Pharmacokinetics of Radiolabeled Polyclonal Antiferritin in Patients with Hodgkin’s Disease

Jing Lai, Syed M. Quadri, Paul E. Borchardt, Leigh Harris, Robin Wucher, Eileen Askew, Louis Scheweichler, and Huibert M. Vriesendorp

Abstract
The objective was to identify pharmacokinetic parameters predictive for tumor response and normal tissue side effects after i.v. administered radiolabeled rabbit antihuman ferritin IgG.

Eighty-two patients with recurrent Hodgkin’s disease received 2 mg of rabbit antihuman ferritin i.v., labeled with 4–7 mCi of In-111 followed by two doses of 0.25, one dose of 0.4 mCi of Y-90-labeled antiferritin per kg of body weight 1 week later. Radioactivity and HPLC measurements of blood and urine samples and liver and tumor volumes identified on sequential whole-body scans provided the data for a pharmacokinetic analysis covering the first 6 days after the administration of the radioimmunoconjugate. Side effects and tumor response were recorded.

Temporary hematological toxicity was noted in all patients. Sixteen patients showed a tumor response. The Y-90 blood level at 1 h after administration correlated with the severity of subsequent hematological toxicity. The rapid blood elimination half-life of radioactivity was 4.4 h. Less than 5% of the administered radioactivity was eliminated in the first 24 h urine. The slow blood elimination half-life was 44 and 37 h for In-111 and Y-90, respectively. One of 12 retreated patients produced anti-rabbit IgG antibodies. The volume of distribution was larger for Y-90 than for In-111-labeled antiferritin (160 versus 110% of estimated blood volume). Accidentally extravasated rabbit IgG was rapidly catabolized in perivascular tissues with an effective half-life of less than 35 h. Slower catabolism was noted for rabbit IgG in blood (t1/2 = 40 h), liver (t1/2 = 62 h) or tumor (t1/2 = 40–80 h). Twelve of 13 patients with an effective tumor half-life > 57 h showed a tumor response.

i.v. administered polyclonal rabbit antihuman ferritin, labeled with In-111 or Y-90 is stable in vivo and targets Hodgkin’s disease. Intravascular Y-90 causes a vascular leak and a larger volume of distribution for antiferritin.

Elevated Y-90 blood levels at 1 h and a tumor half-life of >57 h predict for hematological toxicity and tumor response, respectively.

Introduction
RIT attempts to provide more radiation to cancer tissues than normal tissues by using the cancer seeking properties of the administered immunoglobulin and the limited range of the energy deposition from the radioisotope connected to the immunoglobulin. Ferritin, an iron storage protein, is found in high concentration in tissues containing Hodgkin’s disease (1). Polyclonal rabbit antihuman ferritin IgG labeled with iodine-131 or indium-111 targets Hodgkin’s disease after i.v. administration (2, 3). Yttrium-90 provides higher doses, higher dose rates, and higher dose homogeneity to tumor than iodine-131 (4, 5). The combined use of In-111- and Y-90-labeled antiferritin allows for outpatient treatment of patients with recurrent Hodgkin’s disease (6).

The presence of a radiolabel on a cancer drug simplifies the performance of a noninvasive pharmacokinetic analysis in vivo. This report describes such an analysis in 28 patients with recurrent Hodgkin’s disease treated with polyclonal rabbit antiferritin labeled with In-111 or Y-90 in the Arlington Cancer Center in Texas. Different pharmacokinetic parameters are predictive for normal tissue side effects or tumor responses after radiolabeled antiferritin therapy. The information obtained is helpful to the planning of future clinical RIT trials.

Patients and Methods

Patients
Twenty-eight patients with multiple recurrences of mainly nodular sclerosing Hodgkin’s disease were entered on studies after the physician-sponsored IND BB7139 was obtained. Medium age of patients was 34 years of age (range 20–50). The sex ratio (male:female) was 1.8. Further demographic details can be found in a companion study (7). Consent forms approved by Arlington Cancer Center’s Institutional Review Board and the United States Food and Drug Administration were signed and witnessed prior to study entry. Patients frequently suffered from B symptoms and had extensive nodal and extranodal disease resistant or recurrent after prior chemotherapy, radiotherapy, and/or bone marrow transplantation. Entry criteria included (a) histological diagnosis of Hodgkin’s disease; (b) measurable...
disease; (c) negative HIV titers; (d) Zubrod score 1 or 2; (e) recurrence or persistence of Hodgkin’s disease after at least two treatment regimens; and (f) granulocyte count >1500 per ml and platelet count >50,000 per ml.

