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Advances in Brief

Effective α-Particle-mediated Radioimmunotherapy of Murine Leukemia[1]

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Abstract

The specificity, toxicity, and efficacy of α-particle-mediated radioimmunotherapy of murine erythroleukemia was assessed by use of tumor-specific monoclonal antibody 103A labeled with ^{212}Bi. Forty % of the injected dose/g tissue targeted to neoplastic spleens within 1 h after i.v. injection. When ^{212}Bi-103A was injected on day 13 of disease, a dose-dependent response was achieved, as measured by a reduction in splenomegaly and absence of liver metastasis. Mice treated with ^{212}Bi-103A on day 8 of disease showed no histological evidence of erythroleukemia on day 22 and survived significantly longer (median, 118 days) than mice treated with ^{212}Bi-control IgG (78 days) or untreated mice (63 days), indicating successful specific radioimmunotherapy.

Introduction

Conventional radiation therapy does not use α-particles in cancer treatment because of their scanty penetration into tissues (1). Yet it is precisely this limited radius of effectiveness which makes α-emitters potentially the tumoricidal agent of choice when mAb[3] (2) are used for systemic radioimmunotherapy of vascularized tumors like leukemia (3). Decay of ^{212}Bi produces highly cytotoxic, densely ionizing, high-energy radiation over a 40–100-μm range, limiting cellular destruction to an extremely small area. The short half-life (60.6 min) also minimizes systemic toxicity, and availability from a ^{224}Ra generator facilitates potential clinical use of the isotope (4). Because of the instability of metal chelates heretofore used for labeling (5), radioimmunotherapy with ^{212}Bi-mAb conjugates has been limited to *in vitro* experiments (6) and to *in vivo* delivery systems such as intralymphatic infusion or i.p. injection that are designed to avoid the circulatory system (7–9). Currently, use of the 2-(*p*-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid ligand known to form stable bismuth complex *in vivo* was prevented by slow complex formation kinetics with $^{212}BiI_4^-$ in established labeling procedures (5). To obviate these problems, we have prepared a novel isothiocyanatobenzyl derivative of CHX-A-DTPA, which both rapidly sequesters bismuth and is stable *in vivo* (10, 11). In the present study we have used the Rauscher murine erythroleukemia (12) and mAb 103A directed against its cell surface-associated antigen in BALB/c mice as a model (13–17) for ^{212}Bi-radioimmunotherapy. When untreated, the disease induced by the RLV progresses rapidly, with microscopic foci found in the spleen within a few days and macroscopic foci visible after 6–8 days. By day 20 of the disease, severe splenomegaly with liver and bone metastasis is evident; death due to anemia, splenic rupture, infection, or metastatic disease follows near day 60 (12, 16, 17). We have previously demonstrated that mAb 103A targets *in vivo* to a RLV envelope glycoprotein (gp70) expressed on the surface of RLV-infected cells (5, 13–17). In some of these experiments bovine IgG was used as a control because it does not bind to normal or erythroleukemic cells and it has a tissue distribution over 24 h that is equivalent to that of mAb 103A (5, 16, 17). We now report that in this Rauscher leukemia virus system it is possible to achieve complete clinical and histological remission of disease and prolongation of survival using ^{212}Bi-labeled 103A delivered via the circulatory system.

Materials and Methods

CHX-A-DTPA Conjugation and Labeling of mAb 103A with Bismuth. mAb 103A (13) and nonspecific bIgG were conjugated to CHX-A-DTPA as previously described for other DTPA derivatives (5). The chelate-conjugated antibody was labeled with ^{212}Bi as described previously (5), and a specific activity of 3 μCi/μg was achieved. The preparation was judged to be free of longer-lived radionuclides (^{224}Ra and ^{212}Pb, precursors of ^{212}Bi), because no radioactivity was detected in the protein at 24 h after labeling. Biodistribution experiments used a longer-lived tracer, ^{206}Bi, at a 3:2 molar ratio with ^{205}Bi; these isotopes have physical half-lives of 15.3 and 6.2 days, respectively.

CHX-A-DTPA Conjugation, Measurement of Retention of Activity of Chelate-conjugated Antibodies, and Labeling with Bismuth. To assess the effect of chelation the CHX-A-DTPA mAb and CHX-A-DTPA bIgG were iodinated and injected into normal and day 13 leukemic mice essentially as described previously (14). The spleens were removed 30 min later and the cells were isolated. Spleen cells were washed 3 times with 10 ml of 20 mM sodium phosphate, pH 7.4, containing 150 mM NaCl and 0.2% bovine serum albumin and then counted in a microcytometer. The radioactivity in the cells was measured in an LKB-gamma counter. The uptake ratio was defined as the cpm bound to 1×10^8 leukemic spleen cells divided by the cpm bound to 1×10^8 normal spleen cells.

