Fractionated Radioimmunotherapy of B-Cell Malignancies with $^{131}$I-Lym-1

Gerald L. DeNardo, Sally J. DeNardo, Lois F. O’Grady, Norman B. Levy, Gregory P. Adams, and Stanley L. Mills

Departments of Internal Medicine [G. L. D., S. J. D., L. F. O., G. P. A., S. L. M.], Pathology [G. L. D., N. B. L.], and Radiology [G. L. D., S. J. D.], University of California, Davis Medical Center, Sacramento, California 95817

Abstract

Eighteen patients with Stage 4 B-cell malignancies, which were primarily of intermediate or high grade and progressive despite multiple drug chemotherapy and external irradiation, were treated with fractionated doses of $^{131}$I-labeled Lym-1. Lym-1 is an IgG2a monoclonal antibody that was produced by immunizing mice with Raji cell nuclei that originated from a patient with African Burkitt’s lymphoma. Despite advanced disease, 10 of the patients had objective evidence for a complete or partial remission. Toxicity was very modest except in one patient who developed hypotension. Dose-dependent hepatic uptake of Lym-1 was observed in the patients and in BALB/c mice suggesting receptor-mediated recognition of this murine antibody.

Introduction

B-cell malignancies represent monoclonal expansions of neoplastically transformed normal cells. These malignancies are heterogeneous in terms of their biological behavior. Some lead to death within a few months despite aggressive treatment; others, with little or no treatment, exist for decades with few ill effects. A basic problem with B-cell malignancies is to distinguish tumors of varying prognosis. Tumors derived from different types of lymphocytes show clinically significant differences in behavior. The size of the individual tumor cells is of prognostic value. Small cells about the size of a normal lymphocyte suggest a good prognosis whereas larger cells suggest a bad prognosis (1).

The purpose of this publication is to report the results of treatment of patients with advanced, progressive B-cell malignancies, mostly of large cell type. Fractionated doses of $^{131}$I-Lym-1, an IgG2a murine monoclonal antibody that reacts with membrane antigens found on the malignant cells of most patients with these diseases, were used without other cancer therapy.

Materials and Methods

Patient Selection. Eighteen patients with histologically and phenotypically documented B-cell malignancies were studied. Histological classification was according to the Working Formulation of Non-Hodgkin’s Lymphoma for Clinical Usage (2). Three patients were classified as low grade, 9 patients as intermediate grade, and 6 patients as high grade malignancies. All patients had failed conventional therapy, had progressive and measurable disease at the time of accession to the protocol, and provided informed consent in accordance with institutional and regulatory agencies. Patients received no other treatment for their cancer for at least 4 weeks prior to, or during, treatment with Lym-1. Each of the patients had immunopathological evidence for reactivity of their malignant cells with Lym-1. Imaging evidence for tumor uptake of antibody after administration of diagnostic doses of $^{131}$I- or $^{111}$In-labeled Lym-1 was obtained prior to treatment.

Study Design. Each patient had a history, physical examination, radiographic imaging, complete blood count, urinalysis, chemistry panel, and assay for HAMA prior to entry and during the course of the study. The patients were treated with $^{131}$I-Lym-1 in 30–60-mCi doses at 2–6-week intervals until a cycle of 300 mCi was completed or until death or a HAMA response interrupted treatment. Iodine solution was given to block thyroid uptake of $^{131}$I. Sites of tumor were measured by physical examination and radiography. Two perpendicular diameters for each palpable mass were determined with a caliper. The volume of nonpalpable masses was determined by X-ray, computerized tomography, or magnetic resonance imaging. Responses were classified as: complete remission, the absence of demonstrable disease, including negative bone marrow examinations, for at least 4 weeks; partial remission, a decrease in the sum of the products of all tumor dimensions by at least 50%, or of all tumor volumes by at least 70%, for a minimum duration of at least 4 weeks; steady state, any decrease in tumor dimensions of less than 50%, or tumor volume of less than 70%, and an absence of new lesions, lasting for at least 4 weeks; no response, absence of progression and no development of new lesions while on treatment; progression, increase of at least 25% in the size of any one lesion or the development of new lesions.