Twenty-six patients received their first RIT cycle, and 12 of them went on to receive one or two additional RIT cycles thereafter. Two patients were entered into the program for their fourth RIT cycle, having received the same RIT reagent in another institution previously. After protocol treatment all patients were followed for acute (3 weeks), delayed (within 2 months), and late side effects (>2 months) of the treatment as well as tumor response determinations. Side effects were scored with tables published by Eastern Cooperative Oncology Group and Radiation Therapy Oncology Group. Scores can be an integer number between 0 and 4; 0 indicates no toxicity, and 4 indicates life-threatening toxicity. This report describes follow up of patients till October 1998 between 4 and 18 months after radioimmunoconjugate administration. Tumor responses were recorded for this report combining complete and partial responses as defined in the accompanying paper (7).

In Vitro Quality Control

Immunoconjugate. IgG was isolated from the sera of four different rabbits hyperimmunized with ferritin isolated from a spleen of a Hodgkin’s disease patient. After purification HPLC showed a single peak with an elution time compatible with IgG. Further analysis by SDS-PAGE under reducing conditions showed only two bands compatible with IgG heavy and light chains. The rabbit IgG was conjugated to 2B3M-DTPA with IgG. Further analysis by SDS-PAGE under reducing conditions showed only two bands compatible with IgG heavy and light chains. The rabbit IgG was conjugated to 2B3M-DTPA after its conversion into an isothiocyanatobenzyl derivative. A thioura linkage with the ε amino group of lysine residues secured the chelate to the polypeptide chain of the IgG (8). On average, one chelate molecule was conjugated per IgG molecule. Immunoreactivity was confirmed in immunodiffusion tests, ELISA tests, and affinity chromatography. Precipitating bands were found for human ferritin but not for mouse, rat, or rabbit ferritin in immunodiffusion tests. ELISA tests showed similar immunoreactivity for immunoglobulin and immunoconjugate. The affinity chromatography showed similar immunoreactivity of the immunoconjugate before and after radiolabeling (In-111 or Y-90).

Radiolabeling. Between 4–7 mCi of indium-111 total or 0.25, 0.3, or 0.4 mCi of yttrium-90 per kg of body weight was linked to 2 mg of rabbit IgG per treatment cycle. After DTPA challenge and Sephadex column purification the radioimmunoconjugate was sterile filtered. Endotoxin levels determined by a Limulus assay were required to be <175 Endotoxin Unit/dose. TLC tests were performed prior to administration, always showing that >95% of the radioactivity was bound to the immunoconjugate. Serum stability tests had been performed for each isotope label prior to dispensing of the immunoconjugate batch in individual patient vials. Less than 7% of each isotope dissociated from the rabbit antiferritin in 72 h at 4° or 37°Celsius.

Administration

Diluted in 1–2% (w/v) human albumen/PBS to a total volume of 5–10 ml, the radioimmunoconjugate was adminis-

<table>
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<th>Table 1</th>
<th>Y-90 blood levels at 1 h after administration and subsequent hematological toxicity score</th>
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<tr>
<td></td>
<td>Hematological toxicity score&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y-90 level at 1 h</td>
<td>&gt;3</td>
</tr>
<tr>
<td>&gt;0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
</tr>
<tr>
<td>&lt;0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
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<sup>a</sup> Combined granulocyte and platelet score.
<sup>b</sup> μCi/µCi administered/kg. X<sup>2</sup> of 5.6, 1 df. P < 0.05.

