Clinical Evaluation of Intraperitoneal *Pseudomonas* Exotoxin Immunoconjugate OVB3-PE in Patients With Ovarian Cancer

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OVB3-PE is an immunotoxin composed of a murine monoclonal antibody reactive with human ovarian cancer and conjugated to *Pseudomonas* exotoxin (PE). Twenty-three patients with refractory ovarian cancer were treated intraperitoneally (IP) with escalating doses of OVB3-PE to study toxicity, pharmacokinetics, antiimmunotoxin antibody formation, and antitumor response. Dose-limiting CNS toxicity occurred after repeated doses at 5 and 10 μg/kg. Other non–dose-limiting toxicities included transient elevation of liver enzymes, fever, and gastrointestinal toxicity. Pharmacokinetics of IP and serum OVB3-PE were determined in 16 patients. Peak peritoneal fluid levels exceeded the in vitro median effective dose at all doses tested. At doses of 1 to 2 μg/kg, the immunotoxin concentration in the peritoneal fluid remained constant for up to 8 hours and dropped to negligible levels after 12 hours. At the 5 and 10 μg/kg doses, levels remained high for up to 24 hours (> 100 ng/mL) and then gradually decreased and became undetectable (< 4 ng/mL) after 72 hours. Serum levels of OVB3-PE were also analyzed in 16 patients. At doses of 1 μg/kg and 2 μg/kg, serum levels were not detectable (< 5 ng/mL). However, after doses of 5 or 10 μg/kg, peak serum level occurred at 24 hours after each dose and dropped to negligible levels by 72 hours. Sera from 12 patients were analyzed for anti-PE antibodies and antibodies to mouse immunoglobulin (HAMA). All patients developed antibodies against PE within 14 days of therapy. Domain II of PE appeared to be the most immunogenic portion of the PE molecule. HAMA was detected on day 14 of therapy in nine patients, on day 21 in two, and on day 28 in one patient. No clinical antitumor responses were observed. We conclude that IP OVB3-PE at dose levels of 5 μg/kg (× 3) and 10 μg/kg (× 2) is accompanied by dose-limiting toxic encephalopathy. Neurologic toxicity is likely to be due to crossreactivity of OVB3 to normal human brain tissue, which was not appreciated during preclinical screening.

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OVARIAN CANCER is a common gynecologic malignancy that accounts for the death of 11,600 women per year in the United States.[1] Disseminated peritoneal disease is not amenable to surgical cure, and aggressive chemotherapy has been used as primary treatment. For those patients who fail or relapse after initial cisplatin-based chemotherapy, the overall prognosis remains poor. It is clear that the development of new therapeutic approaches for this disease is needed.

In the past decade, several clinical studies have examined the potential therapeutic value of various monoclonal antibody conjugates with plant and bacterial toxins, radionuclides, or chemotherapeutic agents.[2-5] These efforts have been aimed at testing the concept that monoclonal antibodies could serve as targeting agents to human tumors. We have been investigating the utility of immunotoxins composed of antibodies linked to *Pseudomonas* exotoxin (PE), a 66-kd protein produced by *P aeruginosa*. This protein has been crystallized, and x-ray diffraction analysis has revealed three prominent domains.[6] The amino terminal domain mediates receptor binding (domain Ia); the middle one, translocation (domain II); and the carboxyl terminal domain (domain III), the adenosine diphosphate (ADP) ribosylation and inactivation of

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elongation factor 2 (EF-2), which inhibits protein synthesis and leads to cell death.[7] PE was coupled with OVB3, a murine immunoglobulin G_{2b} (IgG_{2b}) antibody that reacts with all human ovarian cancers tested. The resulting immunotoxin, OVB3-PE, was shown to kill human ovarian cancer cells in cell culture and to prolong the life of nude mice bearing human ovarian tumor xenografts.[8,9] Toxin-conjugates and conventional chemotherapeutic drugs have different mechanisms of action. Therefore, those cancer cells with natural or acquired resistance to chemotherapeutic drugs cannot be crossresistant to toxin-based therapies.