Targeting and Biodistribution of Radiolabeled Antibody. *In vivo* stability of the radioimmunoconjugate was accessed by analyzing its biodistribution compared to that of free bismuth and mAb 103A in normal and leukemic mice using the longer-lived tracer, ^{206}Bi. Normal and 13-day leukemic mice were given injections in the tail vein of 3 μg ^{206}Bi-labeled mAb 103A. The biodistribution of the radiolabeled preparation was determined by removing blood, spleen, kidney, liver, lung, heart, bone, brain, stomach, intestine, and muscle and counting the tissue samples directly in the gamma counter at various time points after injection. Results were expressed as percentage of ID/g.

Toxicity. Groups of normal and 13-day leukemic mice were given i.v. injections of up to 200 μCi of ^{212}Bi-labeled 103A. Toxicity was assessed on day 22 of the disease by histological examinations of kidney, liver, spleen, thymus, lymph node, and bone marrow, looking for signs of cellular damage and repair. The body weight reduction observed in the leukemic mice was also compared to that seen for the controls as evidence of toxicity.

Radioimmunotherapy. Groups of 8- or 13-day leukemic mice were given i.v. injections of varying doses of ^{212}Bi-labeled 103A. Therapeutic efficacy was assessed on day 22 of the disease by determining the reduction in splenomegaly in the treated leukemic mice as compared to

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[3] The abbreviations used are: mAb, monoclonal antibodies; RLV, Rauscher leukemia virus; bIgG, bovine immunoglobulin G; CHA-A-DTPA, *trans*-cyclohexyldiethylenetriaminepentaacetic acid; ID, injected dose.

untreated leukemic and normal mice. Spleen and liver histological sections from treated and untreated leukemic mice were also evaluated for microscopic tumor foci, as described below.

The long-term efficacy of therapy was assessed in 8-day leukemic mice treated with 150 μCi ^{212}Bi-103A or 150 μCi ^{212}Bi-labeled nonspecific antibody. The length of survival of the treated mice and untreated leukemic mice was compared.

Histopathology. Organs were fixed in 10% buffered formalin and embedded in Tissue Prep 2. Tissue sections (6 μm) were stained with hematoxylin and eosin and evaluated without knowledge of treatment status. Spleens were rated on a scale of 1 to 5 for evidence of tumor foci, extramedullary hematopoiesis, and lymphoid follicular atrophy. Evidence of liver metastasis was assessed on a similar scale.

Results and Discussion

Conjugation of 103A and bIgG with CHX-A-DTPA resulted in the attachment of 1.03 molecules per antibody. The addition of CHX-A-DTPA did not significantly affect tumor uptake. At the cellular level, uptake ratios (CHX-A-DTPA 103A bound to leukemic cells as compared to that bound to normal spleen cells) of 14:1 were achieved at 30 min postinjection. This tumor-targeting activity was similar to that previously shown for other chelators conjugated to 103A (5, 13, 16).

To assess the *in vivo* stability of our radioimmunoconjugate we used ^{206}Bi as a tracer for the short-lived ^{212}Bi and compared the biodistribution in normal mice of ^{206}Bi-CHX-A-DTPA-103A, free bismuth, and mAb 103A biosynthetically labeled with ^{35}S. ^{206}Bi rapidly cleared the blood and localized in the kidney. ^{35}S-labeled 103A showed, as expected (5), high levels in the blood and concomitant increases in highly vascularized tissues, such as liver, heart, lung, and kidney. The biodistribution of ^{206}Bi-CHX-A-DTPA-103A was indistinguishable from that observed for ^{35}S-labeled 103A, indicating that the conjugated mAb 103A was stable and that free radiometal was not released *in vivo* (Table 1).

Tumor targetings of ^{35}S-labeled 103A, radioiodinated CHX-A-DTPA-103A, and ^{206}Bi-CHX-A-DTPA-103A were compared to assess the retention of biological activity by the CHX-A-DTPA-modified mAb. In the case of ^{206}Bi-CHX-A-DTPA-103A, 40% of the ID/g localized to tumor-bearing spleens at 1 h after injection, and this level increased with time to 63 and 77% at 6 and 24 h, respectively (Table 1). Very similar results were observed for the ^{35}S- and radioiodine-labeled preparations. In normal mice, the ID/g did not exceed 10% in the spleens at any time. These results demonstrate that the antibody specificity and activity were retained compared to unconjugated antibody. For therapy, a radionuclide must ideally remain linked to its protein carrier for at least five half-lives. The stability we observed *in vivo* for ^{206}Bi-CHX-A-DTPA-103A far exceeded 5 h, predicting that ^{212}Bi-CHX-A-DTPA-103A (^{212}Bi-103A) would effectively deliver therapeutic doses of ^{212}Bi to the tumor.

