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Criteria for the selection of radionuclides for tumor radioimmunotherapy

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Abstract

The potential of utilizing monoclonal antibodies as carriers of radionuclides for the selective destruction of tumors (radioimmunotherapy, RIT) has stimulated much research activity. From dosimetric and other considerations, the choice of radiolabel is an important factor that needs to be optimized for maximum effectiveness of RIT. This paper reviews and assesses a number of present and future radionuclides that are particularly suitable for RIT based on the various physical, chemical, and biological considerations. Intermediate to high-energy beta emitters (with and without gamma photons in their emission) are emphasized since they possess a number of advantages over alpha and Auger emitters. Factors relating to the production and availability of candidate radiometals as well as their stable chemical attachment to monoclonal antibodies are discussed.

INTRODUCTION

Research on radiolabeled monoclonal antibodies (MAb) has experienced an intense surge of interest due to their promise to serve as selective carriers of radionuclides to tumor-associated and other specific antigens in vivo (1). However, even though results so far have been impressive, practical benefits of this approach for imaging and/or therapy applications have not matched the expectations that were raised more than a decade ago. Selective destruction of tumors using radiolabeled MAbs (radioimmunotherapy, RIT) is considered particularly suited for treating tumors not easily amenable to surgical control, and for treatment of small disseminated lesions and/or secondary micrometastases. From various considerations, especially dosimetry, the choice of radiolabel is an extremely important factor that needs to be optimized for a successful exploitation of this technique. Even though I-131 is marginally suited for RIT, most therapy trials have so far utilized this isotope due to: its commercial availability at low cost, the well understood chemistry of iodine, and the experience from its use in treating thyroid disorders. This paper briefly reviews the many present and future radionuclides that appear more suitable for RIT based on both practical as well as theoretical considerations. The problem of stable attachment of various candidate radiometals to antibodies is also addressed.

RADIONUCLIDE SELECTION CRITERIA

The selection criteria of radionuclides for RIT must be based on the physical and chemical characteristics of the radionuclide, its production, and the biological variables governing its in-vivo distribution. The important physical variables include half-life, the type, energy and branching ratio of particulate radiation and the gamma-ray energies and abundances. It is important to match the physical half-life with the MAb in-vivo pharmacokinetics. If the half-life is too short, most decay will have occurred before the MAb has reached maximum tumor/background ratio. Conversely, too long a lifetime would result in unwanted radiation dose to other tissues after the labeled MAb is shed from the tumor. The type of particulate emission is also important to consider. The potent lethality of Auger and low-energy conversion electrons due to induced Coulomb explosions is well documented (2). However, this effect can best be realized with intranuclear localization of the radionuclide, which does not generally occur with radiolabeled MAb. Beta particles are

less densely ionizing and have longer range but lower LET than alphas. Their distribution requirements are, however, less restrictive for RIT. The gamma-ray energies and abundances are important since the presence of gamma rays allows external imaging but also adds to the whole body dose. The various physical properties, in combination with biokinetic data and assumptions about tumor size, etc., can be used to calculate radiation absorbed dose at the cellular level (3-5).

The main chemical variables important in choosing a radionuclide for RIT are the specific activity achievable, radiochemical purity, trace metal contamination, the number of labels per MAb molecule obtainable without compromising immunological activity, and the in-vivo stability of the radionuclide-protein attachment. The specific activity depends primarily on the method of production. The presence of metal ions other than the product is a concern as they can compete for binding sites on chelate-MAb conjugates. It is largely controlled by the selectivity of the chemical separation scheme, which is often not perfect.

These physical and chemical factors must then be viewed in light of available biological information. Substantial variations in antibody uptake, its macro and microdistribution, and its kinetics have been reported. Nevertheless, for many antibodies presently under investigation for RIT, some generalities do emerge. It is generally believed that one half to three days is usually required to reach maximum tumor uptake although optimum tumor to normal tissue contrast may take longer. Despite the availability of numerous antigen sites on cancer cells, present evidence indicates a non-uniform cellular distribution of the MAb in most cases (6). These facts considerably reduce the attractiveness of short-ranged Auger and alpha-emitting radionuclides for RIT. Their role, however may be important in specific situations such as for treating blood tumors and micrometastases. The longer range of beta particles allows more uniform tumor irradiation despite the heterogeneity of radioactivity distribution within the tumor tissue.

