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^{177}Lu-antibody conjugates for single-cell kill of B-lymphoma cells in vitro and for therapy of micrometastases in vivo

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Abstract

Antibodies (Abs) conjugated to ^{177}Lu, a relatively low-energy β-particle emitter, were evaluated in vitro for their cytotoxic activity and in vivo for their therapeutic activity against disseminated B-cell lymphoma xenografts in SCID mice. ^{177}Lu was compared with other β-particle emitters (^{131}I and ^{90}Y), and also with emitters of low-energy electrons (LEEs, meaning Auger and conversion electrons of <50 keV). The Abs used reacted with CD20, CD74 or HLA-DR, and the target cell was the Raji B lymphoma. Like the other β-particle emitters, ^{177}Lu was a potent and specific toxic agent in vitro, when conjugated to Abs recognizing high-density antigens. It appeared to be slightly less potent than ^{131}I per decay, but this difference was relatively small, and would not be a major factor in the selection of the optimal radionuclide for clinical use. The nonspecific toxicity from ^{177}Lu was less than from ^{90}Y, but ^{177}Lu still produced greater nonspecific toxicity in vitro than LEE emitters. The maximum tolerated dose (MTD) of ^{177}Lu-anti-CD74 in SCID mice was 1.81 MBq (49 μCi)/mouse. When this dose was administered on day 5 after tumor inoculation, significant protection was obtained, but considerably less than the protection obtained in previous experiments with LEE emitters ^{111}In and ^{67}Ga. In conclusion, ^{177}Lu has advantages over other available β-particle emitters as a therapeutic agent, but its efficacy in the treatment of micrometastases seems to be less than that of LEE emitters, due to greater nonspecific toxicity. This conclusion, however, may not apply to therapy of macroscopic tumors.

Keywords: Radioimmunotherapy; ^{177}Lutetium; B-cell lymphoma; Micrometastases; β-Particle emitter; Auger electron emitter; Cytotoxicity in vitro

1. Introduction

While cancer therapy with radiolabeled antibodies (Abs) has become increasingly established as a therapeutic modality [1,2], the choice of the optimal radionuclide energy remains uncertain, and probably depends on the nature of the tumor burden. β-Particle emitters, which have been most widely used for radioimmunotherapy (RAIT), are primarily intended to kill cells within macroscopic tumor masses, in which most of the dose to individual cells is from "cross-fire," meaning decays that occur on nearby cells. Due to their relatively long path length in tissue, much longer than a cell diameter, β particles do not appear to be optimal for single-cell kill [3]. In contrast, two other types of radiation have much shorter path lengths in tissue, making them more suitable for single-cell kill: these are α particles and low-energy electrons (LEEs). The optimal electron energy for single-cell kill is approximately 20 keV (for decays on the cell surface or in the cytoplasm) [3–5], and electrons with this level of energy include both Auger electrons and internal conversion electrons, which we refer to collectively as LEEs. α Particles, which are under active investigation [6,7], are extremely potent per decay. Low-energy electrons are much weaker toxic agents than α particles, but may have even less nonspecific toxicity, due to a shorter path length in tissue.

We recently compared β-particle emitters with LEE emitters (^{111}In and ^{67}Ga), in terms of their ability to kill single cells in vitro and to treat micrometastases in an in vivo animal model [8–11]. An anti-CD74 Ab (LL1) conjugated to ^{111}In or ^{67}Ga was an effective therapeutic agent in a SCID mouse model of disseminated B-cell lymphoma, while Ab conjugates with ^{90}Y were much less effective [11], and this difference could be attributed to

