

Comparison of Systemic Toxicities of ^{177}Lu -DOTMP and ^{153}Sm -EDTMP Administered Intravenously at Equivalent Skeletal Doses to Normal Dogs

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Bone-seeking radiopharmaceuticals have been used to effectively treat cancer arising from and metastasizing to bone in humans and dogs. The rate of complete tumor control is low, and the geographic distribution of available compounds is limited by their half-lives. This experiment was done to evaluate in normal dogs the toxicity of ^{177}Lu -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene phosphonate (^{177}Lu -DOTMP) used as a potential therapeutic radiopharmaceutical. **Methods:** Four normal purpose-bred dogs were administered ^{177}Lu -DOTMP at a dose of 8.14 MBq/kg and monitored for 84 d for evidence of toxicity in the bone marrow and vital organs. **Results:** No statistically significant alterations in the biochemical profile, white blood cell count, or platelet count were observed in any dog. Very mild decreases in the red cell count were seen on day 84. No microscopic evidence of toxicity was present at necropsy. **Conclusion:** The dogs receiving ^{177}Lu -DOTMP tolerated the administration and the effects of the compound without apparent clinical toxicity. The results of this experiment support the further evaluation in tumor-bearing dogs of ^{177}Lu -DOTMP as a potential therapy for metastatic bone cancer and primary bone tumors in humans and dogs.

Key Words: ^{177}Lu -DOTMP; dogs; bone tumors; bone-seeking radiopharmaceuticals

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For over a decade, ^{153}Sm -ethylenediaminetetramethylene phosphonate (^{153}Sm -EDTMP) (Quadramet; Cytogen

Corp.) has been successfully used to treat human cancer metastatic to bone and canine primary bone tumors (1–4). The first reported study in bone tumor-bearing dogs resulted in the control of clinical signs of the tumor in 32 of 40 dogs and the total resolution of the tumor in 7 of those dogs (2). Subsequently, a South African study of 9 dogs receiving ^{153}Sm -EDTMP at a dose of 37 MBq/kg reported that one dog experienced a complete tumor response and that 7 of the 9 dogs experienced palliation of clinical pain signs (3). More recently, an Australian group reported the results for a series of 35 dogs that received up to 4 doses of ^{153}Sm -EDTMP at 37 MBq/kg (4). Of 32 dogs with appendicular skeletal tumors, 63% exhibited subjectively evaluated improvement in lameness severity (4). The median survival of 100 d for dogs receiving ^{153}Sm -EDTMP was statistically similar to that of a historical cohort treated with the standard therapy of amputation (4). In a prospective, randomized study of 40 dogs with appendicular osteosarcomas, treatment with ^{153}Sm -EDTMP was palliative and allowed for a delay of amputation, in some cases indefinitely; in these cases, the metastatic pattern appeared to be altered from the typical pattern, with fewer dogs developing pulmonary metastases (5). Delays in administration of chemotherapy were common in these dogs because of the myelosuppressive effect of ^{153}Sm -EDTMP.

A bone-seeking radiopharmaceutical with less impact on bone marrow function and a higher complete response rate is desirable. There is a need to evaluate the properties of other radionuclides that could yield greater tumor control and interface more flexibly with other therapies.

Recently, work performed with a mouse model demonstrated the useful skeletal targeting properties of ^{177}Lu -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene phosphonate (^{177}Lu -DOTMP) (6,7). The polyazamacrocyclic ligand framework may offer a complex more kinetically inert than that exhibited by ^{153}Sm -EDTMP (8).

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Currently, ^{153}Sm -EDTMP is chelated at a ratio of 80 mM EDTMP and 0.3 mM ^{153}Sm (9). The excess ligand results in a risk of chelation of plasma calcium and delivery of nonradioactive phosphonic acid to bone. ^{177}Lu -DOTMP may offer chemical and physical advantages for treating cancers arising in or metastasizing to bone. Biodistribution studies with non-tumor-bearing mice revealed skeletal uptake of approximately 16% of the injected dose of ^{177}Lu -DOTMP per gram of bone, with the remainder of the dose being excreted rapidly in the urine (6,7). This pattern of distribution is like that of ^{153}Sm -EDTMP and lends the drug its skeletal tumor-targeting properties.

With its large cross section (2,100 barns), ^{177}Lu can be produced at adequate specific activities by irradiation of the natural lutetium target in moderate-flux reactors, and its long half-life allows for shipment over long distances to remote regions (10). Additionally, its 208-keV γ -emission (11% abundance) allows for imaging of its distribution to facilitate dose calculation. An effective, safe bone-targeting agent with a half-life of sufficient length for worldwide distribution would be a significant advancement in the therapy of bone-associated cancers.

Dogs with osteosarcomas were previously demonstrated to be important models of naturally occurring disease in humans (11). A demonstration of acceptable toxicity of ^{177}Lu -DOTMP would allow clinical trials to be performed in tumor-bearing dogs. ^{153}Sm -EDTMP at a dose of 37 MBq/kg has been demonstrated to be safe and effective as a therapy for humans with metastatic bone cancer and for dogs with primary or metastatic bone cancer (2,12,13). At this dose, the organ with dose-limiting toxicity is the bone marrow. Circulating white blood cell (WBC) and platelet counts routinely, and often dramatically, are suppressed in dogs after treatment with ^{153}Sm -EDTMP (2–4). The counts predictably return to normal within approximately 4 wk (2,12).