CANDIDATE RADIONUCLIDES

Beta emitters also offer a much wider choice of candidates that possess various particle ranges and chemical properties. Prospective beta emitters can be grouped into two classes: 1) those emitting intermediate energy beta particles and gamma rays suitable for imaging; and 2) those with high-energy beta emission and little or no gamma emission. The use of isotopes in the first group would allow diagnostic low-dose biodistribution experiments before administering a therapeutic dose of the exact same preparation. This constitutes a real advantage because it has been observed that the biodistribution can be influenced by the choice of radionuclide alone, even with the same antibody system (7). Clinically, it will be necessary to image each patient prior to therapy in order to assess antigenic status and to calculate tumor and sensitive tissue doses from the observed biodistribution. A minor disadvantage of this choice is that because of the penetrating nature of the gamma radiation a less than optimum target/nontarget dose ratio may result. The most attractive isotopes of this class are shown in Table 1.

Of these, Cu-67 has been previously identified as being useful for RIT, and is presently under active investigation (8-9). Free Cu-67 produced following antibody processing does not localize in bone, liver, or kidney, in contrast to many other radiometals. Although Sm-153-ethylenediaminetetramethylenephosphonic acid (EDTMP) has undergone investigation as a bone cancer agent, very little has been reported on the use of Sm-153 as an antibody label (10). As can be seen from Table 1 its physical properties do fulfill the various criteria discussed above. Similarly Rh-105 has received some attention (11), and more recently Sc-47 (12) and Au-199 (13-14). Iodine-131 is in the therapeutic class but clearly its long half-life and high abundance of 364 keV photons make it less attractive than the other candidates. Nevertheless, due to its ready availability and ease of labeling, it has found widespread application for RIT (15).

Conversely, the isotopes in class 2 (Table 2) have better target/nontarget dose ratios but imaging studies for biodistribution must be performed with other radionuclides. Unfortunately, scintigraphic resolution from bremsstrahlung (that can be used with these isotopes) may be poor making quantification nearly impossible. In addition, not many good

Table 1. Radionuclides for Radioimmunotherapy: Low to Intermediate β^- Energy; γ Emission Suitable for Imaging (>10% abundance)

Radionuclide	$t_{1/2}$ (d)	E β^- avg., keV	Eγ, keV(%)
Sc-47	3.4	162	159 (68)
Cu-67	2.6	141	185 (49)
Rh-105	1.5	190	319 (19)
I-131	8.0	181	364 (81)
Sm-153	1.9	280	103 (28)
Lu-177	6.7	133	208 (11)
Re-188	0.71	764	155 (15)
Ir-194	0.80	808	328 (13)
Au-199	3.1	86 143*.	158 (37)

*Conversion electron

Table 2. Radionuclides for Radioimmunotherapy: Intermediate to High β^- Energy; Little (<10%) or no γ Emission

Radionuclide	$t_{1/2}$(d)	E β^- avg., keV	Eγ, keV (%)
As-77	1.6	228	239 (1.6)
Y-90	2.7	935	----
Pd-109	0.56	360	88 (3.6)
Ag-111	7.5	350	342 (6.7)
Pr-142	0.80	860	1576 (3.7)
Pm-149	2.2	364	286 (3.1)
Gd-159	0.77	311	363 (8.0)
Ho-166	1.1	666	80 (6.2)
Re-186	3.8	425	137 (8.7)