Pharmacokinetic Studies in Patients. Studies of the pharmacokinetics of Lym-1 was produced in our laboratory from mouse ascitic fluid by precipitation with saturated ammonium sulfate solution and subsequent pH stepwise gradient elution from a protein A-Sepharose affinity chromatographic column (3) or was obtained from Damon Biotech, Inc. (Needham Heights, MA) according to specifications. Each preparation was documented to be virus free in accordance with United States Food and Drug Administration regulations. The hybridoma was originally produced by fusion of splenic lymphocytes from mice immunized with extracted nuclei from cultured Raji cells that originated from a patient with African Burkitt’s lymphoma (4).

Radiodination was performed using chloramine-T and high specific activity sodium $[^{111}]$iodide in 0.05 N sodium hydroxide. Greater than 90% of the radioactivity in the radiopharmaceutical behaved like the antibody by electrophoretic and chromatographic analysis. Immunoreactivity of each preparation was at least 87% that of the unlabeled antibody. The specific activity of the radiopharmaceutical was approximately 5–10 mCi/mg of antibody. Each patient was given 5–50 mg of unconjugated Lym-1 i.v. at a rate of 0.5–2 ml/min after which labeled Lym-1 in the desired amount of radioactivity was administered. All materials were documented to be sterile and pyrogen free.

Toxicity. Complete blood counts, platelet counts, urinalysis, and chemistry panels including renal, hepatic, and electrolyte studies were obtained prior to, 3 days after, and 2 weeks after each treatment. Each patient was clinically evaluated prior to each dose and every 2 weeks thereafter. Vital signs, including pulse, respiration, temperature, and blood pressure, were monitored before and at 15-min intervals for 2 h after administration of the labeled antibody. Serum was assayed for anti-mouse antibodies before each treatment.

Pharmacokinetic Studies in Patients. Studies of the pharmacokinetics of Lym-1 indicated a dose dependency. Four patients with B-cell lymphoma were given 2–4 mCi of $^{131}$I-Lym-1 on each of three occasions within a 1-month interval. On two occasions $^{131}$I was attached to 0.05 or 0.5 mg of antibody. On the third occasion $^{131}$I-Lym-1 was given after unlabeled Lym-1 (4–5 mg) was administered as a preload. In another patient who was in the treatment protocol, the amount of Lym-1 preload was randomly varied between 5, 20, or 50 mg. Blood samples were obtained and counted in a gamma well counter. High performance liquid chromatography (TSK-3000) was performed on each plasma sample in order to size the radioactivity by molecular sieving. Computer modeling corroborated empiric observations that the clearance of radiolabeled antibody from the blood was prolonged when unlabeled antibody was administered prior to the radiolabeled antibody (5).

The abbreviation used is: HAMA, human anti-mouse antibodies.

1 Presented at the "Second Conference on Radioimmunodetection and Radioimmunotherapy of Cancer," September 8–10, 1988, Princeton, NJ. Supported by Department of Energy Grant DE FG03-84ER60233 and NIH Grant NIH PHS CA 47829.

2 To whom requests for reprints should be addressed, at University of California, Davis Medical Center, Section of Radiodiagnosis and Therapy, 4301 X St., FOLB II E, Sacramento, CA 95817.

3 The abbreviation used is: HAMA, human anti-mouse antibodies.
mg of unlabeled antibody as preload were determined to provide the desired pharmacokinetic behavior for the treatment protocol.

**Pharmacokinetic Studies in Mice.** The pharmacokinetic behavior of 0.2 or 20 µg doses of 131I-labeled Lym-1 was studied in nontumored BALB/c mice (mean weight, 24 g). Immunoreactivity of the radiopharmaceutical was 65% of that of unlabeled Lym-1. Blood and liver samples were taken at 1, 24, 48, 72, and 120 h after injection were counted in a gamma well counter to determine the percentage of the injected dose. Radioactivity in the blood and extravascular compartments of the liver was estimated and subtracted from the total hepatic radioactivity to obtain the radioactivity actively accumulated by the liver (6-8). The radioactive content in the hepatic blood of each group of mice was determined using the measured weight of the liver, the measured concentration of radioactivity in the peripheral blood, and the assumption that these mice had hepatic blood volumes equivalent to those published by Dittmer (7) from observations made with labeled RBC and albumin in mice. The radioactive content of the hepatic extravascular compartment was obtained by assuming that the ratios of the extravascular and intravascular compartments of the liver were equivalent to those of the entire mouse. The slow phase of the blood clearance curve was extrapolated to zero time in the conventional manner in order to obtain the ratio of the extravascular and intravascular compartments. This ratio was used to operate on the blood clearance curve in order to obtain concentration of radioactivity in the extravascular compartment at each point in time. While these calculations involved assumptions that may not be completely accurate, the methods are conventional and any errors apply equally to both groups of mice.