Ovarian cancer patients with small-volume residual disease are good candidates for therapeutic evaluation of reagents administered directly into the peritoneal cavity. In addition to our own studies on ovarian cancer,[8-10] Griffin et al[11] have shown that immunotoxins constructed with ricin A chain and given intraperitoneally (IP) have significant tumoricidal activity in nude mice bearing IP human malignant mesothelioma. Pharmacologic modeling[12] suggests that prolonged IP administration of large molecules, such as proteins, results in an increased depth of penetration into normal parietal tissues such as the diaphragm and abdominal wall musculature[13] compared with smaller molecules. Penetration into tumors has been less well characterized but may be no better than that observed after intravenous administration. Although conventional drugs can be cleared from the peritoneal space by transmembrane diffusion into capillaries, macromolecules are limited to clearance via tissue lymphatics. Dissemination of ovarian cancer results in lymphatic obstruction with further impairment of IP clearance. Therefore, IP immunotoxin should have a particularly prolonged half-life in this group of patients. We undertook a phase I study of IP OVB3-PE in patients with ovarian cancer limited to the peritoneal cavity.

PATIENTS AND METHODS

Patient Selection

All patients had histologic confirmation of refractory invasive epithelial cancer of the ovary limited to the peritoneal cavity after platinum-based chemotherapy that could be serially evaluated by noninvasive or invasive techniques. Other criteria for eligibility included Karnofsky performance status greater than 70 and minimum life expectancy of 3 months. Chemotherapy, immunotherapy, or radiation therapy was not allowed within 3 weeks of study entry (6 weeks for nitrosourea or mitomycin). Laboratory criteria included serum creatinine level less than 2 mg/dL, 24-hour creatinine clearance greater than 70 cc/min, serum bilirubin level less than 1.5 mg/dL, serum SGOT level less than 50 IU/L, and prothrombin time less than 14.0 seconds. Patients were required to tolerate Tenckhoff catheter insertion, maintain catheter integrity, and have an adequate peritoneal space to permit IP therapy. Patients with disease outside the peritoneal cavity, including chest masses or effusion, intraparenchymal liver disease, subcutaneous nodules or CNS metastasis, were excluded. Patients were excluded if they had previously received any murine monoclonal antibody or if significant neutralizing antibody to PE was demonstrated before treatment. All patients gave informed consent before study entry. The study was approved by the Clinical Research Subpanel of the National Cancer Institute (NCI), the investigational review board of the NCI-Frederick Cancer Research Development Center (FCRDC), and the Cancer Therapy Evaluation Program of the Division of Cancer Treatment.

OVB3-PE

OVB3-PE is an immunotoxin consisting of the murine IgG_{2b} monoclonal antibody OVB3 coupled with PE. Preclinical activity against human ovarian cancer has been described by Willingham et al[8] and FitzGerald et al.[9] For the production of OVB3, mice were immunized with membranes from the human ovarian cancer cell line OVCAR-3. Immune spleen cells were fused with NS-1 cells, and the resulting hybridoma was selected on the basis of antibody binding to OVCAR cells. OVB3 reacts with a variety of ovarian cancer cell lines, fresh ovarian cancer tissue, and ascites specimens with minimal reactivity against normal tissues. PE was purified from the culture medium of *P aeruginosa* by Swiss Serum and Vaccine Institute, Berne, Switzerland. OVB3 was coupled with PE by a thioether bond. Clinical lots of OVB3-PE were prepared by the Laboratory of Molecular Biology, NCI/National Institutes of Health and by Hybritech Inc, LaJolla, CA. Each lot met the specifications of United States Pharmacopeia sterility, rabbit pyrogenicity, general safety, and murine virus testing. The median effective dose of OVB3-PE for inhibition of protein synthesis against the ovarian carcinoma cell line OVCAR-3 in vitro was 0.5 ng/mL.

Study Design

This was a nonrandomized, fixed, multiple dose-escalation study. The first group of 19 patients received a single cycle of therapy, consisting of a fixed dose of OVB3-PE administered IP via a Tenckhoff catheter on days 1 and 4 (Table 1). Because of two episodes of CNS encephalopathy at the 10 μg/kg dose level, the protocol was amended to reduce the dose of OVB3-PE to 5 μg/kg and to administer

Table 1. Study Design of OVB3-PE IP Therapy

Escalating Dose, Fixed Schedule	Fixed Dose, Escalating Schedule
I. 1 μg/kg, days 1, 4	III-B. 5 μg/kg, days 1, 4
II. 2 μg/kg, days 1, 4	V. 5 μg/kg, days 1, 4, 7
III-A. 5 μg/kg, days 1, 4	VI. 5 μg/kg, days 1, 3, 5, 7
IV. 10 μg/kg, days 1, 4	

an escalating number of doses (Table 1). Spontaneous ascites was drained before each infusion. Before each treatment dose, patients received an IP test dose of OVB3-PE (20 μg total) over 10 minutes, followed by observation over 2 hours. In the absence of systemic reaction to the test dose, treatment doses were given. For each treatment dose, patients received 1 L of normal saline followed by immunotoxin dissolved in 50 cc normal saline with 5% human serum albumin, followed by an additional 1 L of normal saline. Patients were monitored closely for vital signs and fluid status, with support for anaphylaxis available at the bedside. All patients were hospitalized during treatment and for a minimum of 24 hours after the last dose of immunotoxin.

