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Effects of Radiolysis on Yttrium-90-Labeled Lym-1 Antibody Preparations

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The physical half-life of 2.6 days and 2.2 MeV beta emissions of ^{90}Y provide excellent properties for radioimmunotherapy applications. However, the clinically useful beta particles may be a source of radiation-induced damage of ^{90}Y-labeled immunoconjugate radiopharmaceuticals during preparation or short-term storage. The stability of ^{90}Y-labeled Lym-1 antibody was studied in standard radiopharmacy conditions to establish a formulation at which radiolysis is not a problem. **Methods:** Lym-1–2IT-BAD immunoconjugate intermediate was prepared according to our standard procedure, then labeled with ^{90}Y at 1, 2, 4 and 9.4 mCi/mg Lym-1 using 0.5 *M* tetramethylammonium acetate, pH 7, labeling buffer. Each mixture was challenged in diethylenetriaminepentaacetic acid to remove nonspecifically bound ^{90}Y. The ^{90}Y-2IT-BAD-Lym-1 products were purified by centrifuged molecular sieving column chromatography. The radiochemical purity and immunoreactivity of each preparation was monitored daily by high-performance liquid chromatography (HPLC) and solid-phase radioimmunoassay, respectively, for 3 days. The preparation at 2 mCi/mg was also formulated in 4% (wt/vol) human serum albumin (HSA) overall and at 9.4 mCi/mg in five-fold water, 4 and 10% (wt/vol) HSA overall; all were monitored as above. **Results:** The monomeric quality and purity profile of products at 1 and 2 mCi/mg were retained (≥ 80%) as was their immunoreactivity (≥ 75%) over 3 days. The radiochemical purity and immunoreactivity of the product at 4 mCi/mg declined to 65% and 28%, respectively, by 3 days after preparation and in just 48 hr, the product at 9.4 mCi/mg had degraded to 21% in radiochemical purity with only 3% immunoreactivity. The current HPLC data and earlier published chromatographic evidence did not support a compromised radiochemical integrity of ^{90}Y-DOTA complexes by loss of ^{90}Y from the DOTA chelate. **Conclusion:** Radiolysis of ^{90}Y-labeled antibody preparations did not appear to be a problem at ^{90}Y-2IT-BAD-Lym-1 products ≤ 2 mCi/mg. Human serum albumin proved to be an effective radioprotectant as the initial 100% immunoreactivity of the product at 2 mCi/mg was retained for 72 hr. The results underscore the need for appropriate formulations and dilutions of clinical doses of ^{90}Y immunopharmaceuticals immediately after manufacture.

Key Words: yttrium-90; antibody; radiolysis; radioprotectant

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The lethal effect of energetic beta particles from ^{90}Y on tumor cells is fundamental to its use in radioimmunotherapy (RIT). The development of better methods for delivery of ^{90}Y beta particles to tumor cells has continued to attract research interest. This research has included the development of polylactic acid microspheres as carriers for the ionic ^{90}Y matrix (*1*), ^{90}Y-Al-Si oxide glasses (*2*) and the antibody-based ^{90}Y radioimmunoconjugates (*3*). In each of these procedures, the stability of the yttrium preparations, both in vitro and in vivo, is crucial for successful ^{90}Y radionuclide therapy. While several articles are available on diminished efficacy of ^{90}Y radioimmunoconjugates due to ionization of the radiometal from the chelates (*4*), transchelation to proteins (*5*) or radiometal trapping in normal cell lysosomes (*6,7*), articles on radiation damage to the peptide or protein substrates (radiolysis) are scarce.

Encouraging results in clinical therapy protocols involving ^{90}Y-labeled monoclonal antibody (MAb) preparations (*8–10*) may lead to an increase in the use of ^{90}Y radiopharmaceuticals and their injected doses (mCi). Therefore, ^{90}Y radiochemistry has become more efficient by using methods involving minimal MAb, high radioactivity yields and optimum specific activity of final products. At high specific activity, however, ^{90}Y immunoconjugates are particularly prone to radiolysis due to the energetic particulate emissions from yttrium.

