1 murine antibody 520C9 was substantially less than that of 260F9. The development of an antiidiotype response distinguishable from a human anti-mouse antibody response was not clear in the other patients tested. In general, the IgG peak titers substantially exceeded the IgM peak titers. In all patients, a human anticonjugate response was noted with 16 days of the onset of treatment and as early as 8 days following the onset of therapy in patients 1 and 3.

Patient Tumor Studies. In patient 1 an abdominal wall metastasis was biopsied and found to demonstrate the antigen recognized by antibody 260F9. A chest wall metastasis was biopsied at the conclusion of 8 days of treatment and analyzed for antigen expression and persistence of the immunoconjugate. While the malignant cells in this lesion expressed antigen, as measured by the method of immunoperoxidase staining of frozen lesion sections with 260F9 antibody, no persistent immunoconjugate was detectable in the lesion (data not shown). This finding is not unexpected since higher doses have been required to achieve saturation of tumor binding sites in clinical trials with other antibodies (22).

Toxicity Mechanism Studies. The identification of significant capillary leak syndrome toxicities in association with immunoconjugate therapy suggested the possibility that the immunoconjugate was delivering recombinant ricin A chain to unintended targets and that this delivery might be inducing the toxic syndrome. We chose to investigate the possibility that the addition of the ricin A chain had altered the structure of the IgG1 murine antibody 260F9 so that the modified protein was capable of binding to human Fcγ receptors. To test this, human monocytes were incubated in vitro with varying concentrations of immunoconjugate or with 260F9. As shown in Fig. 1, the IgG1 murine antibody 520C9 was substantially less than that of 260F9. The development of an antiidiotype response distinguishable from a human anti-mouse antibody response was not clear in the other patients tested. In general, the IgG peak titers substantially exceeded the IgM peak titers. In all patients, a human anticonjugate response was noted with 16 days of the onset of treatment and as early as 8 days following the onset of therapy in patients 1 and 3.

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To investigate the mechanisms by which this binding may occur, monocytes were incubated with a variety of ICs and other proteins as shown in Table 5. To evaluate the role of the 260F9-binding portion on monocyte binding, monocytes were exposed to an IC identical to 260F9 MAb-rRA except that the antibody portion of the IC was the MOPC-21 myeloma protein. To evaluate the possibility that the specific linker used to conjugate the antibody to ricin A chain was responsible for the observed results (through either disulfide exchange or another linker-specific mechanism), two ricin A chain 260F9 ICs prepared using different thioether linker technologies, designated 260F9 MAb-L1-rRA and 260F9 MAb-L2-rRA, were tested as well. The myeloma protein UPC-10, an IgG2a murine protein which interacts with the Fcγ receptor expressed by human monocytes, was tested as well. Finally, the ability of preincubation with 50 µg/ml pooled human IgG to inhibit IC binding via saturation of monocyte Fcγ receptors was evaluated as well. As shown by the representative experiment depicted on Table 5, all of the ICs bound well to monocytes. Furthermore, the binding of each IC was inhibited to control levels when the monocytes were preincubated with human immunoglobulin. The ICs did not bind to lymphocytes. To further evaluate the Fcγ receptor-mediated uptake of the immunoconjugate, a number of experiments were performed in which human monocytes were preincubated with human immunoglobulin prior to the addition of the IC. As shown in Fig. 2, preincubation with pooled human IgG clearly suppressed binding of the IC to human monocytes.

The clinically used IC preparations contained less than 10% dimers and multimers by nonreducing gel electrophoresis. To further investigate the mechanisms by which this IC may bind to the Fcγ receptor expressed by human monocytes, the ability of chemically constructed SPDP-linked dimers of MOPC-21 to bind to human monocytes was investigated. Fig. 3 demonstrates the results of a series of experiments in which monocytes were incubated with either MOPC-21, 260F9 MAb-rRA, or MOPC-21 dimers at varying concentrations. As expected, the immunoconjugate bound to human monocytes; this binding was inhibited by preincubation with human immunoglobulin. The MOPC-21 dimer bound to monocytes as least as well as the immunoconjugate. Furthermore, this binding was only partially reversed by preincubation with human immunoglobulin. Since the preparation used may have contained higher multimers of MOPC-21, it is possible that these immunoglobulin aggregates possessed substantially higher affinity for Fcγ receptors than did the dimers. These data suggest one possible explanation for the binding of this IC to human Fcγ receptor-bearing cells.

