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


Bone marrow transplantation (BMT) is an effective treatment for a range of hematologic malignancies. 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. The LD50 for external whole-body radiation is approximately 4.5 Gy because of BM failure. 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. Challenges, intrinsic to this approach, in getting selective deposition of therapeutic radionuclide dosages in target tumors are being met.

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 166Ho phosphonate chelate formed with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid) (DOTMP) in dogs. These studies show that 166Ho 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, 166Ho, has little systemic toxicity compared with total body irradiation (TBI). The 166Ho-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 and the spatial aspects of different bone-seeker distributions in skeleton has been characterized experimentally and theoretically for beagles and humans.

MATERIALS AND METHODS

Radiopharmaceutical Preparation

166Ho (t1/2 = 26.78 hours, Eγmax = 1.8 MeV; γ1 = 0.081 MeV (6.2%), γ2 = 1.38 MeV (1%)) was produced by neutron irradiation of 165Ho-oxide targets (University of Missouri Research Reactor, Columbia, MO). The activity was obtained as the 166Ho-carrier added 166Ho (153Ho-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 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, 166Ho-DOTMP. BM biopsies at 7 to 10 months posttransplantation indicate continued normal hematopoietic activity.

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cross check the producers reported activity (specific activity typically 5000 MBq/mg 166Ho-oxide). Radionuclidic purity assays by gamma-ray spectrometry showed 166Ho (t1/2 = 1200 y) and 152Eu (t1/2 = 13.4 y) present in a ratio of ≈1 Bq(166Ho and 152Eu):1012 Bq166Ho at time of administration. Complexation of 166Ho with DOTMP was verified by cation exchange chromatography. Ho-166 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 2.6 × 1010 undifferentiated hematopoietic blast cells per kilogram body weight were obtained. BM cells were cryopreserved at −196°C 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 166Ho-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 support. BM harvest and surgical splenectomy were performed under general anesthesia.

Ho-166 DOTMP dosages, nominally 3,700 MBq (100 mCi) for 10 kg body weight, were adjusted to physiologic pH and infused over a period of 20 minutes into anesthetized beagles via the cephalic vein at a rate less than 1 mL/min. A lactue box with 5-cm walls housed the infusion system and provided effective shielding against the 166Ho β field. Cryopreserved cells were rapidly thawed at 37°C and washed with physiologic saline containing 10% canine serum. The marrow cells (1.7 to 1.9 × 108 cells per kilogram body weight) were infused intravenously (IV) at the fifth day after 166Ho-DOTMP administration. Dogs not transplanted were killed when they were clinically morbid or hematologic parameters indicated severe anemia.

RESULTS

Results Summary

Successful BM ablation in dogs with 370 MBq/kg body weight of 166Ho DOTMP was shown by absence of hematopoietic activity in BM biopsies 5 days post-166Ho infusion (Figs 1A and B), and peripheral pancytopenia (Fig 2). The four dogs not receiving BM transplants did not survive and died with empty marrows. Their terminal blood cell count data are shown in Table 1. Successful autologous BMT (Figs 1C and D) with cryopreserved mononuclear cells administered at 5 days post-166Ho infusion in three beagle dogs is inferred from the gradual increase in numbers of circulating neutrophils and platelets and hemoglobin levels (Fig 3) at 10 to 12 days after the BMT. Marrow biopsies were deferred until 8 months (Figs 1C and D) when hematologic parameters indicated recovery of hematopoietic function. The transient increase in values shown by the curves of Fig 3 at 7 days after BMT reflect single blood transfusions administered to provide temporary support for low platelet counts at 10 to 12 days postholmium infusion.

The radiation doses of 50 Gy derived theoretically,13 for red marrow (Fig 4) regions are based on the average skeletal radioactivity burden and derived average dose (20 Gy; percent standard deviation = 15%) for the nominal 10-kg dog administered 370 MBq (10 mCi) per kg body weight. Direct radiochemical assays of activity in early BM biopsies gave a median value of 6.1 Gy; in good agreement with theoretical expectations for this animal model.

As noted earlier, the LD50 for external whole-body radiation is approximately 4.5 Gy because of BM failure, but doses up to 16 Gy can be tolerated with marrow transplantation if toxicity to lung, liver, gastrointestinal tract, and other organs does not prevent further dose escalation. 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 166Ho 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 166Ho 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 post-transplantation).

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).

Blood Activity (percent ID)

\[ A_{\text{avg}} \exp(-k_{\text{avg}})\text{time(min)} = A_{16}\exp(-k_{16})\text{time(min)} + A_{28}\exp(-k_{28})\text{time(min)} \]

where \( A_{16} = 16.5 \), \( A_{28} = 1.22 \) are the arithmetic mean and geometric standard deviation = antilog \( s_d A_{\text{avg}} \), respectively; \( k_{16} = 0.095 \text{ minutes}^{-1} \) and \( s_d A_{16} = 1.49; A_{28} = 1.27; k_{28} = 0.0088 \text{ minutes}^{-1} \) and \( s_d A_{28} = 1.35 \).

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) = \( A_{\text{tot}}\exp(-k_{\text{tot}})\text{time}(>1.5 \text{ hours}) \), where \( A_{\text{tot}} \) is the time zero extrapolation of the whole body activity fraction data for times greater than 1.5 hours. The mean \( A_{\text{tot}} \) value is 0.47 ± 0.07 SD. The geometric mean of \( kT \) is 0.03 hour\(^{-1}\) and \( s_d A = 0.22 \).

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 radio-lanthanide, 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 radiography 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
The range of cell concentration values versus time are shown by the shaded regions for 4 beagles not subject to autologous BMT after administration of 370 MBq/kg body weight. The age of these dogs span from 16 to 24 months. Measurement times denoted by vertical bars.

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 (ΔA₀) 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 ¹⁶⁶Hao 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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* 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 166Ho-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 RMT 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 153Sm-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 radiopharmaceutical 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 ($E_{\text{max}} = 0.81$ MeV [20%], 0.71 MeV [50%], and 0.64 MeV [30%] relative to $E_{\text{max}} = 1.8$ MeV of 166Ho) and its effects are largely limited to paratrabecular bone, sparing the central core of long bones and much of the BM.
space. However, a prescient study with $^{166}$Ho EDTMP gave encouraging results.22,23 $^{166}$Ho is better suited for marrow-ablative purposes. It is primarily a $\beta$ emitter with a higher energy ($E_{\max} = 1.83$ 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 cytodestruction with resultant aplasia and severe pancytopenia. Hematologic recovery occurred in dogs after autologous BMT, but not in nontransplanted controls.

Forty 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 al23 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.

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Bone marrow transplantation in dogs after radio-ablation with a new Ho-166 amino phosphonic acid bone-seeking agent (DOTMP)

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