Table 1 *Targeting of ^{206}Bi-CHX-A-DTPA-103A*

Results are expressed as the mean of the percentage of injected dose per g of tissue (%ID/g) 15 min and 1, 6, or 24 h after i.v. injection of 1.2 μCi of ^{206}Bi-CHX-A-DTPA-103A into groups of 3 normal or 13-day leukemic mice.

	Normal mice				Leukemic mice			
Tissue	15 min	1 h	6 h	24 h	15 min	1 h	6 h	24 h
Spleen	9.4	7.9	7.5	5.9	25.4	39.9	63.0	77.4
Blood	57.3	37.2	34.5	22.4	45.6	40.4	27.4	16.2
Kidney	12.7	11.0	11.4	11.5	10.5	13.2	10.0	9.1
Liver		12.0		9.3		9.1	8.3	6.4
Lung		18.1		8.2		17.4	8.9	6.4
Heart		10.4		8.0		9.5	7.1	4.9
Bone		2.9		3.0		5.8	5.4	4.3
Brain		0.7		0.5		1.7	1.1	0.9
Stomach		1.8		1.4		1.7	2.1	1.7
Intestine		2.1		2.3		3.3	3.4	2.6
Muscle		0.8		1.7		1.1	1.1	1.3

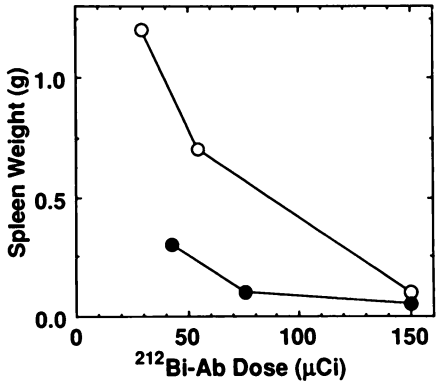

Fig. 1. Dose response of erythroleukemic mice to ^{212}Bi-labeled antibody at day 13 of the disease as measured by reduction in splenic weight. Female BALB/c mice, 5 to 6 weeks of age, were infected i.v. with 250 μl of 1:60 dilution of a 20% suspension of Rauscher leukemia virus. Mice received i.v. injections of ^{212}Bi-CHX-A-DTPA-103A (●) or ^{212}Bi-CHX-A-DTPA bovine IgG (○). Sacrifice was performed on day 22 of disease. *Points*, mean of values from 4 or 5 mice. Doses are precise to within ±10%.