The present experiment was designed to compare ^{177}Lu -DOTMP delivered to normal dogs at a skeletal dose equivalent to the injection of ^{153}Sm -EDTMP at a dose of 37 MBq/kg. It was our hypothesis that ^{177}Lu -DOTMP would be clinically tolerated by the dogs and would result in less myelotoxicity than ^{153}Sm -EDTMP because of the lower β -energy emission of ^{177}Lu (maximum, 0.497 MeV) than of ^{153}Sm (0.810 MeV). These properties would be expected to result in significantly decreased cross fire in the marrow cavity and lower predicted marrow toxicity while still allowing the same dose to be delivered to hydroxyapatite-containing bone. The results of this experiment will serve as the basis for a clinical trial of ^{177}Lu -DOTMP in tumor-bearing dogs.

MATERIALS AND METHODS

The normal-dog toxicity study was conducted in compliance with a protocol approved by the Animal Care and Use Committee of the University of Missouri—Columbia Animal Care Quality Assurance Office.

Radiochemistry

DOTMP was purchased from Macrocyclics and used as received. All reagents were prepared in MilliQ water (Millipore Corp.) unless otherwise mentioned. ^{177}Lu in 0.05 M HCl (~ 215.5 MBq/ μL) was obtained from the University of Missouri Research Reactor. A DOTMP solution at a concentration of 48.9 $\mu\text{g}/\mu\text{L}$ was prepared by dissolving DOTMP (100 mg) in MilliQ water (2 mL) and adjusting the pH to 5.5 with concentrated NaOH (45 μL). The Lu-DOTMP complex was prepared by adding ^{177}Lu (1,724.2 MBq, 8 μL) to an ammonium acetate (0.2 M, pH 9, 200 μL)–buffered solution. DOTMP was added to yield a concentration of ~ 5.2 MBq/ μg (333 μg , 6.8 μL) (Lu:DOTMP ratio, 1:48) or 1.665 $\mu\text{g}/\mu\text{L}$. The reaction mixture was vortexed and heated for 1 h at 100°C. The radiolabeling concentration was ~ 5.2 MBq/ μg (Lu:DOTMP ratio, 1:48).

Quality control was achieved by loading part of the diluted reaction mixture (30 μL , ~ 2.5 MBq) into an SP-Sephadex (40–120 μm ; Sigma) strong cation exchange column (0.4 mL of resin) and eluting it with saline (2×9 mL). The ^{177}Lu -DOTMP complex passed through the column, and any unbound ^{177}Lu was retained. The labeling was calculated as the percentage of the initial activity that was retained on the column and was found to be 99.41%. The reaction mixture was diluted to 92.5 MBq/mL by adding saline (18.30 mL) and adjusting the pH to 7.6 with NaOH (1 M) for administration (6).

Dosimetry

The clinically tolerable dose of ^{153}Sm -EDTMP (37 MBq/kg) has been estimated to deliver a total skeletal dose of approximately 250 cGy in a dog without a skeletal lesion (1–3,14). For comparison with previous evaluations of ^{153}Sm -EDTMP toxicity, the dose of ^{177}Lu -DOTMP delivering a skeletal isodose of 250 cGy was calculated. The assumptions for the calculation were as follows: all β -energy, all Auger electron energy, and 5% of γ -energy would be absorbed within 0.20 cm of the disintegration point; the energy deposition from $^{176\text{m}}\text{Lu}$ and $^{177\text{m}}\text{Lu}$ would be less than 1% of the total; the average β -energy is 34% of the maximum dose (D_{max}); uptake would be approximately 16% of the dose injected into the skeleton (6,7); the density of bone is 1.7 times greater than that of tissue; the skeletal mass is approximately 9% of the total dog body mass; and the radiation dose would be evenly distributed in bone. An uptake of 16% is very conservative compared with uptake in a rat model and relative to reported ^{153}Sm -EDTMP uptake in humans and dogs (9,15,16).

With a formula for calculating total energy deposition within the tissue of the skeleton from all emissions from ^{177}Lu decay, a dose of ^{177}Lu -DOTMP of 37 MBq/kg would be expected to yield a total skeletal dose of 1.1 Gy. The dose in centigrays was calculated as the total energy deposited by use of the megaelectron volts per disintegration, the number of disintegrations per 2 mean lives, the dose in megabecquerels per kilogram of body weight, the percentage uptake, the grams of bone per kilogram of body weight, and the deposited energy per gram of bone. The formula for the calculation of the total skeletal dose was as follows: dose (cGy) = $\{[0.589832 \text{ MeV} \times (37,000,000 \text{ dps} \times 86,400 \text{ s/d}) \times (1/0.693 \times 6.74) \times 2] \times (8.14 \text{ MBq/kg} \times 0.16)\}/(90 \times 62,420,000)$. Therefore, a dose of ^{177}Lu -DOTMP of 8.14 MBq/kg was calculated to deliver a dose of approximately 250 cGy to the skeleton of a normal dog.