Patient Evaluation

Patients were restaged with noninvasive techniques at the end of the first cycle, and those with progressive disease were removed from study. Responding patients were eligible for additional cycles of therapy at 28-day intervals, provided they had not developed anti–OVB3-PE antibodies or experienced unacceptable toxicity.

Pharmacology Studies

Ascitic fluid was collected at 5 and 30 minutes and 1, 2, 4, 8, 12, 24, and 72 hours after each dose. Blood was collected at 4, 8, 24, 48, and 72 hours and immediately before each dose, then every 24 hours until patient's discharge from hospital.

OVB3-PE was measured in serial samples of ascitic fluid and serum by an enzyme-linked immunosorbent assay (ELISA). Ninety-six–well microtiter plates were coated with affinity-purified goat antimouse $F(ab')_2$ (Jackson Immunoresearch Laboratories, West Grove, PA), and nonspecific binding was blocked with 3% gelatin. Test samples were diluted 1:10 to 1:1,000 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and incubated for 1 hour at 37°C. Rabbit anti-PE (1:2,000 dilution) was added for 1 hour and then washed off. Peroxidase-conjugated, affinity-purified goat antirabbit antibody (Jackson Laboratories) was added for 30 minutes. The reaction was developed using 2.2-azino-di-(3-athyl-benzthiazolinsulfonate 6) (ABTS; Boehringer Mannheim, Indianapolis, IN). Optical densities were read at 405 nm in a MicroElisa autoreader (Dynatech, Alexandria, VA). For each plate, a standard curve of absorbance versus concentration of OVB3-PE was generated. The threshold for the peritoneal fluid assay was 5 ng/mL and for the serum assay, 4 ng/mL.

Human Anti-PE Antibody

Anti-PE antibody was also measured in serum by ELISA. Ninety-six–well microtiter plates were coated with whole PE, domain I of PE, domain II plus III (PE-40), or domain III of PE, incubated for 90 minutes at 37°C and then blocked with PBS-containing 1% BSA. Serum samples were added in dilutions beginning at 1:10, incubated, washed, incubated with peroxidase conjugates of goat antihuman IgG (Jackson Laboratories), and developed using ABTS. Results are reported as optical density values of serum specimens diluted 1:100.

Human Antimouse Antibody

Human antimouse antibody (HAMA) was also measured in serum by ELISA. Plates were coated with OVB3 and blocked with 3% gelatin. Serum samples were added in dilutions beginning at 1:10 and incubated for 60 minutes at 37°C followed by alkaline phosphatase conjugates of goat antihuman IgG (Jackson Laboratories), and finally developed with ABTS. The results were read at 405 nm. Background optical density was approximately 5% of the maximum. Positive wells were scored when the optical density was at least twice the background.

RESULTS

Patient Characteristics

Twenty-three patients were entered onto the trial between November 1987 and November 1989. All patients had a history of refractory invasive epithelial cancer of the ovary and had received and failed prior treatment with a platinum-based chemotherapy regimen. Their ages ranged from 39 to 68 years; the mean age was 53 years. Karnofsky performance status ranged from 70 to 90 (median, 90). All patients had disease limited to the peritoneal cavity, except one patient, who had liver metastases at the time of therapy. All patients were screened and found to be negative for anti-PE antibodies before entering the trial. Two patients were initially treated despite low levels of neutralizing activity against PE. In these patients OVB3-PE was immediately neutralized in vivo, and neutralizing activity could not be eliminated by peritoneal lavage. Therefore, additional patients were only treated in the absence of neutralizing activity. Before treatment, patients underwent peritoneoscopy and were found to have an adequate peritoneal space to permit IP therapy. At the time of the procedure, a Tenckhoff catheter (if not already present) was placed for immunotoxin delivery.