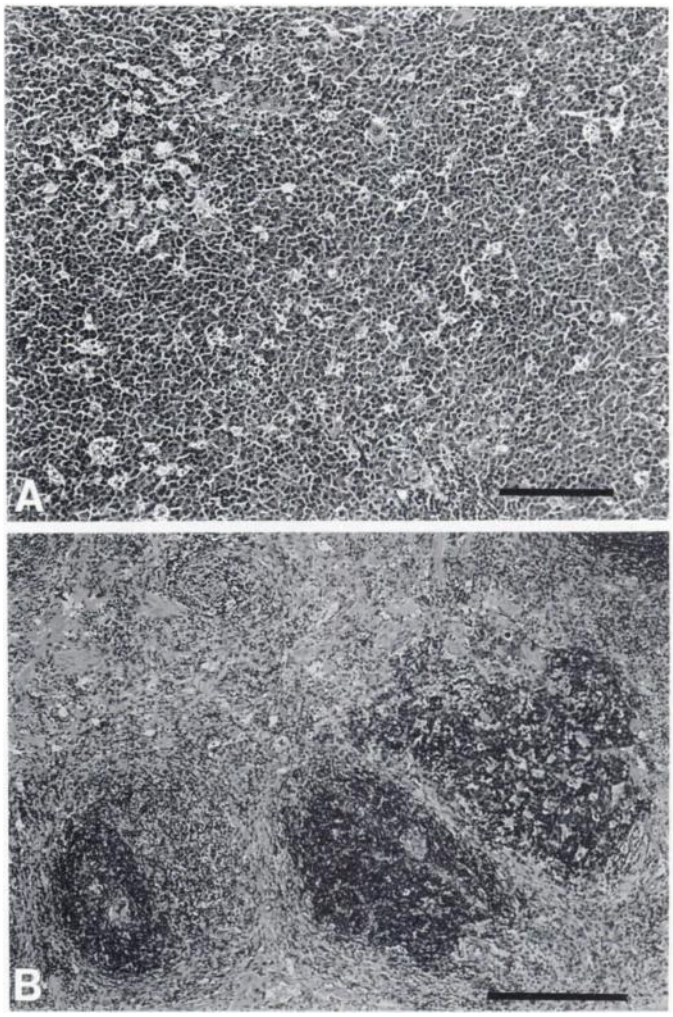

Fig. 2. Histopathology of spleens from treated and untreated erythroleukemic mice at day 22 of disease. *A*, spleen from an untreated mouse. *Bar*, 50 μm. There is marked atrophy of lymphoid tissue and a monotonous infiltrate of erythroleukemic cells. *B*, spleen from a mouse treated with 150 μCi ^{212}-Bi-CHX-A-DTPA-103A. *Bar*, 100 μm. Note the normal lymphoid follicles and absence of erythroleukemic cells. H & E.

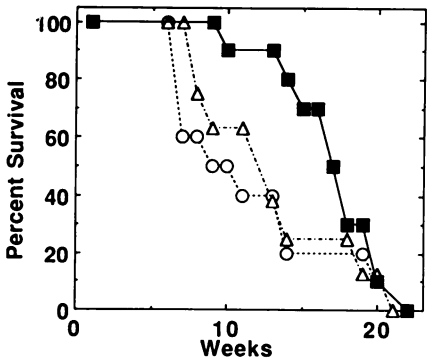

Fig. 3. Survival time of erythroleukemic mice treated on day 8 of disease. Mice were infected with Rauscher leukemia virus as described in Fig. 1. Mice received a single i.v. dose of 150 μCi ^{212}Bi-CHX-A-DTPA-103A (■) or 150 μCi ^{212}Bi-CHX-A-DTPA-bovine IgG (△) or were left untreated (○). At the time of death, complete pathological examinations were performed. Respective median survival times were 118, 78, and 63 days.

Therapeutic response was evaluated by assessing the reduction in tumor, as indicated by spleen weight and by histological examination of spleen, lung, liver, and lymph nodes. Mice at day 13 of the disease were given injections of 40, 75, or 150 μCi of ^{212}Bi-103A or ^{212}Bi-control IgG (nonspecific bovine IgG). At day 22 of the disease, mice were sacrificed and the tumor burden was assessed. Doses of 40 and 75 μCi of ^{212}Bi-103A were highly effective in reducing splenic tumor growth, as compared to the control ^{212}Bi-IgG. With 150 μCi of either specific or control IgG, a reduction in splenic weight was observed (Fig. 1). However, whereas there was no histological evidence of liver metastasis in mice treated with 150 μCi of specific ^{212}Bi-103A, metastases were present in mice treated with the same dose of control ^{212}Bi-IgG. Histological evaluation of spleens treated with 75 and 150 μCi of ^{212}Bi-103A showed near-normal architecture, although several leukemic foci were present (Fig. 2).

Similar experiments were carried out using leukemic mice at day 8 of the disease, when the tumor burden is 50-fold lower. When evaluated at day 22 of the disease, mice treated with 150 μCi ^{212}Bi-103A showed no histological evidence of erythroleukemia, and splenic weights were indistinguishable from those of normal mice.

We assessed toxicity histologically in normal and erythroleukemic mice, directing particular attention to the kidneys and bone marrow. There was no histological evidence of toxicity in animals receiving doses as high as 200 μCi of ^{212}Bi-103A. In addition, there was no difference in the body weights of the treated and untreated mice. Therapy was not dose limited by myelosuppression, as has been observed for other radionuclides (16, 17).

To assess clinical efficacy, including remission of disease, we carried out a long-term survival study. Groups of mice at day 8 of the disease were treated with 150 μCi ^{212}Bi-103A or ^{212}Bi control IgG or left untreated. Median survival times for mice given ^{212}Bi-103A were nearly twice those of untreated controls (118 days *versus* 63 days) ($P < 0.01$), whereas the difference in median survival between mice treated with control IgG and untreated mice (78 *versus* 63 days) was statistically insignificant (Fig. 3). Postmortem examinations of all mice showed evidence of erythroleukemia.

We have demonstrated that clinical and histological remission of erythroleukemia and prolongation of survival can be achieved using specific antibody labeled with α-emitting ^{212}Bi. The recurrence of erythroleukemia in our animal model was expected, since the disease is induced by persistent viral infection and achieving a complete cure requires elimination of both erythroleukemic cells and infective virus (18). The efficacy, lack of toxicity, and high therapeutic index of these preparations suggest that in human leukemias or lymphomas where rapid delivery of radiolabel to target cells is possible (19), effective therapy may be achieved with such ^{212}Bi-labeled constructs.

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