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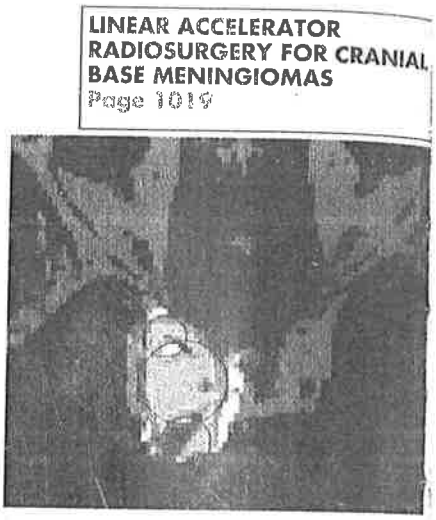
CLINICAL STUDIES



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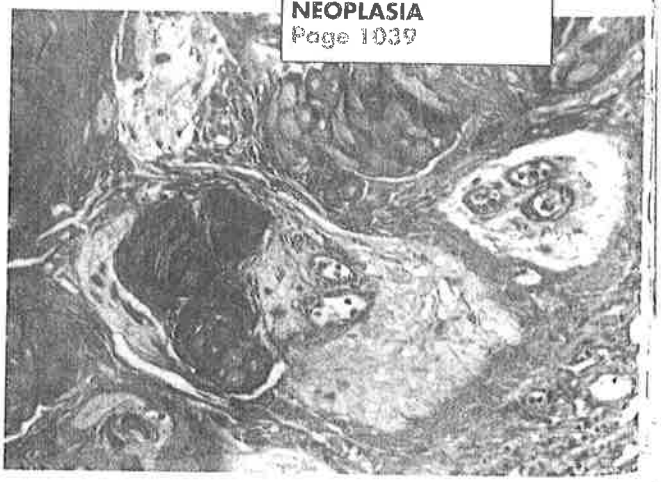

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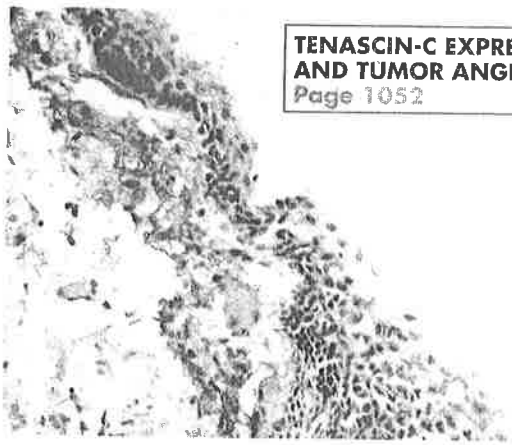

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SURGICAL APPROACHES

CLINICAL STUDIES

Intraventricular Immunotoxin Therapy for Leptomeningeal Neoplasia

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OBJECTIVE: The goals of this clinical trial of intraventricular 454A12-rRA therapy were to identify dose-limiting toxicities, to evaluate the pharmacokinetics of single-dose intraventricular 454A12-rRA, and to detect antitumor activity.

METHODS: We performed a pilot study of intraventricular therapy with the immunotoxin 454A12-rRA in eight patients with leptomeningeal spread of systemic neoplasia. The immunotoxin 454A12-rRA is a conjugate of a monoclonal antibody against the human transferrin receptor and recombinant ricin A chain, the enzymatically active subunit of the protein toxin ricin. Patients were treated with single doses of 454A12-rRA ranging from 1.2 to 1200 μg.

RESULTS: The early phase half-life of 454A12-rRA in ventricular cerebrospinal fluid (CSF) averaged 44 ± 21 minutes, and the late phase half-life averaged 237 ± 86 minutes. The clearance of the immunotoxin was faster than the clearance of coinjected technetium-99m-diethylenetriamine penta-acetic acid, averaging approximately 2.4-fold greater. No 454A12-rRA degradation was detected by Western blot analysis of ventricular CSF for a period of 24 hours, and bioactivity was retained in CSF paralleling the concentration of immunotoxin. No acute or chronic drug toxicity was identified in patients who received less than or equal to 38 μg of 454A12-rRA by intraventricular injection. Doses more than or equal to 120 μg caused a CSF inflammatory response that was associated with transient headache, vomiting, and altered mental status. This acute syndrome was responsive to steroids and CSF drainage. No systemic toxicity was detected. In four of the eight patients, a greater than 50% reduction of tumor cell counts in the lumbar CSF occurred within 5 to 7 days after the intraventricular dose of 454A12-rRA; however, no patient had their CSF cleared of tumor, and clinical or magnetic resonance imaging evidence of tumor progression was demonstrated in seven of the eight patients after treatment.