**DISCUSSION**

This dose escalation phase I clinical trial of bolus IC administration was suspended after four patients had been accrued because patients treated by continuous infusion of the IC in another phase I trial developed severe and unexpected neurologic toxicity. No serious neurotoxicity was observed with the bolus schedule, and we speculate that the continuous infusion schedule may have resulted in an increased area under the curve, permitting observation of the neurotoxicity at equivalent daily IC doses. We found two additional problems which may limit the likelihood of reaching therapeutically useful dosages of this IC. The first, not unexpectedly, is the development of anticonjugate antibodies. This response was seen as early as 8 days following the onset of therapy in our series. This response does not seem to be trivial in that patient 3 experienced a rash and in patient 1, who developed anticonjugate antibodies by day 8, peak serum levels of IC decreased late in treatment. The second problem was the toxic syndrome consisting of edema, hypoalbuminemia, weight gain, and, occasionally, congestive heart failure or pulmonary edema. This toxicity syndrome was not related to the schedule of administration of 260F9 MAb-rRA because similar toxicity was noted in patients treated with a continuous infusion schedule at doses of either 50 or 100 µg/kg daily for 8 days.

This symptom complex has been reported in clinical trials using other ICs incorporating the ricin A chain. Spitler et al.

**Table 5** Ricin A chain immunoconjugates bind to human monocytes via Fcγ

<table>
<thead>
<tr>
<th>Tested protein (25 µg/ml)</th>
<th>Monocytes (% positive)</th>
<th>Lymphocytes (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immunoglobulin</td>
<td>9.7 -*</td>
<td>8.1 -</td>
</tr>
<tr>
<td>260F9</td>
<td>9.3 -</td>
<td>10.9 -</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>3.6 -</td>
<td>10.4 -</td>
</tr>
<tr>
<td>UPC-10</td>
<td>91.4 -</td>
<td>12.0 -</td>
</tr>
<tr>
<td>260F9 MAb-rRA</td>
<td>48.2 -</td>
<td>12.9 -</td>
</tr>
<tr>
<td>MOPC-21-MAb-rRA</td>
<td>42.3 -</td>
<td>12.0 -</td>
</tr>
<tr>
<td>260F9-L1-rRA</td>
<td>29.0 -</td>
<td>11.6 -</td>
</tr>
<tr>
<td>260F9-L2-rRA</td>
<td>64.4 -</td>
<td>8.2 -</td>
</tr>
</tbody>
</table>

* - not done.
cytes with pooled human immunoglobulin at concentrations to human monocytes. However, this lack of detectability may body will require testing (25). Preincubation of purified monocytes with 1C monomers, then these preparations may be adequate saturation. However, if the 1C monomers bind to monocytes, then ICs which incorporate F(ab) or F(ab') fragments of anti valency caused by creation of 1C dimers or multimers (Fig. 3). Further investigation is required to investigate the possibility that this modified pro monocytes at concentrations which were achieved clinically and lysis of human umbilical vein endothelial cells (29, 31). Although we observed little binding of 260F9 MAb-rRA to and lysis of human umbilical vein endothelial cells (29, 31). The clinical observation that this syndrome worsens when autologous activated lymphocytes are added suggests that these cells may be involved in the pathogenesis of the syndrome. Damle et al. (29) have demonstrated that these lymphokine-activated killer cells bind to endothelial cell surfaces, further supporting the association of these cells with the syndrome. Recently it has been demonstrated that endothelial cells from interleukin 2-treated patients express activation antigens, although Fcγ receptors were not evaluated in that study (30). The mechanisms by which such activated cells may induce this syndrome remain unknown, although lymphokine-activated killer cells mediate detachment and lysis of human umbilical vein endothelial cells (29, 31). Although we observed little binding of 260F9 MAb-rRA to human lymphocytes (Table 5), studies which evaluate the interactions of ricin A chain ICs with interleukin 2-activated large granular lymphocytes are required. The recent demonstration that human lymphokine-activated killer cells lyse autologous monocytes (32) suggests that monocyte lysis may be a common mechanism for disparate etiologies and manifestations of the capillary leak syndrome.

The mechanisms by which IC therapy may lead to capillary leak syndrome require further investigation. Understanding the pathogenesis of this IC-induced toxicity may lead to a better appreciation of the mechanisms which induce this syndrome when it is associated with other therapeutic agents.

ACKNOWLEDGMENTS

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L2-RRA, and to Jeffrey Kessel for preparation of the MOPC-21, SPDP-linked conjugate.

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