For our current clinical RIT protocols, several ^{90}Y-MAb conjugates were prepared. We investigated evidence of radiolysis over time in ^{90}Y-labeled 2IT-BAD-Lym-1 conjugate at varying specific activities. The extent of radiolysis on this immunoconjugate was compared, when formulated with or without human serum albumin (HSA), as a possible radioprotectant.

MATERIALS AND METHODS

Lym-1 (Techniclone, Inc., Tustin, CA) is a murine IgG2a MAb specific for membrane antigens found on malignant cells of most patients with B-cell lymphoma (*11*). Yttrium-90 was purchased as a concentrated carrier-free radiochemical grade $^{90}YCl_3$ in 0.05M HCl from Battelle-PNNL Laboratory (Richland, WA). Diethylenetriaminepentaacetic acid (DTPA) was obtained from Fisher Scien-

tific (Fair Lawn, NJ), and a 0.1 M solution was prepared in 0.5 M tetramethylammonium acetate, pH 5, buffer. Saline was obtained from Abbott Laboratories (North Chicago, IL).

The radiochemical purity (RCP) of radiolabeled products was established by high-performance liquid chromatography (HPLC) using a size exclusion TSK-3000 column (Tosohaas, Montgomerryville, PA) at 1 ml/min flow rate and radiometric (Beckman model 170) and UVat 280 nm (model 166, Beckman, San Ramon, CA) detectors.

The immunoreactive property of all ^{90}Y immunoconjugates was assayed following the standard solid phase radioimmunoassay (RIA) procedure (*12*). Briefly, assay solutions of each ^{90}Y-labeled Lym-1 immunoconjugate product containing equivalent of 10 ng/100 μl and 1 ng/100 μl Lym-1 were prepared in 1% bovine serum albumin (BSA), and each added to wells on plastic plates impregnated with partially purified membrane fragments from Raji test cells and irrelevant acute lymphoblastic leukemia CEM control cells. Three replicates of each cell type were used per assay. Each well was incubated at 37°C for 30 min, then rinsed out with 1% BSA, cut out and counted on a gamma well counter along with three replicates of each assay solution as standards. The assay was also performed on a lightly iodinated ^{125}I-Lym-1 standard, which binds as unmodified Lym-1, so that RIA results of ^{90}Y products are expressed in percent immunoreactivities relative to unmodified Lym-1.

Preparation of 2IT-BAD-Lym-1 Conjugate

The synthesis of chelate-carrying BAD, a bromoacetamidobenzyl derivative of DOTA (1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid) was described earlier (*13*). McCall et al.'s (*14*) method was used to conjugate BAD to Lym-1 via 2-iminothiolane (2IT). The conjugation was conducted in 0.1 M tetramethylammonium phosphate pH 8 at Lym-1, 2IT and BAD concentrations of 14.7 mg/ml, 1.27 and 2.54 mM, respectively. The Lym-1–2IT-BAD was purified into 0.1M ammonium acetate pH 5.5 by molecular sieving chromatography using Sephadex G50 (Pharmacia, Piscataway, NJ). The conjugate was stored in 1-ml aliquots in 1.5-ml capacity metal-free plastic Eppendorf tubes at −70°C until needed.

The metal-binding capacity of the conjugate was determined by the ^{57}Co assay of the [BAD]/[Lym-1] ratio using a modified form of the method of Meares et al. (*15*). Standardized cobalt chloride solution containing trace $^{57}CoCl_2$ (ICN Radiochemicals, Irvine, CA) was added in excess to an aliquot of Lym-1–2IT-BAD in 0.1M tetramethylammonium phosphate pH 8. The mixture was incubated for 30 min at room temperature. Aqueous ethylenediaminetetracetic acid was added to a final concentration of 10 mM to chelate any nonspecifically bound cobalt ions. The mixture was incubated for 15 min at room temperature. Trace-radiolabeled ^{57}Co-2IT-BAD-Lym-1 was purified into phosphate buffered saline by centrifuged molecular sieving chromatography using Sephadex G50. Aliquots of this purified solution were counted along with ^{57}Co-traced $CoCl_2$ standards to determine the concentration of ^{57}Co chelate in the product. The concentration of Lym-1 was determined by UV spectroscopy at 280 nm using the absorbance at 1%, 1 cm of 14.4 (*11*). The [BAD]/[Lym-1] ratio was calculated as the ratio of the molar concentration of cobalt chelate and Lym-1.