CONCLUSION: Tumoricidal concentrations of the immunotoxin 454A12-rRA can be attained safely in the CSF of patients with leptomeningeal tumor spread. (Neurosurgery 41:1039–1051, 1997)

Key words: Antibody, Cancer, Cerebrospinal fluid, Pharmacokinetics, Ricin toxin

Leptomeningeal neoplasia occurs in 5 to 20% of all cancer patients and results in a very poor prognosis, with a median survival of only a few months (10, 19, 21, 22, 41, 52, 55, 66). In adults, breast and lung carcinoma cause the majority of cases of leptomeningeal neoplasia (2, 39, 66). Studies indicate that 35 to 40% of all breast cancer patients experience central nervous system (CNS) involvement at some point in their disease and that 5% experience leptomeningeal involvement (54, 61, 66). In addition, primary CNS tumors, including medulloblastomas, ependymomas, pineal tumors, and gliomas, can spread diffusely to the leptomeninges, thwarting efforts at treatment.

Leptomeningeal neoplasia produces a clinical course that is characterized by progressive neurological deficits (21, 41, 62, 66). Cerebral symptoms, which include headache, changes in mental status, and seizures, occur in 50% of patients, cranial nerve symptoms arise in 38%, and spinal nerve root symptoms affect 70%. With leptomeningeal spread of systemic cancers, the median survival in untreated patients and patients unresponsive to treatment is less than 2 months (41, 55). With aggressive treatment, including radiation therapy and intrathecal methotrexate, the average survival is only prolonged to 6 to 7 months (19, 41, 66). However, even with aggressive treatment, 40 to 50% of patients fail to stabilize. Nonetheless, meningeal involvement with cancer is often not a premorbid phenomenon and in two-thirds to three-quarters of patients, it occurs when systemic disease is stable or in complete remission (19, 66). Effective therapy in these patients would significantly extend survival.

Chemotherapy is commonly used for leptomeningeal neoplasia; however, it can have serious side effects. The most well-known and studied chemotherapeutic agents used intrathecally are methotrexate, cytosine arabinoside, and thiotepa (9, 19, 24, 42, 62, 66). Intraventricular methotrexate is the most effective current treatment for meningeal spread of various tumors, including breast cancer (42). However, long-term use of methotrexate, particularly in high doses and in patients who have received radiation therapy, may cause significant CNS toxicity, including necrotizing leukoencephalopathy (62).

The development of monoclonal antibodies that recognize specific tumor-associated antigens provides the possibility of creating more specific therapeutic reagents. Antibodies have been conjugated to radionuclides and various peptide toxins to create new drugs with high tumor selectivity in vitro (7, 20, 25, 31, 33, 57, 60, 67, 68). However, attempts to develop these compounds for clinical use indicate that transcapillary and interstitial barriers limit the delivery of antibodies or antibody conjugates into solid tumors. In leptomeningeal neoplasia, the malignant cells often grow as thin sheets bathed in cerebrospinal fluid (CSF) (41), which reduces the problems of drug delivery and tissue penetration; therefore, leptomeningeal neoplasia is an attractive candidate for intrathecal therapy with antibody-toxin conjugates (immunotoxins).

We conducted a pilot study of intraventricular therapy with the immunotoxin 454A12-rRA, a chemically linked conjugate of a monoclonal antibody against the human transferrin receptor (454A12) and a protein toxin (recombinant ricin A chain [rRA]). Transferrin receptors transport iron-transferrin complexes into cells and are overexpressed on rapidly dividing cells, including cancer cells, reflecting increased iron requirements (15, 17, 18, 32, 51, 57–59). Within the normal adult CNS, transferrin receptors are sparse and are largely restricted to the luminal surface of brain capillaries (3, 12, 26, 28). Ricin, a potent protein toxin consisting of two subunits, binds cells and, when internalized, kills them by enzymatically inactivating protein synthesis (16, 40, 69). Recombinant ricin A chain, the catalytic subunit, lacks the B chain that mediates nonspecific binding and internalization. By linking ricin A chain to certain monoclonal antibodies, potent cytotoxic drugs are produced that are specific for cells bearing the antigen recognized by the antibody (20, 31, 68, 72). The immunotoxin 454A12-rRA demonstrates cytotoxic activity against many tumor cell lines, including breast carcinomas, leukemia, glioblastomas, and medulloblastomas (29, 72).

