Bone Marrow Transplantation in Dogs After Radio-Ablation With a New Ho-166 Amino Phosphonic Acid Bone-Seeking Agent (DOTMP)


β-emitting $^{166}$Ho ($t_{1/2} = 26.78$ hours, $E_{\text{max}} = 1.8$ MeV) complexed with the phosphonic acid chelator, 1.4.7.10 tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid) (DOTMP) at a ligand-to-metal ratio of 1.5:1 binds to bone. This radioactive complex is a marrow-ablating radiopharmaceutical that appears useful for preparation of bone marrow (BM) transplant recipients without the morbidity usually associated with total body irradiation preparatory regimens. We have found with seven splenectomized young adult beagle dogs that a $^{166}$Ho radiopharmaceutical dosage of 370 MBq/kg body weight provides an initial skeletal radioactivity burden of at least 1.5 GBq/kg skeleton and results in complete ablation of hematopoietic marrow cell populations within 7 days. The β particle flux distribution in BM-forming skeletal tissue is not uniform. Red marrow radiation doses varied from 30 to 115 Gy as estimated by direct radioassay and autoradiographic analyses of both bone biopsies and postmortem samples; the median value of 61 Gy agreed with our theoretical expectations. In vivo radioactivity distribution was evaluated with nuclear imaging methods. Apparently, normal hematopoiesis was restored in three dogs with autologous BM transplants performed 5 to 6 days after administration of the marrow ablative radiopharmaceutical, $^{166}$Ho-DOTMP. BM biopsies at 7 to 10 months posttransplantation indicate continued normal hematopoietic activity.

Bone Marrow transplantation (BMT) is an effective treatment for a range of hematologic malignancies.\(^1\)\(^4\) The objective of the procedure is to administer high-dose myeloablative therapy followed by autologous or allogeneic marrow transplantation to restore hematopoiesis. These disorders exhibit a dose-dependent response to radiation. External whole-body high-energy photon radiation is the cornerstone of most preparative regimens for BMT, usually in combination with cyclophosphamide or other chemotherapy.\(^1\) The LD\(_{50}\) for external whole-body radiation is approximately 4.5 Gy because of BM failure.\(^2\) Doses up to 16 Gy can be tolerated with marrow transplantation, but toxicity to lung, liver, gastrointestinal tract, and other organs occurs in many patients, preventing further dose escalation.

Doses of radiation much larger than 16 Gy are necessary for optimal antitumor effects. It is highly desirable to target delivery of radiation to malignant cells and escalate the dose delivered beyond that possible with external radiotherapy while sparing normal tissue from toxicity. This has recently been attempted using a number of techniques. Radionuclides conjugated monoclonal antibodies (MoAbs) directed to tumor cell surface antigens have been used with a goal of localizing β- or γ-emitting radionuclides at the site of the tumor.\(^6\)\(^9\) Challenges, intrinsic to this approach, in getting selective deposition of therapeutic radionuclide dosages in target tumors are being met.\(^10\)

An alternative for malignancies largely limited to the BM is the administration of bone-seeking radiopharmaceuticals that accumulate in the skeleton and deliver radiation to the adjacent marrow. In this study, we evaluate the feasibility of this approach using systemic administration of a new $^{166}$Ho phosphonate chelate formed with 1,4,7,10 tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid) (DOTMP) in dogs. These studies show that $^{166}$Ho chelate effectively localizes to bone and at defined dosage can serve as a myeloablative therapeutic agent before BMT.