Preparation of ^{90}Y-2IT-BAD-Lym-1

The Lym-1–2IT-BAD conjugate in 0.1 M ammonium acetate pH 5.5 was adjusted to a solution of 6 mg/ml in 0.5 M tetramethylammonium acetate pH 7. One milligram of this Lym-1–2IT-BAD solution was added to each of four metal-free plastic Eppendorf tubes containing 2, 4, 7 and 13 mCi $^{90}YCl_3$ solution and the mixtures incubated at room temperature for 45 min. Each mixture was challenged in a DTPA solution at a final DTPA concentration of 10 mM to chelate any nonspecifically bound yttrium ions. The mixtures were incubated at room temperature for 15 min. The ^{90}Y-2IT-BAD-Lym-1 product from each mixture was purified into phosphate buffered saline (PBS), pH 7, by centrifuged molecular sieving chromatography.

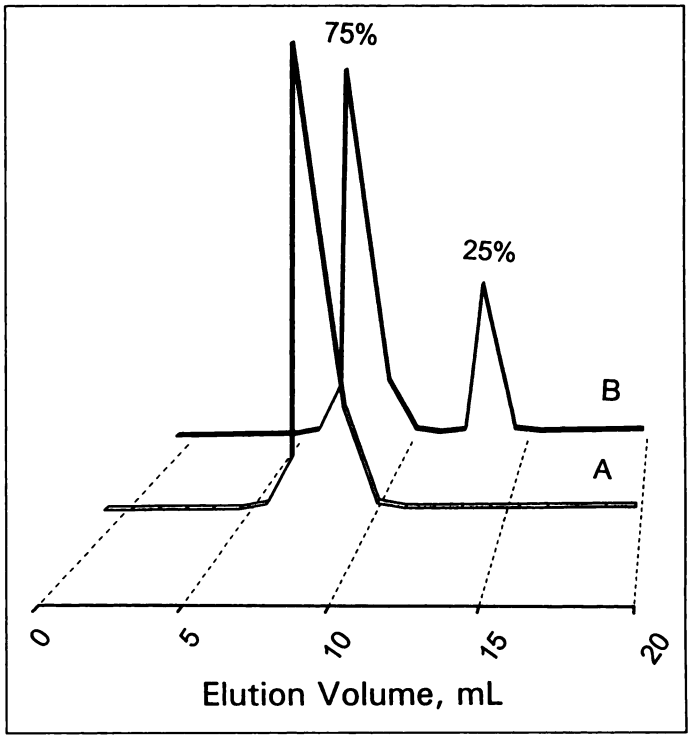

FIGURE 1. HPLC profile of a 4 mCi/mg ^{90}Y-2IT-BAD-Lym-1 preparation at 100% radiochemcial purity (A) and 75% purity (B) due to radiolysis by Day 2 of labeling.

The final preparations were assayed on the dose calibrator to determine the respective radioactivity (mCi) contents. The following final drug products were obtained and stored in 500 μl capacity metal-free Eppendorf tubes at 4–8°C throughout the study: 1 mCi/mg/100 μl, 2 mCi/mg/100 μl, 4 mCi/mg/100 μl and 9.4 mCi/mg/100 μl. The 2 mCi/mg product was formulated in 4% (wt/vol) HSA, while the 9.4 mCi/mg product was formulated in five-fold water, 4% and 10% (wt/vol) HSA, overall, to study the effects of radiolysis at these formulation conditions. These products, similarly stored throughout at 4–8°C, were respectively: 2 mCi/mg/104 μl PBS + 20 μl 25% (wt/vol) HSA, 9.4 mCi/mg/100 μl PBS + 400 μl water, 9.4 mCi/mg/104 μl PBS + 20 μl 25% (wt/vol) HSA and 9.4 mCi/mg/60 μl PBS + 40 μl 25% (wt/vol) HSA. Each preparation/formulation was monitored by HPLC and RIA assays immediately and daily for 4 days (Days 0, 1, 2, and 3). The RIA was determined regardless of the RCP status of the preparation.