Pharmacokinetics

Blood and urine samples were taken at 1, 18, 42, 66, and 138 h after administration. The first and second 24 h urine after administration were collected to determine the %ID in urine. Blood radioactivity levels were converted into μCi/ml without decay correction and plotted on log linear graph paper. The log Y axis is used for radioactivity, the linear X axis is for time in hours. The blood elimination curve was biphasic for all patients. The straight terminal part of the elimination curve can be used to calculate the t<sub>1/2</sub> of the slow elimination process. Extrapolation of this straight line to the Y axis provides a numerical β value. Subtraction of this straight line from the early time points provides a new straight line describing the rapid elimination process from which a t<sub>1/2</sub> can be calculated. Extrapolation of the new line to the Y axis will provide a numerical value for α.

Blood volume was estimated for each patient in ml by multiplying the idealized body weight in kg by 75 (9). Volume of distribution was estimated by dividing the total mCi administered by the mCi per ml in blood 1 h after administration.

Additional Tests

HARA titers were assayed for by a double sandwich ELISA. Blood and urine samples were analyzed by HPLC (Bio-Silect SEC 250-5 size exclusion column). Fractions were collected and counted in a gamma counter.

Gamma Camera Images

After the administration of In-111-labeled antiferritin, patients underwent planar gamma camera scans at 2, 18, 42, 66, and 138 h. A dual head Picker camera with medium energy collimators was used. Machine software allowed for the use of a region of interest analysis for liver and tumor volumes. Serial anterior planar views were used to determine counts per pixel over time in these volumes. Half-lives were determined by plotting counts per pixel on log linear graph paper. A single photon emission computed tomography scan was performed of tumor-containing areas at 43 h after administration to confirm tumor targeting.
Results

In Vitro Quality Control

In vitro quality control information was part of the product description given in the IND submission. Only a brief summary of the data obtained was given in Materials and Methods, as this communication is dedicated to an in vivo quality control analysis. Details on in vitro quality control were reported previously (10).

Radiation Safety

Small amounts of radioactivity were measured in chair pads of patients with B symptoms (sweats) during the administration, presumably due to secretion of radioactivity by sweat glands. When decontamination was necessary, it was easy to perform and successful. Film dosimeters carried by healthy individuals accompanying the patients for the 2–3 weeks they spent in Arlington showed readings less than 20 mRem. Weekly tests with survey meters and wipe tests of surfaces in the radiopharmacy and patient administration room were performed and never above 2× background.

Side Effects

Acute side effects were not observed after In-111 antiferritin administrations. After Y-90 antiferritin administration, some patients complained for 2–3 days of feeling tired and nauseous. Five different late side effects were observed in five
Fig. 2 Two whole-body anterior scans of UPN 017 in January 1998 and May 1998. Approximately 5 mCi of indium-111-labeled antiferritin was administered i.v. on both occasions. Only scans at 2 and 43 h after administration are shown.

different patients: vanishing bile duct syndrome, Sweet’s syndrome, nephrotic syndrome, porphyria cutanea tarda, and myelodysplasia. None were considered to be caused by the radio-labeled antiferritin treatment (7). The first three syndromes are known manifestations of progressive Hodgkin’s disease, which the patients in question were experiencing at the time. The last two late side effects could be due to treatments received by the patients prior to study entry. Delayed side effects were thrombopenia, granulopenia, and anemia. This was reversible in 12–16 weeks with the exception of three patients who experienced bone marrow aplasia for longer periods of time. Blood radioactivity 1 h after Y-90 administrations showed a 3-fold range. A level above 0.09 μCi per μCi administered predicted for more serious hematological toxicity ($P < 0.05$ Table 1).

HARA in a titer of 1:100 were found in one of 12 retreated patients (Fig. 1). The two peaks in the HPLC in Fig. 1B were identified as a high MW peak (>$1,000,000$) and a low MW peak (<30,000). The high MW peak was considered to represent immune complexes consisting of one human IgM or several human IgG molecules attached to one rabbit IgG molecule. The low MW peak was thought to be In-111-labeled peptides removed by HARA action from the rabbit protein. In Fig. 2, the gamma camera scans of the first and second In-111-labeled antiferritin administration are shown for this patient. The second administration led to rapid elimination of radioactivity of the blood pool. Most radioactivity ended up in the liver. The effective $t_{1/2}$ of the liver activity was approximately 35 h. Catabolism of the radioactive protein was confirmed by increased urine elimination, visible on the 43 h scan by visualization of the bladder. The %ID in urine was three times higher for the second administration.