Normal-Dog Toxicity Study

Four (2 male, 2 female) nonanesthetized, healthy, skeletally mature, purpose-bred laboratory dogs purchased from Marshall BioResources were injected intravenously with ^{177}Lu -DOTMP at approximately 8.14 MBq/kg after a 16 d acclimatization period. The dogs were housed in a radiation isolation facility until they met release criteria, according to the radiation safety protocol approved by the University of Missouri Radiation Safety Office; when they met the criteria, they were moved to a standard laboratory animal housing facility. Each animal received a physical examination before being injected. One male dog was treated for 14 d with cephalexin for a superficial pyoderma.

All dogs were monitored twice daily for activity level, appetite, water consumption, urination, and evidence of vomiting or diarrhea. Toxicity in the bone marrow and internal organs was assessed with the following examinations. From each dog, 5 mL of blood were collected for a complete blood count and a plasma biochemical profile before injection with the agent and on days 7, 14, 28, 56, and 84 of the experiment. Urine was collected simultaneously for complete urinalysis. A bone marrow aspirate for cytologic examination and a biopsy sample for histopathologic examination were collected under sedation before administration of the agent and on days 14 and 84. All bone marrow analyses were performed by one pathologist. All dogs were euthanized after blood and urine collection on day 84, and necropsies were performed immediately by one pathologist.

Statistical Analysis

Time points for data collection corresponded to time points from a previous study at the University of Missouri—Columbia of ^{153}Sm -EDTMP administered intravenously to normal beagle dogs at several dosages and cold ^{152}Sm -EDTMP (12). These earlier data, archived at the University of Missouri, were used for a direct statistical comparison of the toxicities of ^{177}Lu -DOTMP and ^{153}Sm -EDTMP at the dose that is administered clinically to both dogs and humans with skeletal neoplasia. The data were analyzed with a mixed-model repeated-measures ANOVA for changes from the baseline within a group of dogs (17). A mixed-model repeated-measures ANOVA with the Fisher least square difference was used to test differences among groups of dogs (17). Differences were considered significant at a P value of ≤ 0.05 .

RESULTS

Physical Examinations

The physical characteristics of the dogs are shown in Table 1. All dogs remained bright and active after the administration of ^{177}Lu -DOTMP. At no time did any of the dogs display behavior suggesting physical discomfort. All dogs displayed

normal appetite and thirst throughout the course of the experiment. Body weight did not change by more than 10% in any dog. No vomiting or diarrhea events were observed in any dog during the course of the experiment.

Urinalysis

There was no evidence of preexisting or developing urinary tract infection in the dogs. Each dog registered a mild degree of proteinuria in the presence of concentrated urine at some point during the course of the experiment. The highest median value for proteinuria (3+ on a 1–4+ scale) was seen on day 7, but this value was not statistically different from the values on the other days ($P = 0.147$). Given the small sample size and a P value of less than 0.15, it must be remembered in future studies that the administration of ^{177}Lu -DOTMP may be associated with proteinuria in the first week after administration, suggesting the possibility that rare nephrotoxicity may occur. The urine specific gravity was greater than 1.025 at all time points except the initial check in one dog and the final check in another dog, suggesting no loss of urine-concentrating ability after the administration of ^{177}Lu -DOTMP. For each dog, serum urea nitrogen and plasma creatinine levels were well within the reference intervals at those time points. No casts were seen on urinalysis for any dog. There was no clinical or urinalysis evidence of primary kidney or bladder damage from the administration of ^{177}Lu -DOTMP.

Plasma Enzyme Values

No statistically significant change from values on day 0 was observed in any plasma biochemical analysis for dogs receiving ^{177}Lu -DOTMP. No alteration from the baseline was seen in the plasma activity of alkaline phosphatase (ALP), an enzyme produced by biliary tract damage, bone metabolism, and corticosteroid induction (Fig. 1). Compared with all dogs receiving ^{153}Sm -EDTMP and control dogs in the previous study (12), dogs receiving ^{177}Lu -DOTMP in the present study had statistically significantly lower plasma ALP activities on day 7 ($P < 0.0001$). Plasma ALP activities were also lower for ^{177}Lu -DOTMP than for ^{153}Sm -EDTMP at both 37 MBq/kg ($P = 0.015$) and 74 MBq/kg ($P = 0.032$) on day 28 and for ^{153}Sm -EDTMP at 74 MBq/kg ($P = 0.044$) on day 84. For dogs receiving ^{177}Lu -DOTMP, there was no observable evidence of biliary tract damage resulting from the administration of the radiopharmaceutical.