RESULTS

The concentration of the Lym-1–2IT-BAD was assayed after purification at 11.9 mg/ml. The [BAD]/[Lym-1] ratio of the Lym-1–2IT-BAD was measured at 2.8. The 2, 4, 7 and 13 mCi/mg radiolabeling mixtures gave final ^{90}Y-2IT-BAD-Lym-1 preparations of 1, 2, 4 and 9.4 mCi/mg specific activities, respectively.

A typical HPLC profile of a ^{90}Y-2IT-BAD-Lym-1 product at 100% RCP immediately after preparation and 75% after radiolytic damage is shown in Figure 1. Monomeric ^{90}Y-2IT-BAD-Lym-1 peaks elute on the HPLC TSK-3000 column at the retention volume of 9 ml, corresponding to 150 kD of Lym-1, while small yttrium species < 500 kD (e.g., ^{90}Y-DTPA) elute at

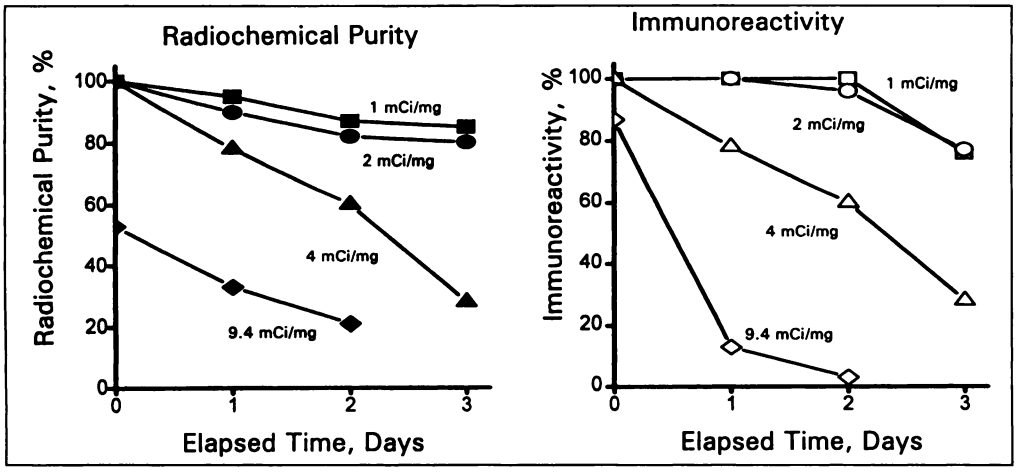

FIGURE 2. Radiochemical purity and immunoreactivity of ^{90}Y-2IT-BAD-Lym-1 at varying specific activities over time.

about 14 ml. On Day 0 of labeling, the radiochemical purity was 100% for all products between 1–4 mCi/mg and 53% for the 9.4 mCi/mg product (Fig. 2). In the 9.4 mCi/mg product, one peak at 150 kD constituted 53%, and a broadband eluting at about 10–12 ml (or 1–10 kD) constituted the remaining 47%.

The trend in immunoreactivities of the preparations was found to follow that observed on the RCP and is also shown in Figure 2. These measurements were not corrected for any loss in RCP due to radiation-induced fragmentation of the whole Lym-1 MAb because works on isolation and identification of such fragments are currently incomplete and not included in this article. The effectiveness of HSA as a radioprotectant for the two preparations (2 and 9.4 mCi/mg) in saline is shown in Figure 3.