BM ablation, accomplished in beagles with bone-seeking DOTMP complexed with the β- and γ-emitting lanthanide, $^{166}$Ho, has little systemic toxicity compared with total body irradiation (TBI).\(^11\) The $^{166}$Ho-DOTMP delivered radiation doses, characterized by an exponentially decreasing dose rate (≈ 1-day half period) and localized to the skeleton, differ both temporally and spatially from TBI. The beagle has been a useful research model for human BMT\(^17\) and the spatial aspects of different bone-seeker distributions in skeleton have been characterized experimentally and theoretically for beagles\(^13\) and humans.\(^14\)

MATERIALS AND METHODS

Radiopharmaceutical Preparation

$^{166}$Ho ($t_{1/2} = 26.78$ hours, $E_{\text{max}} = 1.8$ MeV; $\gamma_1 = 0.081$ MeV (6.2%); $\gamma_2 = 1.38$ MeV [1%]) was produced by neutron irradiation of $^{165}$Ho-oxide targets (University of Missouri Research Reactor, Columbia, MO). The activity was obtained as the $^{166}$Ho-carrier added $^{166}$Ho (65% carrier-added)-chloride salt dissolved in 0.1 N HCl. The Holmium was added to DOTMP at a low ligand-to-metal ratio of 1.5:1 at pH > 10. The phosphonic acid chelator was obtained as a 18-mL kit preloaded with sodium hydroxide for initial pH control (Dow Chemical U.S.A., Freeport, TX. United States Patent No. 4,882,142). Activity greater than 37 MBq (1 mCi) was measured with an ion-chamber dose calibrator that was also used to

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cross check the producers reported activity (specific activity typically 5000 MBq/mg $^{166}$Ho-oxide). Radionuclidic purity assays by gamma-ray spectrometry showed $^{166}$Ho ($\lambda_2 = 1200$ y) and $^{152}$Eu ($\lambda_2 = 13.4$ y) present in a ratio of $\approx 1$ Bq ($^{166}$Ho and $^{152}$Eu):$1 \times 10^6$ Bq $^{166}$Ho at time of administration. Complexation of $^{166}$Ho with DOTMP was verified by cation exchange chromatography. $^{166}$Ho-DOTMP dosages of 370 MBq/kg body weight (10 mCi/kg body weight) were previously found adequate for marrow ablation in beagles with graded dose-response experiments (G.R. Cain, manuscript in preparation; see also Procedures section).

Animal Procedures

Young adult splenectomized beagle dogs used in this study were derived from a purebred colony raised at the University of California, Davis. Colony dogs are provided with comprehensive medical care (deworming, vaccinations, quarterly examinations) by veterinary specialists. All animal facilities and treatment are in accordance with U.C. Davis and American Association for Accreditation of Laboratory Animal Care guidelines. BM was harvested by needle aspiration at 2-week intervals until the day of transplantation. All beagles were splenectomized at least 2 weeks before BM ablation. Extramedullary hematopoiesis in the spleen of beagles with marrow function compromised by radioactive bone seekers or disease processes has been routinely observed (R.G.W.) at our laboratory and has been noted by others. 15-17 In $^{166}$Ho-DOTMP toxicity range-finding experiments (G.C. Cain, in preparation), splenectomized young adult (15 months) beagles, including two given 185 MBq/kg body weight (5 mCi/kg body weight), recovered after transient anemia without any supportive therapy. The subject of this report is our findings with dogs administered twice the maximum radionuclide dosage than that of dogs that survived without supportive therapy. The evidence for red marrow doses of 50 to 60 Gy in these beagles combined with evidence for recovery of normal hematopoietic function suggests that the BM stroma necessary to permit normal repopulation is not incapacitated at radiation doses twofold higher than heretofore examined.

Irradiation of kidney, liver, and other organs by bloodborne activity is transient with the maximum dose rate occurring at the end of the infusion period. The maximum dose rates (Gy/min) in the kidney (and other organs) of nominal 10-kg dogs increased during the first 20 minutes as the activity in the blood pool increased by infusion of the radiopharmaceutical agent. At the end of infusion, the activity in blood clears as described by equation 1. The maximum soft tissue dose rate was to the kidney, which had a median value of 0.018 Gy/min (range 0.012 to 0.030 Gy/min). The liver had the next highest dose rate that was 10-fold lower.