TABLE 1
Characteristics of Dogs on Day of Administration of ^{177}Lu -DOTMP

Dog	Sex	Weight (kg)	Temperature (°C)	Pulse (beats per minute)	Respirations (breaths per minute)	Physical examination findings	Dose administered (MBq)	MBq/kg
1	M	27.4	38.4	110	12	Small, benign dermoid, right eye	228.7	8.35
2	M	26.9	38.8	110	12	Bacterial dermatitis, feet and scrotum	240.1	8.93
3	F	27.4	39.5	95	24	Normal	241.2	8.80
4	F	15	38.7	130	16	Normal	130.6	8.71

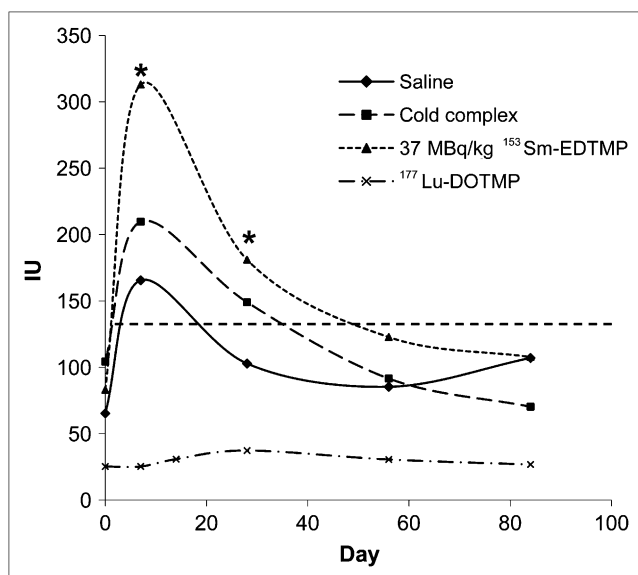


FIGURE 1. Mean plasma ALP activity in control dogs, dogs receiving ^{177}Lu -DOTMP, and dogs receiving ^{153}Sm -EDTMP at 37 MBq/kg. Plasma ALP activity did not vary from baseline for dogs receiving ^{177}Lu -DOTMP. Statistically significant differences in means were present on days 7 and 28 (asterisks). Biliary tract, bone, and corticosteroid-inducible ALP contributed to plasma ALP activity. Horizontal dashed line represents upper limit of laboratory reference interval at time of ^{177}Lu -DOTMP experiment. * $P < 0.05$. IU = international units.

No alteration from the baseline was seen in the plasma activity of alanine aminotransferase (ALT), an enzyme released during hepatocellular damage (Fig. 2). There was no observable evidence of hepatocellular damage resulting from the administration of ^{177}Lu -DOTMP in the normal dogs.

In previous rat studies, ^{177}Lu -DOTMP not deposited in the skeleton was excreted almost entirely in the urine (8). Plasma creatinine and urea nitrogen levels were the most direct indicators of renal function measured in the biochemical panel (Fig. 3). In the 4 dogs receiving ^{177}Lu -DOTMP, all creatinine measurements were well within the reference interval, and the creatinine level did not change by more than 0.1 mg/dL in any dog during the course of the experiment. The plasma urea nitrogen level was also well within the reference range. No dog receiving ^{177}Lu -DOTMP had a plasma urea nitrogen level outside the reference range at any time during the experiment, and there was no statistically significant change in the plasma urea nitrogen level from that on day 0. There was no statistically significant difference between dogs receiving ^{177}Lu -DOTMP and control dogs or dogs receiving ^{153}Sm -EDTMP at 37 MBq/kg in the previous study (12). For dogs receiving ^{177}Lu -DOTMP, no evidence of loss of renal function or renal damage was revealed by the biochemical analysis.

Blood Cell Counts

The total WBC count in dogs receiving ^{177}Lu -DOTMP was not statistically significantly different from that on day

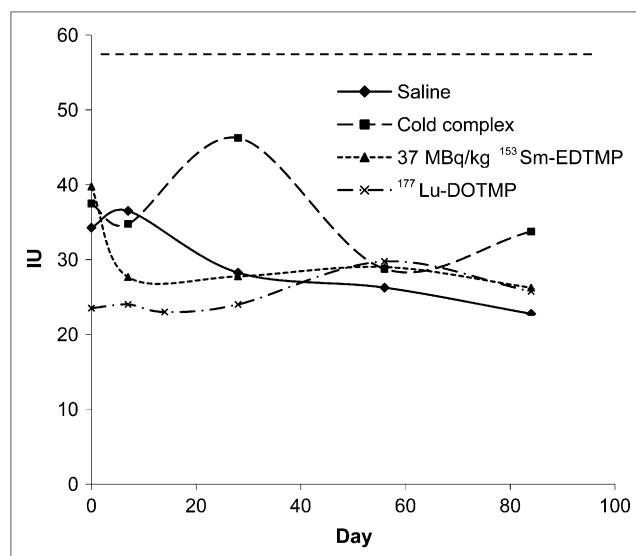


FIGURE 2. Mean plasma ALT activity in control dogs, dogs receiving ^{177}Lu -DOTMP, and dogs receiving ^{153}Sm -EDTMP at 37 MBq/kg. Plasma ALT activity did not vary from baseline for dogs receiving ^{177}Lu -DOTMP, and activity remained within reference interval for all groups. Horizontal dashed line represents upper limit of laboratory reference interval at time of ^{177}Lu -DOTMP experiment. IU = international units.

