High Dose Radiolabeled Antibody Therapy of Lymphoma


Abstract

A trial has been initiated testing the effects of high dose radiolabeled monoclonal antibody administered in conjunction with marrow transplantation for treatment of lymphoma. This study is based on observations in mice demonstrating that radiolabeled antibody against a normal lymphocyte-associate antigen can induce regression of lymphoma masses. These preclinical studies also showed that large amounts of antibody are needed to achieve adequate biodistribution in vivo and that potentially curative doses of radionuclide induce substantial hematopoietic toxicity. Consequently, in patients with recurrent lymphoma, we are first evaluating the influence of dose on the biodistribution of a pan B-cell antibody, MB-1 (anti-CD37). In four patients, the biodistribution studies indicated that at the highest amount of antibody tested $^{131}I$-labeled antibody MB-1 (10 mg/kg) could deliver more radiation to tumor than to normal organs. These patients were treated with antibody MB-1 labeled with 250 to 482 mCi $^{131}I$ estimated to deliver 380 to 1570 cGy to normal organs and 850 to 4260 cGy to tumor. Myelosuppression occurred in all patients and required infusion of cryopreserved marrow in one patient. Complete tumor regressions were observed in each patient. In three other patients with splenomegaly and/or large tumor burdens, biodistribution studies indicated that $^{131}I$-labeled antibody could not deliver more radiation to tumor than to normal organs and these patients were not treated. Thus, tumor burden and spleen size may determine the feasibility of treatment with radiolabeled antibody. Treatment with this antibody labeled with high doses of $^{131}I$ was well tolerated and may prove therapeutically useful. These studies are being continued to determine the maximal doses of radiation that can be tolerated by nonhematopoietic tissues after infusion of $^{131}I$-labeled antibody.

Previous studies in experimental and clinical settings have demonstrated some therapeutic benefit by using monoclonal antibodies against normally occurring differentiation antigens to treat malignancy (1–5). Most responses have been minimal or transient, consistent with our results in murine studies demonstrating that unmodified antibody against a normal differentiation antigen can eliminate limited numbers of lymphoma cells (6). While the factors leading to success or failure of antibody therapy are not completely understood, the limited effectiveness has been attributed at least to two major factors: (a) antibodies require participation of host effector mechanisms which appear limited in their ability to eliminate antibody coated tumor cells (6–9); (b) tumors are heterogeneous in their expression of target antigens, and antibody therapy has resulted in the emergence of variant cells that lack the target antigen (2, 6, 10). Both of these limitations may be overcome by the use of radiolabeled monoclonal antibodies which can be directly cytotoxic. Since cell killing results from emitted radiation, a radionuclide antibody conjugate bound to the surface of one cell may also kill adjacent cells lacking the target antigen.

We have recently initiated a trial of high dose radiolabeled antibody therapy of human lymphoma, a disease that is known to be radiosensitive. Treatment with 1000–1575 rad total body irradiation with or without chemotherapy followed by bone marrow transplantation can cure a significant portion of lymphoma patients, although most relapse (11–13). Consequently, an aim of our clinical studies is to use radiolabeled antibodies to deliver higher doses of radiation to tumor without increasing irradiation to critical normal tissues. Expected hematological toxicity can be overcome by infusing cryopreserved autologous marrow.

Our approach stems from murine studies demonstrating that radiolabeled antibody against a normal lymphocyte-associated antigen can be therapeutically useful (14, 15). These studies suggested that (a) relatively high doses of antibody would be needed to achieve adequate biodistribution in vivo and (b) potentially curative doses of radionuclide would induce substantial hematopoietic toxicity. These studies also demonstrated elimination of antigen-negative variant tumor cells that are present within a lymphoma mass. This observation favors the use of immunoconjugates prepared with radionuclides rather than cytotoxic drugs or toxins. In the latter situations, heterogeneity of antibody binding would severely limit overall efficacy. In recent clinical trials the ability of radiolabeled antibody to induce regression of lymphomas and hepatomas has been demonstrated (16–20).