The integrated kidney dose (Fig 5) derives primarily from activity with an approximate 4-minute residence time in the nephrons. The maximum median dose to kidney is about 1 Gy. The variation about values normalized for a 10-kg dog receiving 370 MBq/kg (10 mCi/kg) of activity, derives from the variation in blood clearance and skeletal uptake; the range about the plotted median values is -0.65 to +1.7 of the median as expected for log normally distributed data. The median cumulative dose maximum for liver (Fig 5) is less than 0.1 Gy. The dogs continue to have normal clinical hematologic parameters 9 to 12 months after transplantation.

Clinical Observations

All dogs not transplanted developed severe neutropenia and thrombocytopenia by 7 to 10 days after $^{166}$Ho administration (Fig 2). Red blood cell (RBC) counts also decreased during this period but at a less precipitous rate. Dogs not transplanted were killed by day 22 because of sepsis and hemorrhage consequent to failure of granulocyte and thrombocyte regeneration (empty marrows). The 5-day period after $^{166}$Ho infusion was characterized by normal evalu-
ations of body temperature, state of hydration, peripheral capillary perfusion and integrity, and appetite demeanor for all dogs exposed to approximately 370 MBq/kg of 166Ho-DOTMP.

Two of the three dogs following autologous BMT remained clinically normal before and after BM engraftment. However, a single animal became ill 24 hours after BMT; liver uptake of activity estimated at ≤10% of skeletal burden was observed in gamma camera images. Significant clinical signs were marked depression, anorexia, pale mucous membrane (without evidence of petechia), hematuria, hyperpyrexia (body temperature 105.4°F). A complete blood count (CBC) indicated an RBC of 4.98 × 10⁶ cells/μL, marked granulocytopenia (156 cells/μL), and lymphopenia (432 cells/μL). Serum analysis (SMA-12) indicated a moderate elevation of alkaline phosphatase (255 IU/L). All other serum chemistry values were judged to be within normal limits. Antibiotic therapy in response to clinical and pathologic findings was immediately instituted. Progressive clinical improvement in appetite, attitude, and body temperature was noted over the succeeding 6 days without concomitant change in hematologic parameters. All physical signs of disease had subsided after 6 days. Peripheral blood counts 2 days later (9 days post-166Ho DOTMP infusion) reflected the onset of successful BM engraftment, and this trend continued until all hematologic parameters returned to normal (49 days posttransplantation).

Activity Distribution

166Ho DOTMP blood levels were typically 1% to 2% of injected dosages (ID) at 120 minutes. From two exponential fittings of blood clearance during the first 24 hours in each dog, we obtained an expression for mean blood activity (percent ID) (equation 1).

\[
\text{Blood Activity (percent ID) } = A_{166} \exp(-k_{166}\text{time(min)}) + A_{28} \exp(-k_{28}\text{time(min)})
\]

where \( A_{166} = 16.5 \), \( A_{28} = 1.22 \) are the geometric mean and geometric standard deviation = antilog s_{166} and s_{28}, respectively; \( k_{166} = 0.095 \text{ minutes}^{-1} \) and \( k_{28} = 1.27 \); s_{166} = 1.27; s_{28} = 0.0088 minutes^{-1} and s_{28} = 1.35.

Dogs were housed in metabolic cages. Urinary excretion accounted for 95% or more of the activity excreted. Feces samples collected up to 10 days after exposure yielded less than 3% of total injected 166Ho-DOTMP activity. The time course of residual whole body activity is described by equation 2: Whole Body Fraction of Injected Dose(Skeleton) = \( A_{\text{tak}} \exp(-k_{\text{tak}}\text{time(min)>1.5 hours}) \), where \( A_{\text{tak}} \) is the time zero extrapolation of the whole-body activity fraction data for times greater than 1.5 hours. The mean \( A_{\text{tak}} \) value is 0.47 ± 0.07 SD. The geometric mean of \( k_{\text{tak}} \) is 0.03 hour^{-1} and \( s_{\text{tak}} = 0.22 \).