This study initiates the evaluation of immunotoxin therapy for human CNS tumors. The objectives of this study were to identify dose-limiting toxicities for intraventricular 454A12-rRA therapy, to evaluate the pharmacokinetics of immunotoxins in CSF, and to detect in vivo antitumor activity against human tumors.

PATIENTS AND METHODS

Patients

Patients who were more than 18 years of age and not pregnant were eligible for this study if their CSF prepared by cytocentrifugation contained malignant cells, they had a confirmed tissue diagnosis of cancer, and their Karnofsky performance score was greater than or equal to 30 (30). Patients were excluded if they suffered obstructive hydrocephalus requiring a shunt or if they received previous therapy with immunotoxin conjugates, murine monoclonal antibodies, or ricin. The protocol was approved by the institutional review board of the National Institutes of Health (National Institute of Neurological Disorders and Stroke protocol No. 90-N-36). Written informed consent was obtained from all patients or their responsible next-of-kin.

Drug preparation

Immunotoxin 454A12-rRA was supplied by Cetus Corporation (Emeryville, CA). This immunotoxin is composed of a murine immunoglobulin (Ig)G1 subclass monoclonal antibody, 454A12, covalently coupled to recombinant ricin A chain via a disulfide linkage. Monoclonal antibody 454A12 binds to the human transferrin receptor (7). The immunotoxin was a mixture of antibody coupled to recombinant ricin A chain in a 1:1 molar ratio, a 1:2 molar ratio, and a 1:3 and higher molar ratios. The percent composition of the immunotoxin was 27% (1:1), 32% (1:2), 22% (1:3), and 18% greater than 1:3. There was no detectable free antibody or recombinant ricin A chain. The composition, purity, and reproducibility of the immunotoxin were approved by the Food and Drug Administration in an investigational new drug application. The immunotoxin 454A12-rRA was supplied in vials containing 4.4 mg of lyophilized drug formulated with human serum albumin and

was reconstituted with 2.2 ml of 0.9% sodium chloride, preservative-free. Subsequent dilutions were prepared with 0.1% human serum albumin in normal saline.

Trial design

Pretreatment evaluation included baseline physical examination, Karnofsky performance score determination, and gadolinium-enhanced magnetic resonance imaging (MRI) of the head and complete spine. Patients were required to have liver and renal function values of less than two times normal values, normal metabolic parameters, and a normal coagulation profile.

A right frontal reservoir (Ommaya type) was attached to a catheter with the tip in the right frontal horn of the lateral ventricle, if not already present. Ventricular and lumbar CSF samples were obtained before therapy for cell count with differential, total protein, glucose, lactate dehydrogenase (LDH) isoenzymes, and cytology (5, 6, 22, 41, 44, 61, 62, 64, 70). An estimate of the number of malignant cells in the lumbar CSF was determined. Before treatment, all patients underwent technetium-99m-diethylenetriamine penta-acetic acid (^{99m}Tc-DTPA) ventriculocisternography to evaluate flow within the CSF pathways (see *Fig. 1*) and, 12 to 18 hours before intraventricular injection of 454A12-rRA, a lumbar subarachnoid drain was placed for frequent lumbar CSF sampling. Treatment consisted of a single dose of 454A12-rRA delivered through the Ommaya reservoir in a constant injection volume of 10 ml, and then barbotage of the reservoir was performed four times.

The initial dose of 454A12-rRA was 1.2 μg (to yield an estimated CSF concentration of 5.6×10^{-11} mol/L if uniformly distributed over the predicted CSF volume of approximately 120 ml). This dose was chosen because it was calculated to result in an expected CSF concentration 2000-fold lower than that which produced toxicity in preclinical animal studies and because the calculated CSF concentration was within the lower range in which human tumor cells were killed in vitro (38, 72). The first patient received a total of three doses that were increased by 1/2 log increments; subsequent patients (two patients per dose level) received single doses that were increased by 1/2 log increments. A very low initial dose and extensive preclinical pharmacokinetic and toxicity data (38) supported the use of the rapid dose-escalation scheme.