On the basis of these studies, we have initiated studies of radiolabeled antibody therapy in humans. We have first evaluated the influence of the antibody dose on biodistribution to tumor and normal tissues. Second, patients in whom antibody can deliver greater amounts of radiation to tumor than to normal tissue have been treated with high doses of $^{131}I$-labeled antibody. These radionuclide doses were expected to induce hematological toxicity which can be overcome by infusion of cryopreserved marrow purged of lymphoma cells. By escalating the radiation doses delivered $^{131}I$ the dose-limiting toxicity of $^{131}I$-labeled antibody in nonhematopoietic tissues can be determined.

In this report, we review our studies in mice and then describe the initial results of our trial using $^{131}I$-labeled antibodies to treat lymphoma in humans. Results of the clinical trial will be reported in detail elsewhere.

Murine Studies

We have used murine monoclonal antibodies reactive with murine lymphoma cells to treat transplanted T-cell lymphomas in settings where the antibody was tumor specific and where it reacted with normal T-cells in addition to tumor. To examine tumor-specific antibodies, we used $^{131}I$-labeled murine monoclonal antibodies which react with the Thy-1.1 antigen to treat established AKR/J Thy-1.1+ tumor masses in congenic AKR/Cum (Thy-1.2+) mice (14). These studies were designed so that the infused Thy-1.1 monoclonal antibody would react solely...
with the Thy-1.1-bearing tumor cells and not with the normal T-cells of AKR/Cum mice which are Thy-1.2+. In these biodistribution experiments, $^{311}$I-anti-Thy-1.1 antibody specifically localized to a s.c. tumor with a mean of 6.5% of the infused dose measured per g of tumor at 24 h after infusion (compared to a mean of 1.5% for the control antibody). Concentrations of $^{131}$I-anti-Thy-1.1 antibody that were achieved in tumor were calculated to result in delivery of a mean of 1600 rad following infusion of 500 $\mu$Ci of $^{131}$I-labeled anti-Thy-1.1 antibody. Estimated doses to normal tissues ranged from 250 to 675 rad. In comparison, 500 $\mu$Ci $^{131}$I-labeled irrelevant antibody was estimated to deliver a mean of 380 rad to tumor.

In therapeutic studies, infusion of 500–1000 $\mu$Ci of $^{131}$I-labeled antibody induced complete or partial regressions of a palpable lymphoma mass (0.5–1 cm diameter) in 36 of 61 mice. Under these conditions unmodified antibody was ineffective (as was $^{131}$I-labeled irrelevant antibody). Hematopoietic suppression was dose limiting because animals receiving more than 1000 $\mu$Ci died of bone marrow aplasia and infection.

In order to examine antibodies reactive with a normal differentiation antigen, we used the same $^{131}$I-labeled anti-Thy-1.1 antibody but treated AKR/J (Thy-1.1+) mice bearing the syngeneic SL2 (Thy-1.1+) lymphoma (15). In this setting, infusion of the same dose of radiolabeled antibody led to relatively lower concentrations of $^{131}$I-labeled antibody within the tumor because of binding to splenic T-cells. In order to inhibit this initial binding to splenic lymphocytes, mice were pretreated with varying amounts of unlabeled antibody and, 24 h later, were treated with labeled antibody. With this approach it was possible to achieve antibody biodistribution that approximated that seen in the AKR/Cum mice as described above. Specifically, an infusion of 1000 $\mu$g unlabeled antibody followed by an infusion of 500 $\mu$g antibody labeled with 1500 $\mu$Ci resulted in delivery of an estimated mean dose of 2400 rad to tumor compared to 750–1350 rad to normal organs and induced regression of tumor nodules in 14 of 17 mice. Again, hematological toxicity was limiting and all mice died with marrow aplasia. Thus, radiolabeled antibodies against differentiation antigens may be useful for therapy in spite of binding to normal cells. In addition, since they bind to normal lymphocytes, radiolabeled antibodies may also eliminate small numbers of lymphoma cells present within an area of normal lymphoid tissue.