0 at any time during the experiment (Fig. 4). The starting WBC count on day 0 was statistically significantly lower in dogs receiving ^{177}Lu -DOTMP than in dogs receiving ^{153}Sm -EDTMP at 18.5 MBq/kg ($P = 0.005$), 74 MBq/kg ($P < 0.0001$), and 37 MBq/kg (4 doses) ($P = 0.001$). The WBC count on day 56 in dogs receiving ^{177}Lu -DOTMP

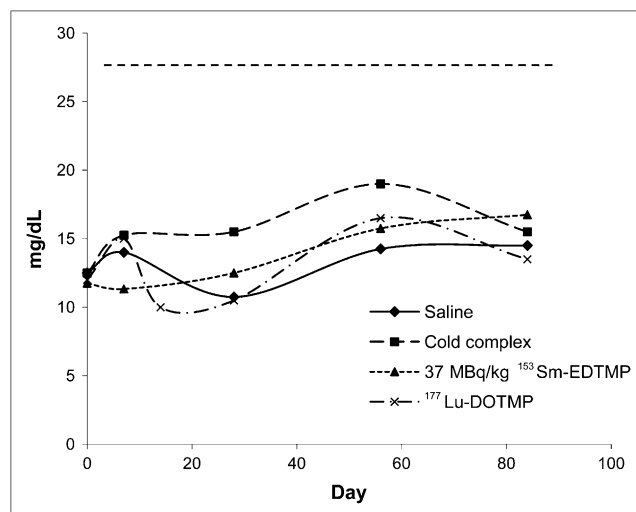


FIGURE 3. Mean plasma urea nitrogen levels in control dogs, dogs receiving ^{177}Lu -DOTMP, and dogs receiving ^{153}Sm -EDTMP at 37 MBq/kg. Plasma urea nitrogen activity did not vary from baseline for dogs receiving ^{177}Lu -DOTMP, and activity was within reference interval for all groups. Horizontal dashed line represents upper limit of laboratory reference interval at time of ^{177}Lu -DOTMP experiment.

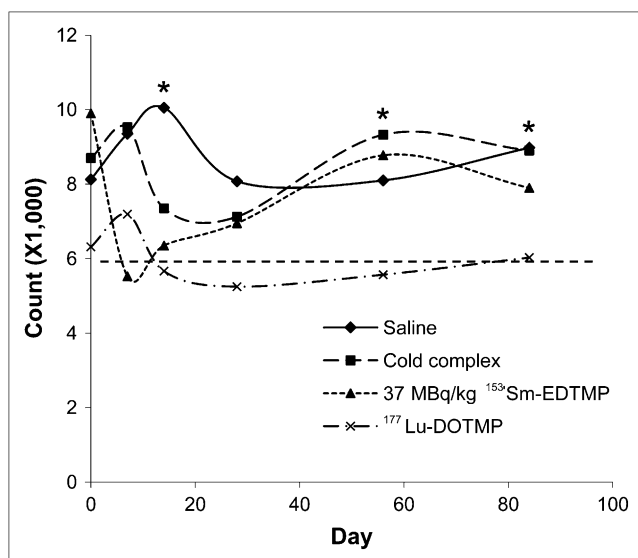


FIGURE 4. Mean WBC counts in control dogs, dogs receiving ¹⁷⁷Lu-DOTMP, and dogs receiving ¹⁵³Sm-EDTMP at 37 MBq/kg. WBC counts did not vary from baseline for dogs receiving ¹⁷⁷Lu-DOTMP, but statistically significant differences in means for other groups were present on days 14, 54, and 84 (asterisks). Horizontal dashed line represents lower limit of laboratory reference interval at time of ¹⁷⁷Lu-DOTMP experiment. **P* < 0.05.

was significantly lower than that in dogs receiving a cold complex (*P* = 0.010) or ¹⁵³Sm-EDTMP at both 37 MBq/kg (*P* = 0.028) and 74 MBq/kg (*P* = 0.018). In addition, the WBC count on day 84 in dogs receiving ¹⁷⁷Lu-DOTMP was lower than that in dogs receiving saline (*P* = 0.044) or a cold complex (*P* = 0.049). Overall, unlike ¹⁵³Sm-EDTMP, ¹⁷⁷Lu-DOTMP caused no significant decrease in the circulating WBC count.

Similarly, no statistically significant differences from the baseline in the circulating platelet count were observed in dogs receiving ¹⁷⁷Lu-DOTMP (Fig. 5). The platelet count in one dog was always below the reference interval, the count for one dog dropped slightly below the reference interval on day 14, and the count for one dog dropped below the reference interval on day 14 and remained below the reference interval. The only statistically significant difference in the platelet count between dogs receiving ¹⁷⁷Lu-DOTMP and dogs receiving ¹⁵³Sm-EDTMP or control dogs was a lower mean platelet count on day 56 in dogs receiving ¹⁷⁷Lu-DOTMP than in dogs receiving ¹⁵³Sm-EDTMP at 37 MBq/kg (*P* < 0.0001).

Red blood cell production, as measured by the hematocrit, may have been mildly impaired by the administration of ¹⁷⁷Lu-DOTMP (Fig. 6). Compared with dogs receiving ¹⁵³Sm-EDTMP, dogs receiving ¹⁷⁷Lu-DOTMP had a higher hematocrit at all time points until day 84; at that time, a statistically significant decrease in the hematocrit was noted for dogs receiving ¹⁷⁷Lu-DOTMP (*P* = 0.0033). On this day, no group had a hematocrit lower than the reference interval.