Pharmacokinetic Analysis

Blood. In Fig. 3, HPLC analyses are given for sera of UPN 025 at different time points after the administration of In-111- or Y-90-labeled rabbit antiferritin. Stoichiometrically one would expect serum ferritin as Ag ($300$ ng/ml and MW 440,000) and rabbit antiferritin as Ab ($400$ ng/ml and MW 160,000) to form one-on-one immune complexes (Ag$\cdot$Ab). This indeed appeared to be the case as evidenced by the early peak on HPLC corresponding to a MW 600,000 structure. For the In-111-labeled proteins, a separation was visible between the first Ag$\cdot$Ab$\cdot$ peak on HPLC and the second, larger Ab peak at MW 160,000. This separation was not observed for
Fig. 3  A, HPLC of serum samples of UPN 025 at different time points after In-111-labeled antiferritin administration. Three peaks are shown, at MW 600,000, 160,000, and <30,000. B, HPLC of serum samples of UPN 025 at different time points after Y-90-labeled antiferritin administration. Compare to A; there is no separation between MW 600,000 and 160,000. All peaks show radioactive decay over time. The peaks at MW 600,000 and <30,000 decayed faster than the peak at MW 160,000.

The Y-90-labeled proteins in this and three other patients tested (Fig. 3B).

The mean $t_{1/2\alpha}$ and $\beta$ and the $\alpha/\beta$ ratios of the 28 patients studied are shown in Table 2. The low ratios indicate the predominance of the slow elimination process for the radiolabeled antiferritin in the circulation. The duration of the effective $\beta$ half-life between 36 and 44 h reflects the stability of the radioimmunoconjugate in blood. The biological half-life of the radioimmunoconjugate in blood was calculated from the mathematical relationship between physical, effective and biological half-lives, i.e.:

$$T_{eff} = \frac{T_{biol} \times T_{phys}}{T_{biol} + T_{phys}}$$

The rabbit IgG had a biological blood half-life in patients with Hodgkin’s disease similar to the one in rabbits, i.e., 100 h. This is shorter than the half-life of most human IgG subtypes in human patients (11). The $t_{1/2\beta}$ of In-111- and Y-90-labeled antiferritin were significantly different: 44 versus 37 h ($P < 0.02$ in Student’s t test). The larger volume of distribution found for Y-90 in comparison to In-111 (medians of 160 versus 110% of estimated blood volume) explained the difference in $t_{1/2\beta}$ values. Y-90 derived irradiation of endothelial cells caused a capillary leak and an extension of the volume of distribution into perivascular tissues. In four patients with large size Hodgkin lesions and intense B symptoms, $t_{1/2\beta}$ for Y-90 was under 30 h. Early
gamma camera scans of such patients showed a grainy background for interstitial tissues. Their median survival time was 61 days (range 25–80). Cause of death was progressive Hodgkin’s disease. In 19 patients who received two Y-90 administrations per cycle, no differences were observed in blood pharmacokinetics between the first and second Y-90 administration (Table 2). In general, pharmacokinetics of repeat RIT cycles were similar to the pharmacokinetics of the first cycle of that patient with the exception of patient UPN 017 (Figs. 1 and 2).

**Bone Marrow.** In most patients, bone marrow was not targeted (see Figs. 2 and 4), although ferritin is known to be present in bone marrow parenchyma. Patients with extension of red marrow into long bones of their extremities or patients recently treated with granulocyte colony-stimulating factor do show symmetric bone marrow uptake. Patients with known involvement of their bone marrow with Hodgkin’s disease were not included in this study.

**Urine.** HPLC results on urine samples demonstrated radioactive moieties below MW 30,000 (data not shown). This represents chelated radioisotope with or without small peptide fragments attached. The first and second 24 h urine elimination was less than 5%ID per day for both Y-90 and In-111, confirming the in vivo stability of the radiolabeled antiferritin.