FIGURE 1. ^{99m}Tc-DTPA ventriculocisternography (left lateral planar gamma camera image). Image obtained in Patient 1 30 minutes after intraventricular injection of 265 μCi of ^{99m}Tc-DTPA (injection was administered into an Ommaya reservoir and barbotage was performed four times), which demonstrates satisfactory distribution through the foramen magnum (*arrow*) into the spinal subarachnoid space (*arrowheads*).

Pharmacokinetics

To compare the CSF pharmacokinetics of 454A12-rRA to bulk CSF flow, 200 to 550 μCi of ^{99m}Tc-DTPA was coinjected with the immunotoxin through the Ommaya reservoir (4, 11, 25, 36, 38). Using an aseptic technique, CSF samples of 2.0 ml were obtained from the Ommaya reservoir and the lumbar drain 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours after injection. The 454A12-rRA concentration was determined by an enzyme-linked immunosorbent sandwich assay (36), and measurement of ^{99m}Tc-DTPA was performed by gamma counting (Packard B5003; Hewlett Packard, Avondale, PA). The amount of ^{99m}Tc-DTPA in each sample was determined after correction for the rapid isotope decay (half-life of 6 h). Additional information on the clearance of the labeled tracer was obtained by sequential gamma camera imaging. Gamma camera images (Siemens Orbiter Camera, low energy all purpose collimator; Siemens Medical Systems, Hoffman Estates, IL; and Elscint Apex Computer; Elscint, Inc., Hackensack, NJ) were obtained 0, 0.5, 4, and 24 hours after injection of ^{99m}Tc-DTPA.

Biexponential decay curves of the form Concentration = $\alpha \exp(-\lambda_1 t) + \beta \exp(-\lambda_2 t)$ were fit to the ventricular (^{99m}Tc-DTPA and 454A12-rRA) data of each patient using IMSL subroutines (IMSL Inc., Houston, TX). The IMSL subroutines perform nonlinear regressions by means of a finite difference Levenberg-Marquardt algorithm. The ratio R, equal to $\alpha/(\alpha+\beta)$, represents the fractional contribution of the early or α-phase. The half-lives of the two phases, $t_{1/2\alpha}$ and $t_{1/2\beta}$, are equal to $(\ln 2)/\lambda_1$ and $(\ln 2)/\lambda_2$, respectively. Clearances were calculated as dose/$AUC_{ventricles}$, where $AUC_{ventricles}$ is the area under the curve (AUC) for the best-fit curve to the ventricular data. AUC_{lumbar} was calculated by the linear trapezoidal method. The AUC beyond the last sampling point was estimated by exponential extrapolation. The apparent volume of distribution, V_d, was calculated by dividing the dose by the concentration-intercept of the ventricular curve.

In vitro bioassays

In vitro testing was used to assess the bioactivity of each patient's CSF after intraventricular injection of 454A12-rRA and to assess the sensitivity of each patient's tumor cells (harvested from the CSF) to the immunotoxin.

Bioactivity of 454A12-rRA in CSF of patients

Samples of CSF obtained from the Ommaya reservoir and the lumbar drain at timed intervals after treatment were tested at various dilutions in protein synthesis assays with the K562 erythroleukemia cell line, as described previously (29, 72). The results are expressed as a percentage of ^{14}C-leucine incorporation in mock-treated control cultures.

Sensitivity of tumor cells in CSF of patients

In three patients, CSF tumor cells were harvested from 25 to 35 ml of lumbar CSF by centrifugation 3 to 5 days before treatment and 2 to 3 weeks after treatment. The cytotoxicity of serial dilutions of 454A12-rRA was then tested against these tumor cells based on the protein synthesis assay described previously (29, 72).

Western blot analysis of CSF of patients for 454A12-rRA

Ventricular CSF samples were obtained from Patients 6 and 8 at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after 454A12-rRA injection and run on 6.5% nonreducing sodium dodecyl sulfate polyacrylamide gels. Each lane was loaded with 20 μl of CSF or conjugate control and 10 μl of loading buffer. Gels were transferred to nitrocellulose filters, incubated with avidin-labeled goat antimouse antibodies to IgG, IgA, and IgM (heavy and light chains) (Zymed Laboratories, South San Francisco, CA), and then detected with biotin-horseradish peroxidase.