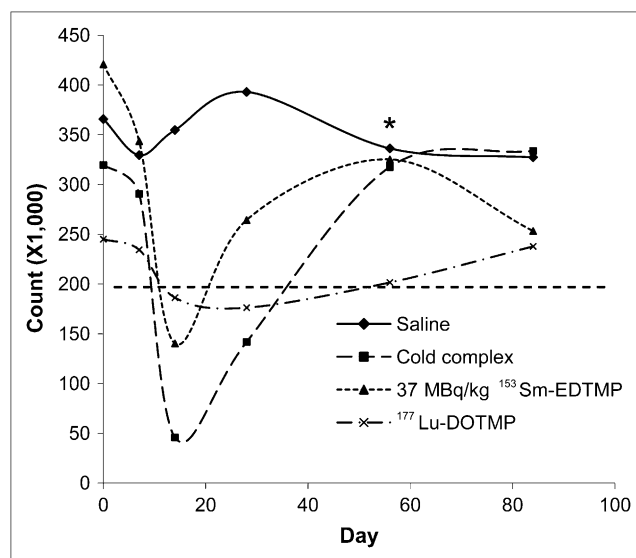


FIGURE 5. Mean platelet counts in control dogs, dogs receiving ¹⁷⁷Lu-DOTMP, and dogs receiving ¹⁵³Sm-EDTMP at 37 MBq/kg. Platelet counts did not vary from baseline for dogs receiving ¹⁷⁷Lu-DOTMP, but statistically significant differences in means for other groups were present on day 54 (asterisk). Horizontal dashed line represents lower limit of laboratory reference interval at time of ¹⁷⁷Lu-DOTMP experiment. **P* < 0.05.

No other alterations in biochemical parameters were identified in any of the dogs receiving ¹⁷⁷Lu-DOTMP (Fig. 7). The compound appeared to be well tolerated, with no organ toxicity being observed in plasma biochemistry, and caused minimal myelosuppression.

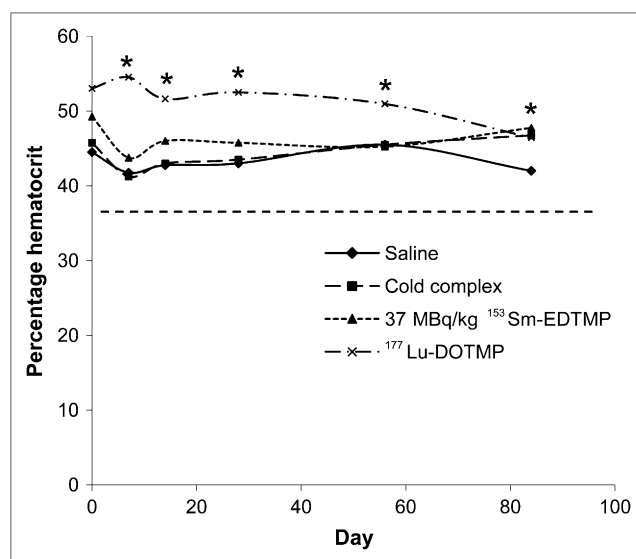


FIGURE 6. Mean hematocrit in control dogs, dogs receiving ¹⁷⁷Lu-DOTMP, and dogs receiving ¹⁵³Sm-EDTMP at 37 MBq/kg. Hematocrit decreased significantly from baseline for dogs receiving ¹⁷⁷Lu-DOTMP only on day 84 and was statistically significantly higher than that in other groups at all other time points (asterisks). Horizontal dashed line represents lower limit of reference interval for laboratory at time of ¹⁷⁷Lu-DOTMP experiment. **P* < 0.05.

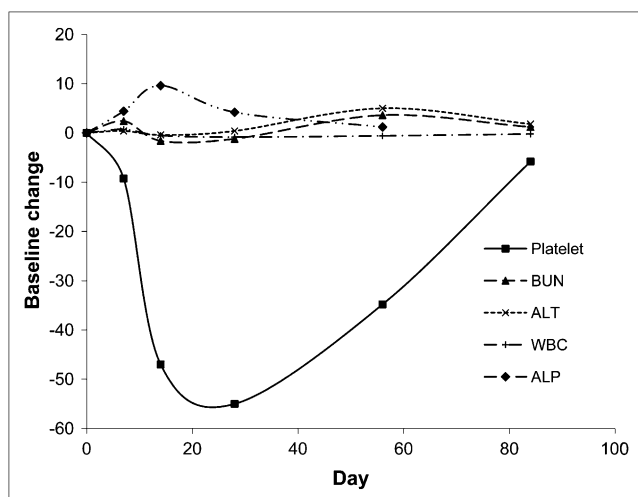


FIGURE 7. Changes from baseline for plasma platelet and WBC counts and ALT, ALP, and plasma urea nitrogen (BUN) levels in dogs receiving ^{177}Lu -DOTMP over course of experiment. No change was statistically significant, supporting minimal toxicity and myelosuppression resulting from tested dose of ^{177}Lu -DOTMP.