imaging analogs are available for the beta emitters of class 2. Because of its high-energy beta particle, suitable half-life, good chelation properties and availability, several groups are currently studying the use of Y-90 as an RIT radiolabel (16). Since Y-90 is unsuitable for quantitative imaging, many groups are utilizing In-111 biodistribution data to predict dose from Y-90 administrations. However, even though there are similarities in tumor uptake, blood clearance and other tissue uptakes, often there are substantial differences in whole body retention and clearance from kidney and the reticuloendothelial system. For example, recently it has been shown that although intravascular kinetics in patients are similar for Y-90 and In-111 labeled T101 antibody using isothiocyanatobenzyl DTPA, the two preparations differ in their tumor uptake and tissue biodistribution properties (17). A similar approach has been taken for the pair Tc-99m and Re-186, the former for imaging, the latter for therapy, again both of which can be attached to antibodies via similar chemistry (18).

RADIONUCLIDE PRODUCTION

The possible production techniques (Tables 3 and 4) and resultant specific activity are very important. An adequate supply of suitable quality I-131 is commercially available. Copper-67 is produced by high energy spallation reactions in the Brookhaven Linac Isotope Producer (BLIP) at Brookhaven National Laboratory (19) and the Los Alamos Meson Physics Facility (LAMPF) at Los Alamos National Laboratory and is available from these

institutions most of the year. Large quantities of Sm-153 can be produced very simply by thermal neutron activation because of its large neutron capture cross section and epithermal resonance integral. A similar situation exists for Lu-177 and Ir-194. However, adequate specific activity can only be achieved at nuclear reactors with neutron fluxes of greater than $2x10^{14}n/cm^2$-sec (e.g., the High Flux Beam Reactor at Brookhaven National Laboratory, High Flux Isotope Reactor at Oak Ridge National Laboratory, and U. Missouri Research Reactor).

There are two possible routes for the production of Au-199. Double neutron capture reaction on natural gold leads to high yield because of the enormous cross section of Au-198, but the specific activity is inadequate for RIT. Thus the indirect reaction on Pt-198 followed by beta decay to Au-199 has recently been investigated (20) and appears to be practical. A similar method can be used for Rh-105. For both these radionuclides, production at a high flux reactor will be advantageous. Re-188 is also interesting because it can be prepared in high specific activity from a convenient W-188/Re-188 generator system (21). The W-188/Re-188 system could be considered a therapeutic analogue to the Mo-99/Tc-99m generator since the chemistry of Re in many ways is similar to that of Tc.

Table 3. Possible Production Reactions of Radionuclides Suitable for Radioimmunotherapy (β^- + γ Emitters)

Radionuclide	Nuclear Reactions
Sc-47	natTi(p,2p)
Cu-67	Zn-68(p,2p);Zn-67(n,p)
Rh-105	natRu(n,γ)Ru-105$\rightarrow\beta$
I-131	U-235(n,f)
Sm-153	Sm-152(n,γ)
Au-199	Pt-198(n,γ)Pt-198$\rightarrow\beta$
	Au-197(n,γ)Au-198(n,γ)
Lu-177	Lu-176(n,γ)
Re-188	W-186(n,γ)W-187(n,γ)W-188$\rightarrow\beta$
Ir-194	Ir-193(n,γ)

Table 4. Possible Production Reactions of Radionuclides Suitable for Radioimmunotherapy (Pure β^- Emitters)

Radionuclide	Nuclear Reactions
As-77	Se-80(p,α)
Y-90	U(n,f)Sr-90$\rightarrow\beta$
Pd-109	Pd-108(n,γ)
Ag-111	Pd-110(n,γ)$\rightarrow\beta$
Pr-142	Pr-141(n,γ)
Pm-149	Nd-148(n,γ)Nd-149$\rightarrow\beta$
Gd-159	Gd-158(n,γ)
Ho-166	Ho-165(n,γ)
Re-186	Re-185(n,γ)

DEVELOPMENT OF RADIOIMMUNOCONJUGATES

The convenience, efficiency and gentleness of various radiolabeling procedures as well as the stability of the radionuclide attachment to the antibody are all important factors and are under active investigation (22). The use of radiometals in RIT requires a stable

attachment of the radiometal to the MAb since free radiometal may target normal tissues thus increasing normal organ and whole body doses. Radiolabeling techniques range widely from simple direct labeling of Re-186, to the use of general purpose bifunctional chelating agents such as the cyclic anhydride of DTPA (DTPA-CA) for Y-90, Pd-109 and Sm-153, to the use of more structurally complex in-house synthesized bifunctional chelating agents for Re-186, Cu-67, Sc-47 and Y-90.