**Liver.** In Fig. 4, whole-body scans of a patient show the entrapment of the radioimmunoconjugate in the liver at the earliest time point. Liver radioactivity disappeared with a monophasic half-life of 62 h close to the physical half-life of In-111 (68 h), indicating the slow catabolism of the radioimmunoconjugate in this anatomical location. Four times higher liver uptake was noted in the patient with HARA. Some patients eliminated a small amount of radioactivity through bile into the intestinal tract.

**Perivascular Interstitium.** The veins of Hodgkin’s disease patients accessible for the administration of chemotherapy develop chemical vasculitis after multiple administrations. This compromises venous access in patients with recurrent Hodgkin’s disease and can lead to inadvertent extravasations of indium-labeled antiferritin. The survival of bare Ab (not connected to ferritin) in perivascular tissues can be visualized on planar whole-body scans and is short due to rapid catabolism (data not shown).

**Tumor.** All patients showed tumor targeting on planar whole-body gamma camera images by 18 h after administra-

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Fig. 4  Serial anterior planar views of UPN 028 after In-111-labeled antiferritin administration. Shown is the tumor uptake in left shoulder and left anterior chest wall. Heart, blood pool, liver, oro/nasopharynx, and testes show increased radioactivity; the spleen was surgically absent.
tion, which was confirmed on single photon emission computed tomography scans at 43 h after administration. The difference in the rate of uptake between tumor and liver is well illustrated by the images in Fig. 4. Uptake was slower for tumor for this as well as all other patients. A region of interest analysis was performed on the elimination of radioactivity from the tumor. A wide range in tumor residence times was found (40–80 h half-lives). In Table 3, tumor half-life is compared to tumor response in the 24 patients in whom tumor half-lives could be determined for their first RIT cycle. Patients with a tumor response after Y-90-labeled antiferritin had slower tumor elimination times of In-111-labeled antiferritin than patients without a response (P < 0.001). The software necessary for tumor dosimetry was not available at Arlington Cancer Center. In all, 16 patients showed tumor shrinkage. It was complete in two patients. Median response duration was 6 months.

Discussion

The in vivo results illustrate the stability of the radioimmunoconjugate. Only a small proportion of the injected radioactivity is eliminated in urine, the α/β ratio for blood elimination is low, and the effective and biological t1/2 values in blood are long (Table 2). Instability of the radioimmunoconjugate is dangerous if due to dechelation free ionized Y-90 is released (12). The bone seeking tendency of free Y-90 has deterred some investigators from using Y-90 due to the anticipated extra bone marrow toxicity. In this study, an extra preventive measure was taken by the addition of a 1000-fold molar excess of free DTPA to the radioimmunoconjugate prior to column chromatography purification. The procedure removed 1–5% of presumably weakly bound Y-90 prior to patient administration (data not shown). Free Y-90 or bone uptake of Y-90 was not measured in this study. Patients were rested with a bone marrow aspirate and biopsy prior to study entry. Hodgkin’s disease was not observed. Bone marrow biopsies were not repeated after the patients received Y-90, as we did not want to expose the patient again to the pain and inconvenience caused by such procedures. A comparison of In-111- and Y-90-labeled 2B3M-DTPA immunocomplexes administered to nude mice or beagle dogs showed low amounts of radioactivity for each isotope in bone or bone marrow (13, 14). The in vivo stability of the radioimmunoconjugate used in this study and in preceding animal studies predicts that only small quantities of Y-90 will be incorporated in bone of treated patients.

Small differences were noted between the in vivo behavior of Y-90- and In-111-labeled immunocomplexes (Table 2 and Fig. 3). The Table 2 data need to be included in future dosimetric calculations that use indium signals for the prediction of a subsequent yttrium biodistribution. The observed pharmacokinetic differences between Y-90 and In-111-labeled antiferritin are due to the short range of energy deposition around Y-90 atoms. The high amount of energy absorbed induces a capillary leak syndrome due to irradiation of endothelial surfaces (Table 2). The most pronounced capillary leaks occur in patients with large and progressive Hodgkin’s disease (t1/2 β < 30 h). This suggests that the disease condition can aggravate the effects of Y-90 on endothelial surfaces, for example by the spill over of BRMs, such as interleukin-2, from tumor into the circulation. The production of BRMs by tissues containing Hodgkin’s disease is well-documented (15).