Bone Marrow Cytology

Overall, the findings of bone marrow cytologic examinations were unremarkable and appeared to be from normal animals. The cellularity was within the limits for a normal adult dog; only a mild decrease in overall cellularity was observed on day 14. On day 84, 2 dogs showed evidence of very mild dysplasia in the erythroid line, characterized by mild megaloblastic changes and late-stage mitosis. This finding coincided with the decrease in the hematocrit observed at the same time. The changes were very mild and were judged to be clinically insignificant but may reflect a mild regenerative response. Bone marrow histopathologic analysis did not reveal any further changes.

Necropsy Results

No lesions attributable to the administration of ^{177}Lu -DOTMP were seen on necropsy. The kidneys, liver, heart, lungs, thyroid, spleen, ureter, bladder, stomach, small intestine, large intestine, pancreas, adrenal glands, fat, skeletal muscle, gonads, uterus, nerve tissue, and bone marrow were examined. No pathologic lesions were seen in any of these tissues.

DISCUSSION

Dogs receiving ^{177}Lu -DOTMP tolerated the administration and effects of the compound without apparent clinical toxicity. No instances of vomiting or diarrhea, clinical lethargy, or behaviorally evident effects were observed. In contrast to the study of ^{153}Sm -EDTMP in normal beagle dogs, urinary tract infection was not observed in the cohort of dogs in the present study. It is possible that the longer half-life of the compound evaluated in the present study makes it less irritating to the bladder mucosa or less

suppressive of local mucocutaneous immunity, but the small cohort examined makes any potential differences purely speculative. There was no evidence of renal damage on urinalysis for any dog. All dogs maintained normal urine-concentrating ability, and no casts that would suggest tubular damage were present.

None of the dogs receiving ^{177}Lu -DOTMP had any statistically significant alteration from the baseline in plasma biochemistry. In contrast to dogs receiving ^{153}Sm -EDTMP, dogs receiving ^{177}Lu -DOTMP did not have an elevated plasma ALP level after the administration of the radiopharmaceutical. In the previous study of ^{153}Sm -EDTMP in normal beagle dogs, the increase in the ALP level was attributed to an increase in the level of the bone isoenzyme, although the latter was not measured (12). The lack of change in the ALP level after the administration of ^{177}Lu -DOTMP suggests that the source of the ALP in the original study may have been the biliary tract. Several other lines of evidence support this possibility. Aminobisphosphonates, similar in structure to EDTMP, were administered to normal dogs and resulted in a decrease in measured bone ALP activity (18). Furthermore, significant liver uptake was identified in an imaging biodistribution study of a single normal dog (15). Given the statistically significant difference in ALP levels between dogs receiving ^{153}Sm -EDTMP and those receiving ^{177}Lu -DOTMP, it is possible that the level of elimination of ^{177}Lu -DOTMP by the liver was lower. This topic should be addressed in imaging biodistribution studies of tumor-bearing dogs. No clinical toxicity resulting from biliary tract irritation has been reported for ^{153}Sm -EDTMP in humans or dogs. There was no statistically significant difference in plasma ALT activity after the administration of ^{177}Lu -DOTMP or ^{153}Sm -EDTMP. Neither compound appeared to cause hepatocellular damage.

There was no measurable effect of ^{177}Lu -DOTMP administration on renal function. Although minor differences were detected between dogs receiving ^{177}Lu -DOTMP and those receiving ^{153}Sm -EDTMP at 37 MBq/kg, neither group had plasma urea nitrogen measurements outside the reference interval. The presence of proteinuria may suggest possible renal glomerular or tubular damage, but no measurable alteration in function was observed. This finding should be carefully evaluated in future studies. These chelated lanthanide bone-seeking radiopharmaceuticals appear to cause no significant renal impairment at the current dose, even though renal elimination accounts for the vast majority of elimination of these agents from the body (10).

The administration of the tested dose of ^{177}Lu -DOTMP caused no apparent suppression of WBC lines in the dogs. Even when we adjusted the level of significance by accepting *P* values of less than 0.15, no significant decreases in WBC levels from the baseline were observed. This finding differs from the findings of the previous study evaluating ^{153}Sm -EDTMP in normal beagle dogs; all doses of ^{153}Sm -EDTMP resulted in statistically significant de-

creases in WBC counts from the baseline in that study (12). Although there were differences between dogs receiving ^{177}Lu -DOTMP and dogs receiving ^{153}Sm -EDTMP, dogs receiving ^{177}Lu -DOTMP began the experiment with lower WBC counts overall, and the fact that they did not change from the baseline, as the dogs receiving ^{153}Sm -EDTMP did, was most telling. This result supports our hypothesis that the lower β -energy of ^{177}Lu -DOTMP would cause less myelosuppression at a skeletal dose equivalent to a dose of ^{153}Sm -EDTMP of 37 MBq/kg. This hypothesis was further supported by the lack of suppression of platelet lines in dogs receiving ^{177}Lu -DOTMP. Although dogs receiving ^{153}Sm -EDTMP at 37 MBq/kg had significant decreases in their platelet counts, dogs receiving ^{177}Lu -DOTMP did not.