Routine in vivo biodistribution studies after administration of 166Ho-DOTMP were performed on day 1 and day 4 with a gamma camera imaging system to evaluate skeletal uptake and soft tissue clearance. In separate dynamic studies with dogs infused while in the view field, kidney images of the 81-keV (6.2% abundant) gamma rays were immediately visualized followed by distinct skeletal images in the first 90 minutes. Comparative studies with another radionuclide, samarium-153 (103-keV photon; 28% abundant) conjugated to DOTMP showed higher resolution images and also that the skeleton was the predominant site of activity. In all dogs, 50% to 60% of the 166Ho-DOTMP cleared via the kidneys with similar times.

Dosimetry

Skeletal dosimetry. Normal beagles injected IV with radio-pharmaceutical doses of 370 MBq 166Ho-DOTMP/kg body weight have a mean skeletal dose of about 18 to 20 Gy (Fig 4) as determined from whole-body activity counting with metabolic measurements. Nuclear imaging and radioassay of skeletal tissue from dogs that were not transplanted were used to verify skeletal deposition. The skeletal distribution of 166Ho-DOTMP is similar to other bone seekers for which existing theoretical descriptions of deposition patterns allow prediction of average radiation dose to trabecular bone and included marrow. The predicted mean dose to trabecular bone and red marrow for the beagles in this study having a nominal 1,000-g skeleton is approximately 50 Gy (Fig 4) for administered dosages of 370 MBq/kg.

Direct estimates of radiation dose from six transverse BM biopsies of the humerus have been obtained from radioactivity assays. The range is 33 to 115 Gy with a median value of 61 Gy. Similar nonuniform dose distribution was found by digital autoradiography of 900 μm transverse sections through the proximal humerus of dogs not transplanted. The mean skeletal dose rate, cumulative skeletal dose, dose rate to red marrow, and cumulative dose to red marrow in the nominal 1,000-g beagle skeleton are summarized in Fig 4.

Soft tissue dosimetry. Soft tissue radiation doses to liver and kidneys are of interest. The radiation doses to kidneys and liver were derived primarily from blood (equation 1). The maximum specific activity median value normalized for a 10-kg dog with 850 mL of blood was 1.3 MBq(0.035 mCi)/mL (range: in MBq is ca 1 to 2 MBq/mL; in mCi is approximately 0.027 to 0.056 mCi/mL). In these procedures, the dose rate increases to a peak (approximately 0.02 Gy/min) during the 20-minute infusion period (maximum blood specific activity) and then declines in direct proportion to the blood activity. The cumulative dose in these organs increased slowly after the first 2 hours (Fig 5) and

Fig 1. (A, B) Representative photomicrograph of BM biopsies taken at 5 days postinfusion of beagles with 370 MBq of 166Ho-DOTMP/kg body weight. Edematous BM stroma and scattered committed hematopoietic cells indicate BM ablation and the absence of hematopoietic activity. Scale bar in (A) equal 100 μm, in (B) 50 μm. (C and D) Representative photomicrograph of BM biopsy of same beagle as in A and B after single autologous BMT with cryopreserved mononuclear cells given at 5 days postinfusion. The biopsy taken 8 months post-166Ho-DOTMP ablation and subsequent transplantation illustrates recovery of normal hematopoiesis. Scale bar in (C) equal 100 μm, in (D) 50 μm.
asymptotically approaches a maximum that was about 1.25 times the value at two hours.