Measurement of antitumor efficacy

Tumor response was assessed directly by the determination of CSF tumor cell counts. CSF tumor cell counts were obtained by averaging the tumor cell counts of three high-power fields after a fixed volume (4 ml) of lumbar CSF was filtered through a 5-μm small Millipore filter (Millipore Corporation, Bedford, MA) (25-mm diameter). Absolute tumor cell counts often exceeded 300 cells/ml. Leptomeningeal neoplasia alters CSF protein, glucose, and LDH isoenzymes, and these parameters were also used as a measure of disease. LDH isoenzyme 1 (LD1) is the predominant form in CSF from healthy patients (44). LDH isoenzyme 5 (LD5) is the most common form to be elevated in malignancy (70). The ratio LD5/LD1 in CSF serves as a marker of leptomeningeal disease when it is greater than 0.15 (62). Indirect measures of tumor response included serial neurological examinations and gadolinium-enhanced MRI scanning of the brain and complete spine.

Detection of anticonjugate antibody formation

Patient serum and CSF before treatment and 1, 2, 3, 6, and 16 weeks after treatment were assayed for antibodies to 454A12-rRA or either of its components (454A12 or rRA) using a secondary radiolabeled antibody in an immunosorbent assay (63).

RESULTS

Eight patients with leptomeningeal spread of systemic neoplasia (six patients with breast carcinomas, one patient with melanoma, and one patient with leukemia) received a total of 10 treatments of intraventricular immunotoxin covering a 1000-fold increase in drug dose from 1.2 to 1200 μg. The patient data is presented in *Table 1*.

Pharmacokinetics

The calculated volumes of distribution of ^{99m}Tc-DTPA and 454A12-rRA ranged from 5.4 to 58.1 ml (*Tables 2* and *3*). ^{99m}Tc-DTPA is an extracellular marker with low capillary permeability, and, therefore, its clearance from CSF approximates bulk CSF flow (47). The early phase (α-phase) half-life of ^{99m}Tc-DTPA in ventricular CSF averaged 49 $\pm$ 19 minutes, and the late phase (β-phase) half-life averaged 304 $\pm$ 78 minutes. In seven of the eight patients, the clearance of ^{99m}Tc-DTPA was less than 18.3 ml per hour (*Table 2*), which is the rate of bulk flow of CSF in healthy patients (65). This finding suggests that there was some obstruction to CSF flow before treatment in most of our patients.

The clearance curves for ^{99m}Tc-DTPA and 454A12-rRA, obtained simultaneously, are illustrated in *Figure 2* for two patients. In all patients tested, the clearance of the immunotoxin was more rapid than the clearance of ^{99m}Tc-DTPA, averaging approximately 2.4-fold greater (*Table 2*). The α-phase half-life of 454A12-rRA in ventricular CSF averaged 44 $\pm$ 21 minutes, and the β-phase half-life averaged 237 $\pm$ 86 minutes. The bioactivity of CSF samples in inhibiting protein synthesis of K562 erythroleukemia cells in culture qualitatively tracks the concentration of immunotoxin. Good inhibi-

TABLE 1. Patients Treated with Intraventricular 454A12-rRA[a]

Patient No.	Diagnosis	Age (yr)	KPS	Previous Therapy	Immunotoxin Dose (μg)
1	Breast cancer	66	40	MRM, 5-FU, RT, IT MTX	1.2
					3.8
					12.0
2	Breast cancer	52	30	MRM, 5-FU, cytoxan, adriamycin, IT MTX, IT thiotepa	38.0
3	ALL	27	80	L-asparaginase, daunorubicin, vincristine, prednisone, mitoxantrone, Ara-C, RT, IT MTX	38.0
4	Breast cancer	44	40	MRM, 5-FU, cytoxan, adriamycin, IT MTX	120.0
5	Breast cancer	63	60	Tamoxifen, IT MTX	120.0
6	Breast cancer	52	40	MRM, 5-FU, RT, tamoxifen, MTX	380.0
7	Melanoma	27	80	Interleukin-2	380.0
8	Breast cancer	58	70	MRM, 5-FU, cytoxan, adriamycin	1200.0

[a] KPS, Karnofsky performance score; MRM, modified radical mastectomy; 5-FU, 5-fluorouracil; RT, radiation therapy; IT, intrathecal; MTX, methotrexate; ALL, acute lymphocytic leukemia; Ara-C, cytosine arabinoside.

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