Tumor Cell Variants That Lack the Target Antigen. We have also demonstrated that $^{131}$I-labeled anti-Thy 1.1 antibody could deliver therapeutic amounts of radiation to antigen negative variant cells within the tumor mass. This ability was demonstrated by treating mice inoculated with a mixture of $2 \times 10^7$ SL-2 (Thy-1.1+) cells plus $2 \times 10^7$ Thy-1.1− variant SL-2 cells, a number of variant tumor cells capable of establishing tumors in vivo. Infusion of 1000 $\mu$Ci of $^{131}$I-labeled anti-Thy-1.1 antibody led to elimination of all viable tumor cells in three of the five mice inoculated with the mixture. In contrast, treatment with radiolabeled control antibody did not induce regressions of the tumor cell mixture, nor did treatment with radiolabeled anti-Thy-1.1 antibody induce tumor regressions in animals inoculated with $2 \times 10^7$ antigen-negative cells in the absence of SL-2 cells. Thus, $^{131}$I-labeled antibodies have the potential for eliminating antigen negative cells within a tumor mass.

Taken together, these results provided a major impetus for clinical trials using radiolabeled antibodies that react with both normal and malignant lymphocytes to treat patients with lymphoma. Furthermore, they demonstrated the importance of antibody dose and the necessity of using high doses of $^{131}$I to obtain maximal therapeutic effects. Finally, they indicated that substantial hematological toxicity will occur following high dose treatment with radiolabeled antibody.

Clinical Studies

We are evaluating patients with recurrent B-cell lymphoma as outlined in Table 1. The antibody used (MB-1) is an IgG1 which recognizes CD37, a M, 42,000 glycoprotein expressed mainly on normal B-cells and most lymphoma specimens (20). The clinical study involves two parts. In the first, the biodistribution of different doses of trace labeled antibody is evaluated at serial time points. In the second, patients estimated to receive a greater amount of radiation to tumor than to normal organs are treated with antibody labeled with high doses of $^{131}$I designed to deliver a prescribed amount of radiation to normal organs. These doses to normal organs are to be escalated during the study. Each of these phases are discussed separately below. As shown in Table 1, marrow is first cryopreserved in anticipation of hematological toxicity due to the radionuclide.

Biodistribution Study. To study the influence of antibody dose on biodistribution, up to three studies at weekly intervals are performed following infusion of 0.5, 2.5, or 10.0 mg/kg of antibody trace labeled with $^{131}$I. As a control, $^{131}$I labeled antibody of irrelevant specificity but identical isotype is also infused. Biodistribution is then evaluated by assessment of blood and urine clearance, by image analysis of normal organs and tumor, and by tumor biopsy where possible. Image analysis is obtained daily and involves generation of region of interest time-activity curves over each major organ and tumor mass. For calculating dosimetric estimates, organ radioactivity uptake is determined by quantitative planar imaging from opposing view (21). Daily whole body counts are also obtained for assessment of whole body disappearance of radioactivity.

Studies of seven patients have shown that the distribution of antibody is influenced by both the dose infused and the presence and size of the spleen and tumor mass. The importance of antibody dose is perhaps best illustrated in one of the asplenic patients. The clearance of $^{131}$I-labeled antibody MB-1 from the blood was rapid as compared to control antibody at the low dose but approximated that of the control antibody at the highest dose (Fig. 1). Imaging studies revealed rapid uptake by liver, lung, and kidney and a clearance rate approximating that in blood at each dose (Fig. 2). Tumor uptake occurred at a slower rate than organ uptake. At the lowest antibody doses, label cleared from tumor at a rate approximating that seen in organs. In contrast, at the highest antibody doses, prolonged retention of label in tumor was observed. A similar result was observed in the second asplenic patient.

These observations suggest that infusion of antibody doses sufficient to maintain blood concentration over time is required to achieve adequate penetration and retention of antibody in tumor. The rapid clearance from blood of $^{131}$I-labeled MB-1 antibody compared to the $^{131}$I-labeled control antibody at low doses likely reflects binding to easily accessible antigen-bearing cells. When this compartment was essentially saturated, MB-1...
clearance in blood approached that of the control antibody allowing penetration and retention of antibody in tumor.

Studies in three patients with massive splenomegaly and a relatively large tumor burden showed that antigen load may influence antibody biodistribution. In these two patients, clearance of MB-1 from blood was more rapid than that of the control antibody even at the highest dose tested, and retention of label in tumor was not observed. In two other patients with slightly enlarged spleens, clearance of MB-1 at the highest dose did approximate that of control and tumor uptake and retention was observed. In addition to these seven patients, the biodistribution studies were discontinued in an eighth individual who experienced thrombocytopenia after the initial antibody infusion.