Affinity column purification of antiferritin performed previously by some of us indicated that the AgAb bond between ferritin and antiferritin is stronger than the bond between ferritin subunits. Eluted antiferritin has a higher MW, because it contains one or more extra ferritin subunits (MW 19,000 or 21,000). The increased radioactivity observed between the MW 600,000 and 160,000 peak in serum HPLC after Y-90 administration reflects the development of truncated ferritin molecules, which remain connected to rabbit IgG. The radiolytic effects of the Y-90 β emissions break the relatively weak bonds between the MW 19,000 and 21,000 subunits of ferritin (Fig. 3B), but not the stronger Ag/Ab complex or the bonds between chelate and radioisotope. Most of the energy deposition of In-111 atoms is at distance and bypasses the ferritin bound to the radiolabeled immunoglobulin and truncated ferritin molecules are not generated, allowing for a separation of the first and second peak in Fig. 3A. The decrease in peak heights in Fig. 3 reflects radioactivity decay as well as elimination of the products. The latter is more pronounced for peak 1 and 3. High MW peak 1 is probably filtered out from the circulation in the liver; the low MW peak 3 is eliminated in urine.

The small amount of protein administered in this study, the small Ag,Ab₁ complex formed in circulation, and the low incidence of HARA formation all indicate that immune complex disease, Arthus reactions, and serum sickness are unlikely events in this patient population with this radioimmunoconjugate (16, 17). These complications were not observed in these

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Table 2

<table>
<thead>
<tr>
<th></th>
<th>α/β ratio</th>
<th>t1/2(α) (h)</th>
<th>t1/2(β) (h)</th>
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<tr>
<td>Diagnostic RAF (In-111)</td>
<td>2.06 ± 0.55</td>
<td>4.7 ± 1.0</td>
<td>44.2 ± 2.8</td>
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<tr>
<td>Therapy 1 (first Y-90)</td>
<td>1.88 ± 0.26</td>
<td>4.4 ± 1.1</td>
<td>37.2 ± 6.0</td>
</tr>
<tr>
<td>Therapy 2 (second Y-90)</td>
<td>2.16 ± 0.55</td>
<td>4.1 ± 1.2</td>
<td>36.7 ± 6.8</td>
</tr>
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</table>

*First RIT cycle of 28 patients. Nineteen patients received two Y-90 administrations per cycle.

*RAF, rabbit antiferritin.

Table 3

<table>
<thead>
<tr>
<th>Tumor response after Y-90 RAF</th>
<th>Tumor half-life after In-111 RAF</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>&gt;57 h</td>
<td>&lt;57 h</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>−</td>
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<td>9</td>
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<td></td>
<td>13</td>
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*RAF, rabbit antiferritin. *P < 0.001 in a double-tailed Fisher’s exact test.

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4 J. Lai and S. M. Quadri, unpublished observations.
patients or other patients treated previously on similar antiferritin protocols.