Toxicity

All patients entered on the protocol were evaluated for toxicity (Table 2). Nineteen patients developed peritoneal or abdominal pain after therapy (83%), seven of which required parenteral narcotics for pain control. There was no evidence of inflammatory or hemorrhagic peritonitis on the basis of peritoneal fluid cell counts (data not shown). One patient was found to have abdominal infection after the first dose, and therapy was discontinued. Twelve patients (52%) developed mild nausea and vomiting (grade 1 to 2) easily

Table 2. Toxicity

Toxicity	No. of Patients	%
Abdominal pain	19	83
1	12	63
2	7	36
Nausea and vomiting	12	52
1	4	33
2	8	67
Fever	5	22
2	5	100
Chemical hepatitis	12	52
Elevated transaminases	8	34
1	4	50
2	4	50
Elevated LDH	4	17
1	2	50
2	2	50
Elevated Alk phosp	9	59
1	8	89
2	1	11
Elevated bilirubin	2	9
1	2	100
Neurocortical toxicity	3	13*
3	2	66
4	1	33

Abbreviations: LDH, lactate dehydrogenase; Alk phos, alkaline phosphatase.

*Neurocortical toxicity occurred after 10 μg/kg × 2 (two patients) and 5 μg/kg × 3 doses (one patient).

controlled with antiemetics. Eight patients (35%) had transient mild elevations of SGOT, SGPT, or alkaline phosphatase. Five patients had low-grade fever with no infection source.

Dose-limiting central neurologic toxicity was documented in three patients. Two incidents of encephalopathy occurred among five patients treated at the 10 μg/kg dose level and were characterized by confusion, apraxia, and dysarthria beginning several hours after the second dose. Both patients eventually recovered after several months; one patient has no residual neurologic deficit, and one has minimal residual apraxia and dysarthria. Cranial computed tomographic (CT) scans were normal in two patients, whereas gadolinium-enhanced magnetic resonance imaging (MRI) in the third patient showed focal inflammatory abnormalities in the pons and midbrain (Fig 1). There was no evidence of metastatic tumor or carcinomatous meningitis in any of the three patients. No neurologic toxicity occurred among six patients who received two doses, two patients who received three doses, and one patient who received four doses, at 5 μg/kg. Fatal neurotoxicity was seen, however, in one patient (no. 16) after a third dose of OVB3-PE at the 5 μg/kg dose level. The patient became progressively confused and, within 6 hours of treatment, developed a severe acute encephalopathy characterized by myoclonus, disorientation, apraxia, and dysarthria. By 12 hours after treatment, she developed petit mal seizures and an upper gastrointestinal bleed. This was followed by coma and a grand mal seizure requiring both diphenylhydantoin and phenobarbital for control. She was transferred to the intensive care unit and intubated. Lumbar puncture revealed a CSF protein of 700 to 800 mg/dL and WBC count of 2/μL to 3/μL; no OVB3-PE was detected in the CSF by ELISA. CT scan without contrast was negative; MRI revealed inflammatory abnormalities in the brain stem, cerebellum, deep nuclei, and deep white matter. The patient remained comatose. Five days after treatment, CT scan showed increased edema with sulcal and cisternal effacement. Electroencephalogram showed persistent seizure activity. Aggressive life support was discontinued, and the patient died 12 days posttherapy of respiratory failure. An autopsy was not performed. This toxicity necessitated immediate termination of the study.

Response

No objective partial or complete antitumor responses were noted in any of the patients. Peritoneal fluid cytology remained positive in all

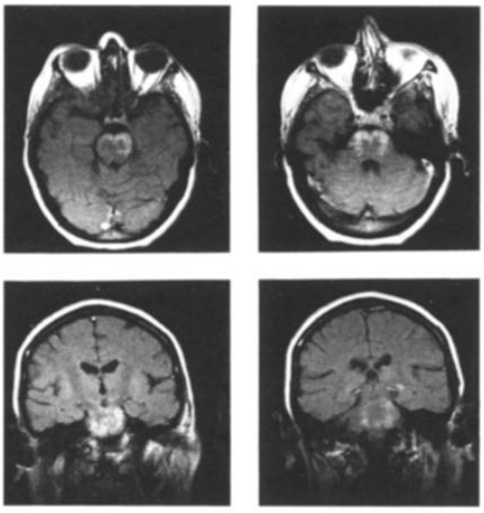

Fig 1. Gadolinium-enhanced MRI of patient no. 12, showing increased signal intensity in the pons and midbrain.

patients in whom positive cytology was present before therapy.