Dosimetric estimates were made for each patient by using the Medical Internal Radiation Dose Committee (MIRD) formulation in which the values were adjusted for each organ based on computerized tomographic measured volumes (22). In four of the patients, dosimetric estimates suggested that $^{131}$I-MB-1 would deliver more radiation to tumor than to normal organs. In the three patients with massive splenomegaly, the biodistribution studies did not indicate that antibody would deliver more radiation to tumor than normal organs and the patients were not treated.

Phase I Trial of Radiolabeled Antibody Therapy

In this part of the study, patients are treated with the amount of antibody estimated to deliver the greatest amount of radiation to tumor compared to liver, lung, or kidney. This amount of antibody is labeled with an amount of radionuclide ($^{131}$I) estimated to deliver a set radiation dose to normal organs, with the plan of escalating this radiation dose in groups of three patients until dose-limiting toxicity is observed. As noted earlier, the expected first toxicity, myelosuppression, is not considered to be limiting since this can be overcome by infusing cryopreserved...
autologous marrow. Whether the second dose-limiting toxicity will be gastrointestinal, pulmonary, or otherwise remains to be determined.

Patients were treated with a single dose of antibody MB-1 labeled with 250 to 482 mCi $^{131}$I estimated to deliver 850–4260 rad to tumor and up to 1000–1500 rad to liver, lung, or kidney. Table 2 shows the estimated radiation doses per infused mCi for various tissues in a patient who was treated with 482 mCi of $^{131}$I bound to a 10-mg/kg dose of antibody MB-1. Toxicity was limited mainly to the hematopoietic system with each patient exhibiting significant myelosuppression. The peripheral blood counts of the patient illustrated above are shown in Fig. 3. After reaching the nadir in the granulocyte and platelet counts, there was spontaneous recovery of hematopoietic function. Importantly, there was no significant nonhematological toxicity.

While the objective of this study was to obtain phase I toxicity data, the patients studied to date have experienced complete responses, although two cases have recurred at 4 and 6 months after treatment. This result is encouraging since the doses of $^{131}$I-labeled antibody used thus far have not caused toxicity other than expected marrow suppression. As the dose of $^{131}$I-labeled antibody is escalated, we expect that infusion of cryopreserved marrow will be necessary for hematopoietic recovery and the duration of complete responses is expected to increase.

Conclusions

Our studies in humans have demonstrated the important influence of antibody dose on in vivo biodistribution in both tumor and normal tissues. Amounts of antibody that maintain serum concentrations at adequate levels were necessary to overcome rapid binding to accessible normal and malignant B-cells and to achieve adequate penetration and retention of antibody in tumor. When this occurs, equivalent or greater amounts of radiation were able to be delivered to tumor as compared to liver, lung, and kidney.

Treatment of patients with antibody labeled with relatively high amounts of $^{131}$I induced no significant toxicity other than myelosuppression. With autologous bone marrow support this hematological toxicity is not dose limiting and significantly higher amounts of $^{131}$I can be infused. Moreover, nonhematopoietic tissues may tolerate higher doses of radiation when delivered from radiolabeled antibodies than from external beam radiation because the radiation is delivered at a low dose rate (1–2 cGy/min for radiolabeled antibody compared to 8–200 cGy/min for external beam therapy). Such low dose rates may preferentially spare nonhematopoietic tissues which can repair radiation damage in a manner similar to conventional fractionation schemes. The cytotoxic effect on hematopoietic tissues and tumor cells should be maintained because they lack substantial repair capacity. Thus, our future studies will determine the dose limit of radiation delivered by $^{131}$I-labeled antibody to nonhematological tissues and define the nature of the toxicity. When the maximum tolerated doses of radiation delivered by $^{131}$I-labeled antibodies are determined, phase II studies can be undertaken to determine the therapeutic value of this approach.

References

HIGH DOSE RADIOLABELED ANTIBODY THERAPY OF LYMPHOMA


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Cancer Res 1990;50:1017s-1021s.

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