DISCUSSION

The need for thorough investigations on the effect of radiolysis on ^{90}Y radiopharmaceuticals at high specific activities was signaled by a few posters at the 43rd Annual Meeting of the Society of Nuclear Medicine (*16,17*). The results of this study on Lym-1 (a lymphoma antibody) are an extension of those presented on the ^{90}Y immunoconjugate of chimeric L6 (a breast tumor antibody) at this meeting (*16*).

Radiochemical Purity

Formation of 1–10 kD degradation products precludes free ^{90}Y radiometal formed by loss of ^{90}Y from DOTA or incomplete challenging in DTPA. Generation of a free radiometal by either of these mechanisms would give radioactive species that normally elute further up in retention volume at about 14 ml on the TSK column. Indeed, earlier articles have indicated that the ^{90}Y-DOTA complex remains stable for several days both on standing and when incubated in serum (*13,15*). This implies that radiation-induced damage due to the energetic beta particles of ^{90}Y could be responsible for degradation of the ^{90}Y-2IT-BAD-Lym-1 immunoconjugate at 9.4 mCi/mg. The radiochemical integrity of the 1 and 2 mCi/mg preparations remained ≥80% for 3 days, but radiolytic decomposition had decreased the radiochemical purity of the 4 mCi/mg product to 65% by the third day and that of the 9.4 mCi/mg product to 21%.

The results in Figure 3 proved that radiolysis of ^{90}Y immunoconjugates could be arrested if the product is formulated in 4% HSA. As formulation was done immediately after radiolabeling, the initial 100% radiochemical purity of the 2 mCi/mg product and the marginal purity of the highly damaged 9.4 mCi/mg product were retained throughout the 3 days of study.

Immunoreactivity Property

The decline in immunoreactivity of the products followed the trend in the depreciation of their monomeric quality (Fig. 2). Much of the immunoreactivity of Lym-1 in the ^{90}Y products was retained (≥ 75%) for 3 days when the specific activity of the ^{90}Y-2IT-BAD-Lym-1 preparation is 2 mCi/mg or less. At higher specific activities, immunoreactivity fell rapidly along with radiochemical quality; the immunoreactivity was 28% on Day 3 for the 4 mCi/mg product with 65% radiochemical purity and a mere 3% for the 9.4 mCi/mg product with 21% radiochemical purity within 48 hr of preparations.

Retention of immunoreactivity of products formulated in 4% HSA followed the same trend (Fig. 3). The initial 100% immunoreactivity of the 2 mCi/mg product was retained for 3 days. Even the highly damaged 9.4 mCi/mg product retained an average immunoreactive property of about 50% over 3 days

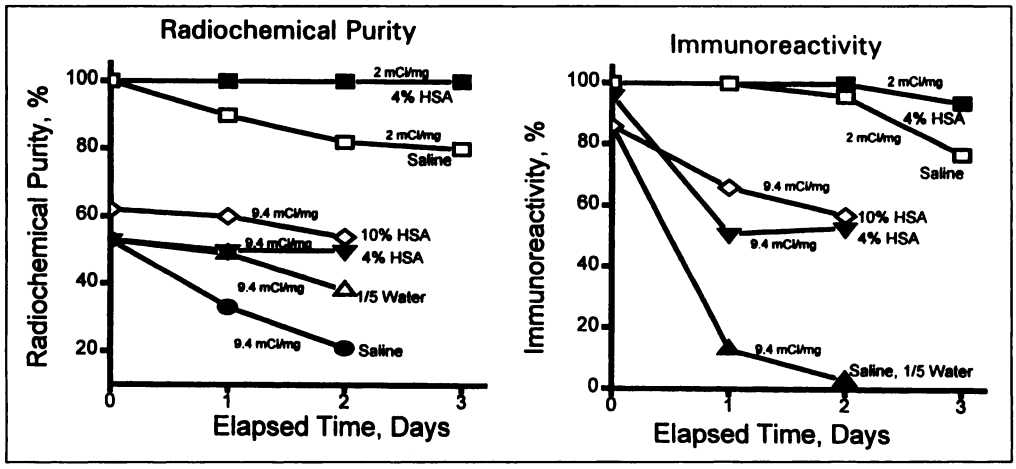

FIGURE 3. Radiochemical purity and immunoreactivity of ^{90}Y-2IT-BAD-Lym-1 at 2 mCi/mg formulated in saline and 4% HSA and at 9.4 mCi/mg formulated in saline, 5-fold water, 4% and 10% HSA.

when in HSA. Preservation of product RCP and immunoreactivity was slightly better in 10% HSA compared with 4% HSA, but diluting the product fivefold in water did not prevent any loss in immunoreactivity.