Pharmacokinetics

Peritoneal fluid. Peritoneal fluid from 16 patients was analyzed for the concentration of intact OVB3-PE using a sandwich ELISA. At the dose level of 1 μg/kg, the concentration remained stable up to 4 hours after infusion, then gradually decreased to undetectable levels (< 5 ng/mL) 12 to 24 hours after each dose (days 1 and 4). At the 2 μg/kg dose level, immunotoxin concentration remained high for up to 8 hours and became undetectable 12 to 24 hours after the first and second doses. At the dose level of 5 μg/kg, four of six patients had high concentrations of OVB3-PE (> 50 ng/mL) in the peritoneal fluid 24 hours after the first dose; it became undetectable after 72 hours. Figure 2 shows a pharmacokinetic curve of patient no. 7, who received 5 μg/kg of immunotoxin on days 1 and 4. Two patients received a third dose of OVB3-PE on day 7, and the pattern of clearance did not differ greatly from the first two doses. Patient no. 16 received three doses of OVB3-PE at 5 μg/kg/dose on days 1, 3, and 5. The concentration of OVB3-PE remained greater than 100 ng/mL 24 hours after each dose and greater than 50 ng/mL before each dose. It became undetectable only 96 hours after the last dose (Fig 3). At the 10 μg/kg dose level, concentrations of OVB3-PE remained greater than 100 ng/mL up to 12 hours after each infusion, but gradually decreased to insignificant levels after 72 hours in all cases. Before each dose, there was no detectable

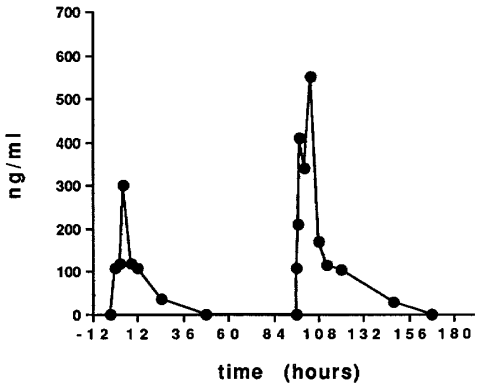

Fig 2. Concentration of OVB3-PE in ascitic fluid of patient no. 7, who received OVB3-PE 5 μg/kg IP on days 1 and 4.

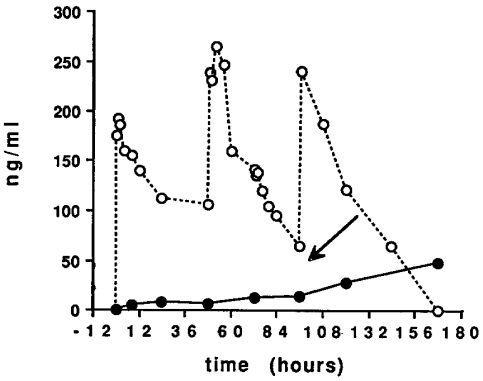

Fig 3. Ascitic fluid (○) and serum levels (●) of OVB3-PE of patient no. 16, who received immunotoxin 5 μg/kg on days 1, 3, and 5. Levels in CSF were undetectable. Arrow indicates the onset of neurotoxicity after the third dose of immunotoxin.

immunotoxin present in the peritoneal cavity. Table 3 shows the peak immunotoxin levels in the peritoneal fluid of these patients after each dose.

Serum concentration of OVB3-PE. Concentrations of OVB3-PE in the serum were determined in 16 patients. At the dose level of 1 and 2 μg/kg, no significant amounts of intact OVB3-PE (ie, < 4 ng/mL) were detected. At the dose level of 5 μg/kg, six of six patients who received IP infusions

Table 3. Peak OVB3-PE Level in Peritoneal Fluid

Schedule/Patient No.	First Dose	Second Dose	Third Dose
1 μg/kg days 1 and 4			
1	45	35	—
2	35	25	—
3	28	25	—
2 μg/kg days 1 and 4			
4	90	92	—
5	60	92	—
6	90	110	—
5 μg/kg, days 1 and 4			
7	120	410	—
8	210	560	—
9	400	550	—
10 μg/kg, days 1 and 4			
10	430	310	—
11	346	324	—
12	500	380	—
13	342	338	—
5 μg/kg, days 1, 4, 7			
14	400	400	500
15	250	360	400
5 μg/kg, days 1, 3, 5			
16	182	265	240

NOTE. Values indicate ng/mL at 2 to 4 hours after IP administration.

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