Due to the chemical similarity between Tc and Re, strategies for labeling MAbs with Re-186 have directly paralleled those for Tc-99m. Direct labeling with Re-188 has been demonstrated utilizing free sulfhydryl groups on the MAb (23); these groups can be generated either by chemical reduction of MAb disulfide bonds or by the reaction of lysines on the MAb with 2-iminothiolane. A more selective approach involves chelation of Re-186 to a N_3S-amide mercaptide ligand (MAG_2-GABA) prior to conjugation to MAbs (18). While less convenient, this approach allows more control over radiolabeling and may have wider applicability with various MAb systems. Antibodies have been labeled with Y-90 using DTPA-CA; however, in clinical trials these preparations showed high bone uptake of Y-90. Substantially reduced bone uptake in mice was shown using p-isothiocyanantobenzyl DTPA (the coordination sites on this ligand are 8 compared to 7 for DTPA-CA); however, it was still higher than what is generally observed with the corresponding In-111 labeled MAb (24). In mice the bone uptake of Y-90 has been reduced to the levels of In-111 using the macrocyclic bifunctional chelating agent p-bromoacetamidobenzyl-DOTA (25). Biodistribution studies in mice of Sc-46 labeled MAb prepared using DTPA-CA have shown high levels of radioactivity in the liver (26). Recently, carrier-free Sc-47 was prepared and successfully attached to 17-1A MAb (26) using the new semi-rigid bifunctional chelating agent 4-isothiocyanato-cyclohexyl EDTA (4-ICE) (27). Using this preorganized ligand the biodistribution in normal mice of the Sc-47 labeled 17-1A IgG was identical to that of the corresponding In-111 labeled antibody. Since In-111 labeled antibodies prepared using 4-ICE have shown higher tumor uptake with a three to four-fold reduction in the retention of In-111 in the liver compared to DTPA-immunoconjugates in mice (28), we expect similar results with Sc-47.

Copper labeled DTPA-immunoconjugates are not stable in serum. Even though the serum stability of Cu labeled 4-ICE-immunoconjugates is substantially higher they are still unstable in-vivo and produce high nonspecific retention of Cu-67. Stable Cu labeled immunoconjugates result only by using derivatives of the macrocyclic polyaminocarboxylates p-aminobenzyl-TETA (29) and DOTA (30) or derivatized cyclic polyamines (cyclams) (31-32). Preliminary studies in patients with pharmacological doses of Cu-67 labeled Lym-1 MAb prepared using parabromo-acetamidobenzyl-TETA have shown tumor regression (33). Very little work has been done on Sm-153 as an antibody label. In one study in mice Sm-153 labeled K-1-21 murine IgG (labeled using DTPA-CA) gave a slightly lower tumor uptake with higher bone and liver uptake compared to I-131 or In-111 labeled K-1-21 (10). The use of bifunctional chelator 4-ICE did not improve the Sm-153 labeled 17-1A biodistribution compared to Sm-153 labeled DTPA-17-1A. This may be due to the fact that since Sm is a lanthanide with f valence electrons having no specific coordination geometry, it does not need a preorganized chelation cavity. A higher number of coordination sites on the ligand may be more important for Sm. For example, the macrocyclic polyaminocarboxylate DOTA (eight coordination sites) forms extremely stable complexes with the lanthanide Gd-153 (34). Samarium (+3) whose size and charge are similar to Gd (+3) should also form stable DOTA complexes. For this reason we are currently synthesizing these and other functionalized macrocyclic polyaminocarboxylates for use with Cu-67, Y-90, Sm-153, and other radiometals.

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