The presence of HSA in ^{90}Y-2IT-BAD-Lym-1 formulation served to increase the total protein content in the radioimmunoconjugate solution. The preponderant HSA molecules at the 4% level (compared to Lym-1 at 0.5%) could, therefore, effectively shield the mAb molecules from bombardment by the beta particles of ^{90}Y, thereby preserving immunoreactivity throughout the period of study. Incidence of radiation-induced damage to Lym-1 was expected at high specific activity of mCi ^{90}Y per mg Lym-1. At the 2.2 MeV energy of some of its beta particles, ^{90}Y could easily break most chemical bonds including the disulfide (S-S) bridge [bond strength, 4.4 eV (*18*)] within various fragment pairs in the Lym-1 molecule. Since the beta particle load on Lym-1 is lower at 1 mCi ^{90}Y/mg MAb than at 4 mCi/mg, and if evidence of radiolysis was indicated even at 2 mCi/mg on Day 3 after labeling, then a pronounced effect would be expected at 9.4 mCi/mg. The fact that a 9.4 mCi/mg product with 53% radiochemical purity was 86% immunoreactive (Fig. 2), further suggests that radiolysis of ^{90}Y immunoconjugates is a continuous process that probably involves arbitrary splitting of the MAb. Other techniques, such as polyacrilamide gel electrophoresis (PAGE), thin layer chromatography (TLC) and competitive binding assay experiments, are ongoing to obtain further proof of radiolysis for these ^{90}Y Lym-1 immunoconjugates.

Our results have been discussed in terms of ^{90}Y load (mCi) per milligram of Lym-1 for simplicity. There are 86 known hit sites for 2IT-DOTA conjugation per molecule of Lym-1 (*19,20*). Since 1 mg of Lym-1 contains about 4×10^{15} molecules and about 10^{13} yttrium atoms are in a mCi of ^{90}Y, some 400×86 potential sites will be available to house one atom of ^{90}Y in a 1 mCi/mg preparation. This implies a low bystander radiation effect of a hot Lym-1 molecule on a cold neighbor molecule, but which nonetheless increases as specific activity increases. The actual bystander effect is much higher when the 2.2 MeV maximum energy deposit and short range of ^{90}Y beta rays are considered. Because there is the probability of any fragment carrying a DOTA, a radiolabeled fragment could be immunoreactive but constitute a radiochemical impurity in situ with the whole ^{90}Y-2IT-BAD-Lym-1 antibody radioconjugate.

The clinical implication of these findings is in their usefulness as a tool to design the radiochemistry of ^{90}Y radiopharmaceuticals. The limiting effects of radiolysis should be considered when developing conjugation methodologies or radiolabeling chemistry of ^{90}Y for the preparation of radioimmunoconjugates at high specific activities. Preparations at 4 mCi/mg specific activity appear to be the threshold above which it is difficult to obtain monomeric ^{90}Y immunoconjugates. Even at this threshold, radiolysis could be noticeable over time unless the product is radioprotected in a suitable formulation such as HSA. It is strongly recommended that the stability profile and shelf-life of ^{90}Y immunoconjugate drug products are known before clinical evaluation.

CONCLUSION

Radiolysis of ^{90}Y-2IT-BAD-Lym-1 depends on specific activities of products. Products at $\leq$ 4 mCi/mg of Lym-1 in specific activities suffer little radiolysis over 2 days, but retention of radiochemical integrity and immunoreactivity for 3 days is possible when formulated in 4% HSA.

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