A summary of the pharmacokinetic information obtained is presented in Fig. 5. Each box in Fig. 5 gives information on the indicated compartment in terms of MW, AgAb configuration, and half-life. In the first In Vitro box, the radioimmunoconjugate is described. New information in this box is the percentage of immunoconjugates that carry a radioisotope (1% for In-111 and 5% for Y-90; specific activity, < 15 mCi/mg). Higher specific activities for Y-90 were not used after preliminary tests showed radiolysis (10). If radiolysis can be prevented, higher specific activities can be used leading to a higher percentage of immunoconjugates carrying yttrium-90 and increased potency of the reagent. The In Vivo part of Fig. 5 starts with the circulatory system (blood). The hematological toxicity observed is due to radiation of hemopoietic stem cells in bone marrow by radioimmunoconjugate in blood circulating through bone marrow (4, 12). Bone marrow targeting by antiferritin is low due to the presence of a blood-marrow barrier and the high hydrostatic pressure in bone marrow (4). Liver uptake of polyclonal antiferritin was determined in previous patient and animal studies as approximately 20% of the administered activity (4). It is clear from the scans shown in Figs. 2 and 4 that the venous endothelium is the initial site of contact between radioimmunoconjugate and liver. Uptake of a MW 160,000 molecule in liver parenchymal cells would take longer than 3 h. The assumption is that the rabbit IgG through its Fc part obtains access to the so-called Brambell reservoir (18, 19). Ferritin/antiferritin IgG complexes can also be taken up in the reservoir, but Ab is not catabolized and returns intact to the circulation. This protection of Ab from catabolism has only been described for IgG. Brambell et al. (19) made the original observations in their studies of transfer of maternal plasma IgG to fetus across the placenta and transfer of IgG in breast milk to neonate across small intestinal mucosa. The Brambell reservoir is also responsible for the inverse relationship between plasma IgG levels and IgG $t_{1/2}$ (19). HARA immune complexes are not taken up in the Brambell reservoir [perhaps due to the participation of IgM in this immune complex or the size of the AgAb complex (Fig. 1B)] but are removed by other cells in the liver, presumably macrophages (Kupffer cells) located close to the endothelium. HARA complexes show a 4-fold higher liver uptake than anti-ferritin or ferritin/antiferritin complexes. The lack of tumor targeting in patients with HARA is readily understood. Eighty % of the administered activity is trapped and catabolized in normal liver tissues. Accumulation of radioactivity in the intestinal lumen can occur through biliary secretion of radioactive moieties in the liver. This activity can be differentiated from abdominal Hodgkin's disease by its movement over time in distal direction and the resemblance of its configuration to an intestinal outline.

The catabolism of Ab is influenced by its interactions with Ag. Without Ag, catabolism of Ab is rapid as shown in the perivascular tissue box in Fig. 5 ($t_{1/2} < 35$ h). In tumor, the greatest range in effective half-lives is found. The configuration of the radioimmunoconjugate in tumor is expected to be an Ag$_2$Ab$_3$ complex with a combined MW > 1,000,000. This has not been verified yet in animal or human patient tumor samples. Transportation of such a large complex through or out of the tumor will be slow. The speculation is that Hodgkin's disease patients with larger tumors produce more BRMs in their tumors. BRMs can dissociate Ag from Ab, leading to more rapid catabolism of Ab and shorter tumor residence times. Alternatively, BRMs might damage tumor blood vessel endothelium, allowing for the exportation of radioimmunoconjugate (with or without Ag) out of the tumor. Either of the two processes suggested would explain why large masses of Hodgkin's disease receive less radiation from RIT and are less likely to respond to RIT (5).

The new insights obtained from the pharmacokinetic and in...
vivo quality control analysis performed in this study should be applied to future clinical RIT studies. External beam radiation of large tumor masses immediately after RIT might provide a method to prevent egress of radioimmunoconjugate from the tumor. The induction of thrombosis in tumor blood vessels after RIT provides another option (20, 21).

In Table 1, hematological toxicity appears to be correlated to blood levels at 1 h after Y-90 administration. In addition a 3-fold difference is noted in blood radioactivity levels between patients receiving the same prescription in mCi/kg. This suggests that some hematological toxicity might be prevented in the future by giving a small amount of Y-90-labeled immunoglobulin first, followed by blood radioactivity measurements at 1 h. Next, only that amount of extra Y-90-labeled immunoglobulin should be administered that will keep the blood radioactivity level just below a previously determined critical toxicity level.

Liver uptake of radiolabeled rabbit IgG appears to occur by at least two different mechanisms: the Brambell reservoir and Kupffer cells. Previously higher liver uptake was noted in patients receiving radiolabeled mouse monoclonal antiferritin IgG and prevented effective tumor targeting (5). This might be due to one of the two mechanisms identified above for liver uptake or to a third, still unidentified, mechanism. Further studies in normal beagle dogs, which show high normal liver uptake of IV administered radiolabeled mouse IgG, similar to the observations made in human patients, may be helpful (4, 13). The therapeutic ratio of radiolabeled antiferritin would improve if uptake of radioactivity in normal tissues, such as liver, can be decreased. This might be possible in the future with modified monoclonal antiferritin. The examples provided illustrate that new mechanistic information and hypotheses can be obtained from pharmacokinetic studies of radioimmunoconjugates. More rapid progress in the development of clinical RIT can be achieved by the continued application of this methodology.

Acknowledgments

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References


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