The primary indicator of the myelotoxicity of ^{177}Lu -DOTMP was reflected in the red cell lines. A statistically significant decrease in the hematocrit was measured on day 84. No other changes observed in the dogs explained this decrease, and cytologic examination of the marrow revealed dysplastic changes in the red cell lines at that time. Although there were statistically significant differences between dogs receiving ^{177}Lu -DOTMP and dogs receiving ^{153}Sm -EDTMP, all hematocrit measurements were within the reference range at all times. Neither compound displayed significant red cell line toxicity in these studies. However, future studies should include careful observation of participants for anemia developing late after ^{177}Lu -DOTMP administration.

There was no significant evidence of toxicity of the ^{177}Lu -DOTMP compound, either cytologically on bone marrow aspiration or histologically on necropsy. The only minor abnormality observed in bone marrow aspirates and biopsies was the mild red cell dysplasia on day 84. At necropsy, there was no evidence of damage to any of the organs of excretion, and histopathologic evaluation did not reveal any unexpected organ toxicity. These findings support the lack of documented toxicity in organs or marrow from the complete blood count, plasma biochemical analysis, and urinalysis. The skeletal dose reported here was intended to be very conservative to establish a minimum safe dose for veterinary clinical studies. The skeletal uptake of 16% is very conservative, and the uptake may, in fact, be much higher, delivering a higher total skeletal dose (9,15,16). Nevertheless, this dose was very well tolerated by the dogs in the present experiment.

There may be therapeutic advantages in the chemical and physical properties of ^{177}Lu -DOTMP. Polyazamacrocyclic ligand frameworks have been demonstrated to form more kinetically inert complexes than their acyclic analogs because of the macrocyclic effect (8). Unlike their acyclic counterparts, these ligands provide a degree of rigidity to the resulting metal complexes, thereby providing a higher degree of kinetic inertness and thus a lower level of metal release and toxicity in normal tissues. Because of the instability associated with the acyclic compounds, it is necessary to use excess ligand to prevent uptake of the radiocolloid in the

liver. The concentration of the excess ligand is critical, because an amount too low will result in high doses to the liver and an amount too high will result in calcium chelation and instant heart failure. The stability of the polyazamacrocyclic ligands for lanthanides means that no excess ligand is required and thereby simplifies the overall formulation of complexes and the development of standard protocols that can be easily translated globally. As supported by the data reported here, the physical properties of ^{177}Lu appear to result in diminished myelotoxicity. The mechanism for this feature may be the lower β -energy causing less cross fire damage to the active marrow or increased sublethal and potentially lethal damage repair in marrow cells resulting from the longer half-life of ^{177}Lu . In either case, it is possible that ^{177}Lu -DOTMP can be combined with chemotherapy to maximize tumor control and therapeutic flexibility.

Although the longer half-life of ^{177}Lu may result in the need for longer radiation isolation than is used with ^{153}Sm , other benefits may be realized with the novel, Lu-containing bone agent. First, transport to underserved areas is facilitated by the longer half-life. In many parts of the world, ^{153}Sm -EDTMP is not a viable treatment option, simply because the 46.3-h half-life of ^{153}Sm results in significant decay with long shipping times. Such decay would not be a problem with the 6.74-d half-life of ^{177}Lu . Additionally, many doctors are deterred from using ^{153}Sm -EDTMP because it is currently available for clinical use only from Wednesday through Friday. Administration may result in a flare response—an acute exacerbation of painful clinical signs—which often occurs over the weekend because of the dosing schedule. Furthermore, a reported low dose–rate reassortment phenomenon may provide a therapeutic advantage to longer-lived radionuclides against tumors in radiopharmaceutical cancer therapy (19,20). Although the dose calculated in this safety study is quite similar to an ^{153}Sm -EDTMP dose of 37 MBq/kg, the rate at which the dose is delivered is dramatically lower. As a result, a potential radiobiologic advantage may be gained as cancer cells are redistributed through the cell cycle and reoxygenated and as normal cells repair the sublethal damage. Both the early study of the bone tumor response to ^{153}Sm -EDTMP and the study of dogs with appendicular osteosarcomas were performed at the University of Missouri; a novel clinical study could be directly statistically compared with those studies (2,5). The results would provide strong preclinical data with which to pursue the evaluation of a product that is a follow-up to ^{153}Sm -EDTMP for human osteosarcomas and metastatic bone cancer.

CONCLUSION

The results of the present experiment support the further evaluation of ^{177}Lu -DOTMP in tumor-bearing dogs. No statistically measurable decreases in WBC or platelet counts occurred, and only late and minor effects on red cell numbers were observed. No toxicity in organs of excretion

was apparent on biochemical analysis or necropsy. The optimal dose would need to be established, and imaging parameters would need to be defined for patients with skeletal lesions. Skeletal uptake is known to occur at a higher level in patients with osteoblastic skeletal lesions, likely necessitating a higher dose than that reported here to be effective (1). Also, the minimal myelosuppression observed in the present experiment may suggest that a relatively higher radioactive dose to the skeleton would be well tolerated. Therefore, ^{177}Lu -DOTMP may offer more optimal tumor dose characteristics and combination therapy choices than the currently available ^{153}Sm -EDTMP.

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Comparison of Systemic Toxicities of ^{177}Lu -DOTMP and ^{153}Sm -EDTMP Administered Intravenously at Equivalent Skeletal Doses to Normal Dogs

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