In the case of kidneys, about 90% of the activity was in the nephrons and about 10% was in the vasculature of the kidney. Dynamic studies of distribution kinetics after a bolus injection were performed with a gamma camera. This allowed the time from the peak in the blood curve to the peak of the kidney activity curves taken from regions of interest situated over the heart and the kidneys to be measured. A 3.5- to 4-minute filling time of the dog nephrons was observed. Thus, the residence time for a given blood activity decrement ($\Delta A_b$) by renal clearance is about 4 minutes also. Repeated measurements of blood-specific activity and urinary activity over the first 120 minutes from end-of-infusion gave a median renal extraction value for $^{166}$Ho introduced as DOTMP of 0.15 (range 0.10 to 0.27). Assuming a 4 mL per second flow rate through the kidneys and a total blood volume of approximately 850 mL for the nominal 10-kg beagle, the time for total blood volume to circulate through the kidneys once was estimated to be a minimum of 3.5 minutes, corresponding to our observed nephron filling time.

For purposes of kidney dosimetry, we integrated the time-dependent blood activity function; then calculated the dose to 50-gram kidneys from 10 mL of blood in vascular space. This was combined with the dose from predicted time-dependent activity in the nephrons calculated from an observed integral amount of urinary activity collected in a known amount of time where the nephronal residence time was assumed to be, conservatively, 4 minutes. The amount of activity extracted from blood and predicted to appear in urine with the parameter estimates given and that actually observed was within a factor of 0.5 to 1.5. The reciprocal of this procedure, ie, starting with known urinary activity produced in a given time and generating a predicted blood activity median value for the time period is equally useful and has the same agreement factors of 0.5 to 1.5.

Liver dose (Fig 5) was estimated by the straightforward calculation of the time-dependent activity in a nominal 375-gram liver. A vascular volume of 10 mL/100 g of liver

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**Table 1. Terminal Cell Count Values for Beagles Not Subject to Autologous BMT After Administration of 370 MBq/kg Body Weight of $^{166}$Ho**

<table>
<thead>
<tr>
<th>Dog ID</th>
<th>Day CBC</th>
<th>Platelets/µL</th>
<th>WBC/µL</th>
<th>RBC x 10^6/µL</th>
<th>Terminal Day</th>
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<tr>
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<td>200</td>
<td>4.72</td>
<td>13</td>
</tr>
<tr>
<td>8BE27L</td>
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<td>6,000</td>
<td>500</td>
<td>1.69</td>
<td>22</td>
</tr>
<tr>
<td>8BE27Z</td>
<td>6</td>
<td>250,000</td>
<td>900</td>
<td>5.77</td>
<td>10*</td>
</tr>
<tr>
<td>9BL23C</td>
<td>20</td>
<td>11,000</td>
<td>900</td>
<td>3.52</td>
<td>21</td>
</tr>
</tbody>
</table>

* Spontaneous death, no gross lesions observed at necropsy.
and an extravascular volume of 22.5 mL (approximately 6\% by weight of a 375 g liver mass). The total volume of 60 mL was assumed to have the same specific activity as blood. This is an upper limit as the extravascular fluid specific activity is expected to be lower or equal to the blood-specific activity.

In the case of the one dog observed to have a liver shadow by gamma camera imaging, an estimate of 10\% of the initial skeletal burden (170 to 190 MBq) in the liver leads to a dose estimate of 6 to 7 Gy if the radiation dose is uniformly distributed over the liver. However, if the source of the liver shadow is \(^{166}\)Ho-labeled material that localizes directly in liver macrophages (Kupffer cells)\(^{19}\) the local cellular dose may be much higher than 6 Gy. Radiation damage to the Kupffer cells that clear the portal stream of potentially infectious agents acquired transmurally from the gut is a possible explanation for the onset of fever observed in this dog.

DISCUSSION

The basis of BMT for hematologic malignancies is to administer high doses of myeloablative therapy followed by infusion of autologous or allogeneic BM to restore hematopoiesis. External whole-body radiation is a major component of many pretransplant treatment regimens, but despite maximally tolerated dose, relapse of the underlying malignancy remains a major problem.