**Results**

Ten of the 18 patients had a complete or partial remission; 2 of these patients had a complete remission. Three patients had a steady state despite suboptimal therapy because treatment was interrupted by serious infection or renal failure. One patient was classified as having progressed because one lesion progressed and his health generally deteriorated despite regression of other lesions during the cycle of treatment. Four patients did not respond, but each received only one treatment dose of 131I-Lym-1, and death or a HAMA response interrupted treatment.

Radiation toxicity occurred in only two patients and was manifested by thrombocytopenia of 1–2 months duration without bleeding. A fistula occurred in two patients. Surgical correction was required in one, and subsequent pulmonary infection led to death in the other. The most serious reaction to the antibody was transient hypotension in one patient. Two patients developed a HAMA response that led to interruption of treatment. Both of these patients had almost completed the treatment cycle and had achieved a partial remission. In another instance HAMA occurred after a single dose of 131I-Lym-1 in a patient who died shortly afterwards from lymphomatous renal failure. Transient fever and chills occurred on five occasions and rash on one occasion but were readily managed. An inflammatory reaction over sites of superficial tumor first appeared 4–6 h after treatment and lasted for 24–48 h in five patients.

Blood clearance was not altered in patients by increasing the amount of administered antibody from 0.05 to 0.5 mg (Fig. 1). The blood clearance of the labeled Lym-1 was rapid and associated with substantial liver uptake unless unlabeled Lym-1 was administered as a preload. Blood clearance and liver uptake were profoundly decreased by preload administration of mg amounts of antibody. However, an increase in the preload from 5 to 50 mg did not further alter the blood clearance (Fig. 2). We also observed that the blood clearance was influenced in the extreme by the volume of tumor that was present (Fig. 3).

Blood clearance of labeled Lym-1 in mice that were given 20 µg of Lym-1. High performance liquid chromatography of the plasma indicated that the radioactivity eluted at a size corresponding to 150,000 daltons. The fraction of the 0.2-µg dose that was actively accumulated by the liver was greater than that observed for the 20-µg dose at all times (P > 0.001) (Fig. 4).

**Discussion**

B-cell lymphomas and leukemias are relatively common malignancies and the malignant cells are more accessible to i.v. administered antibody than for many solid cancers. These cells are known to be radiosensitive, so that the rapid and substantial regression of tumors observed in our patients is not surprising. While treatment was interrupted by HAMA response in several instances, the frequency of this complication was not high and
also true of a sixth patient in whom a HAMA response led to interruption of treatment after a single dose.

The pharmacokinetic behavior of some murine monoclonal antibodies has been observed to vary in patients with the amount of administered antibody (5, 9–11). It has been assumed that this reflects human recognition of the foreign mouse protein. We found that the amount of administered Lym-1 antibody influenced pharmacokinetic behavior in patients and also in mice of the same species in which the antibody was produced. The major differences occurred in the blood and the liver of both patients and mice (12). Active and rapid uptake of antibody by the liver occurred when a small amount of Lym-1 was administered. Dose-dependent recognition of murine monoclonal antibody both by patients and by the same mouse species in which it was produced suggests a receptor-mediated mechanism. Furthermore, it is interesting to observe that this recognition phenomenon occurred in mice and patients with comparable amounts of antibody when adjusted for weight. The amount of antibody that was required was less than that reported for some other antibody systems (9–11). While observation of a dose-dependent antibody influence on blood clearance hepatic uptake in patients is not unique, it has not been described in mice by others.

References

Fractionated Radioimmunotherapy of B-Cell Malignancies with \textsuperscript{131}I-Lym-1

Gerald L. DeNardo, Sally J. DeNardo, Lois F. O'Grady, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/3_Supplement/1014s