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greater nonspecific toxicity of the ^{90}Y conjugate. The maximum tolerated dose (MTD) for the LEE emitters was approximately 10-fold higher than that for ^{90}Y. But ^{90}Y, with its very high-energy β particle, is expected to be less effective in this type of a challenge than radionuclides emitting lower energy β particles, such as ^{131}I and ^{177}Lu. The advantage of lower energy β particles would be manifested in two ways: First, nonspecific toxicity, at distances far from the site of decay, would be less. Second, the subcellular *S* values are somewhat higher [4]. For a cell the size of Raji B-lymphoma cells, which is the target cell that we routinely use, the *S* values (in units of 10^{-4} Gy/(Bq s), for irradiation of the nucleus) for ^{177}Lu are 1.32 for decays on the cell surface and 2.03 for decays in the cytoplasm. Analogous values for ^{131}I are 1.06 and 1.54, respectively, and for ^{90}Y, 0.48 and 0.68, respectively. As we have discussed at length previously [10], the cytoplasmic *S* values probably provide the best estimate of the dose delivered, even for decays that occur primarily on the cell surface. Thus, the *S* value for ^{177}Lu is 3.0-fold higher than that for ^{90}Y. ^{177}Lu and ^{90}Y were recently compared in RAIT of relatively small (1- to 3-mm-diameter) intraperitoneal colon carcinoma xenografts: at the MTD, ^{177}Lu was significantly more effective than ^{90}Y [12]. While ^{131}I and ^{177}Lu are similar in their radioactive decay properties, the key difference between them is that ^{177}Lu is a residualizing label, meaning that catabolites are retained, primarily within lysosomes, after catabolism of the Ab to which the radionuclide was attached [13]. The factor of Ab catabolism is significant for all Abs, but is especially important for work with LL1, the Ab primarily used in our therapy experiments, since this Ab is very rapidly internalized and catabolized [8]. Another limitation of our previous study was that the ^{90}Y-Ab had a relatively low specific activity, of approximately 185 MBq/mg (5 mCi/mg). While a specific activity in this range is typical for ^{90}Y-Ab therapy experiments, and similar to the specific activity of the ^{67}Ga-Ab conjugates used, it is much lower than the specific activity of the ^{111}In-Ab conjugates used. Until recently, ^{177}Lu was available only from the MURR reactor, with a radioactive abundance of approximately 25%; however, higher purity ^{177}Lu (approximately 50%), prepared by an indirect method, is now available from both MURR (University of Missouri reactor, Columbia, MO) and Nordion (Ottawa, Canada).

Therefore, using ^{177}Lu, we have now more thoroughly evaluated the efficacy of β-particle emitters in our experimental models. ^{177}Lu has been conjugated to Abs with both DOTA and DTPA, but we selected DTPA for these studies because binding of ^{177}Lu to DTPA was shown to be both faster and more efficient than binding to DOTA [14]. The superiority of benzyl-DTPA over DOTA as a chelator for ^{177}Lu was also reported by Milenic et al. [15]. This can probably be attributed to the open nature of the DTPA chelator. The Abs used were anti-CD20 (1F5), anti-HLA-DR (L243) and anti-CD74 (LL1), all of which react with high-density antigens on Raji B-lymphoma target cells. By quantitation of the uptake per cell and use of a clonogenic assay to measure high levels of cell kill, we compared the potency per decay and the specificity of ^{177}Lu with other β-particle emitters, as well as with LEE emitters.

2. Materials and methods

2.1. Cells, Abs and radiolabeling

The Raji B-lymphoma cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured as described [11]. The Abs used were anti-CD20 (1F5, an IgG2a), anti-CD74 (LL1, an IgG1) and anti-HLA-DR (L243, an IgG2a), all of which were previously described in detail [9]; they were purified from hybridoma ascites. The negative control Abs used were isotype matched and were either MN-14, an IgG1, or MX352a, an IgG2a. Labeling with ^{131}I was by a conventional chloramine T procedure, as described [16], using ^{131}I from Perkin-Elmer (Boston, MA). The specific activity was approximately 555 MBq/mg (15 mCi/mg). Ab conjugates with benzyl-DTPA were used for all of the radiometals used in this study. Conjugates were prepared as described previously [10], using isothiocyanatobenzyl-DTPA from Macrocyclics (Richardson, TX). The molar ratio of chelator/Ab was approximately 1–2:1. ^{177}Lu was obtained from either MURR or Nordion. The desired amount of activity was mixed with three volumes of 0.05 M ammonium acetate buffer, pH 5.3, and 0.1 mg Ab in phosphate-buffered saline was added. After 60 min at room temperature, 1/10 volume of 10 mM DTPA was added, followed by a 10-min incubation at room temperature. Specific activities are given in Results. ^{90}Y labeling was done similarly, with ^{90}Y from Perkin-Elmer, except that 1/35 volume of 25% (wt/vol) ascorbic acid was added to the reaction mixture before addition of the Ab, as a precaution to prevent radiation damage to the Ab, and the incubation was for only 20 min. We did not determine whether the ascorbic acid had any effect on ^{90}Y labeling and have no evidence that it did. Specific activities were approximately 1480 MBq/mg (40 mCi/mg), except as noted. Samples were generally run over a PD10 gel filtration column (Pharmacia, Piscataway, NJ) in tissue culture medium, primarily for the purpose of buffer exchange, but this also removed most of the unconjugated radionuclide that might be present. Products were analyzed by instant thin-layer chromatography (ITLC), and sometimes, by gel filtration high-pressure liquid chromatography (HPLC), by methods that were described in detail [17], to test for unbound radioactivity, which was always <10% of the total, and usually <5%. Aggregation, as seen in the HPLC profile, was usually not detected, and always <5% of the total. Immunoreactivity of the radiolabeled DTPA conjugates was determined by binding to an excess of Raji or RL cells, as described previously [10]: with ^{111}In conjugates, immunoreactivity was 74.8% for 1F5, 56.3% for L243 and 60.6% for LL1,