An alternative approach is IV administration of a radionuclide-phosphonate chelate, which localizes in bone with delivery of high doses of radiation to the adjacent marrow. This concept was explored by earlier workers with radiosamarium as \(^{153}\)Sm-ethylene diamine tetramethylene phosphonic acid (EDTMP).\(^{20}\) This agent largely localizes to areas of remodeling bone such as osteoblastic metastases and is presently under study for treatment of bone metastases from prostate cancer; preliminary data are encouraging.\(^{21}\)

Samarium-153 EDTMP is not an ideal therapeutic radio-pharmaceutical for hematologic malignancies that diffusely infiltrate the BM and require relatively high-dose radiation throughout the BM cavity. Samarium-153 has a relatively low beta energy (\(E_{\text{max}}\) max 0.81 MeV [20\%], 0.71 MeV [50\%], and 0.64 MeV [30\%] relative to \(E_{\text{max}}\) = 1.8 MeV of \(^{166}\)Ho) and its effects are largely limited to paratrabecular bone, sparing the central core of long bones and much of the BM.
However, a prescient study with $^{166}$Ho EDTMP gave encouraging results.\textsuperscript{22,23} $^{166}$Ho is better suited for marrow ablative purposes. It is primarily a $\beta$ emitter with a higher energy ($E_{\text{max}} = 1.83$ MeV) and a relatively short half life of 26.78 hours. It also has a minor $\gamma$ component (81 keV) suitable for imaging. In this study, we explored the biologic effects of systemic administration of a new $^{166}$Ho-DOTMP phosphonic acid chelate in dogs. The dogs were previously splenectomized to obviate extramedullary hematopoiesis. Administration of 370 MBq/kg weight resulted in diffuse marrow cytoreduction with resultant aplasia and severe pancytopenia. Hematologic recovery occurred in dogs after autologous BMT, but not in nontransplanted controls.

Fifty to fifty percent of the injected dose was deposited in the skeleton with delivery of ca 50 Gy to the red marrow. Approximately 50% of the blood activity was cleared directly into the urine within 90 to 120 minutes with the remainder essentially completely eliminated over the next 24 hours. There was normally no detectable localization or uptake in extraskeletal tissue; thus, the agent was well tolerated clinically. There was no symptomatic toxicity to bone or other adjacent structures including muscle and the central or peripheral nervous system. Dogs have been observed greater than 14 months without deterioration of hematopoiesis or long-term adverse clinical signs.

Further studies are required to determine the maximally tolerated dose of $^{166}$Ho-DOTMP. It is anticipated that toxicity to the marrow stroma, destroying support for hematopoiesis will be dose-limiting, but the dose required to produce this effect may be considerably higher than with external radiation, given the difference in radiation quality, dose rate, and distribution with this approach. Our early observation in an ongoing radiation dose-tolerance study is that 9- to 10-month-old beagles typically have greater than 50% initial retention of radioactivity in the skeleton. In our experiments with these younger beagles and dosages of $^{166}$Ho-DOTMP threefold higher than the 370 MBq/(10 mCi)/kg weight described in this report, we found fibrotic marrow at 6 weeks postablation, analogous to that described by Appelbaum et al\textsuperscript{23} with $^{166}$Ho-EDTMP, which reverts to normal by 6 months. Thus, radioactivity dosages higher than what we find ablative in beagles appears possible without irreversible damage to BM stroma. Additional long-term adverse effects must be evaluated, particularly the risk of late secondary malignancies.

These studies suggest that $^{166}$Ho-DOTMP is an effective bone-seeking agent for delivery of radiotherapy to the BM with little systemic toxicity. Limited systemic toxicity follows in part from the fact that chelation of the holmium is accomplished with only a 2:1 ligand to metal ratio and in part, from the limitation of radiation to primarily skeletal tissue. This approach may be useful for treatment of patients with hematologic malignancies primarily involving the BM, ie, multiple myeloma and leukemias. Given the minimal systemic toxicity, $^{166}$Ho-DOTMP can likely be combined with chemotherapy, external radiotherapy, or other therapeutic agents to treat malignant cells outside of the marrow cavity.

REFERENCES


