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Significant Antitumor Effect from Bone-seeking, α-Particle-emitting ^{223}Ra Demonstrated in an Experimental Skeletal Metastases Model[1]

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ABSTRACT

The therapeutic efficacy of the α-particle-emitting radionuclide ^{223}Ra ($t_{1/2}$ = 11.4 days) in the treatment against experimental skeletal metastases in rats was addressed. Biodistribution studies, involving measurement of ^{223}Ra in bone marrow samples, were performed in rats after i.v. injection. To study the therapeutic effect of ^{223}Ra, an experimental skeletal metastases model in nude rats was used. Animals that had received 10^6 MT-1 human breast cancer cells were treated with ^{223}Ra doses in the range of 6–30 kBq after 7 days. The biodistribution experiment demonstrated that ^{223}Ra was selectively concentrated in bone as compared with soft tissues. The femur content of ^{223}Ra was 800 ± 56% of injected dose per gram tissue times gram body weight (b.w.; mean ± SD) 1 day after the injection and 413 ± 23% of injected dose per gram tissue times gram b.w. at 14 days. The femur:kidney ratio increased from (5.9 ± 2.0)·10^2 at 1 day to (7.2 ± 3.0)·10^2 at 14 days, whereas the femur:liver ratio increased from (6.2 ± 0.2)·10^2 to (9.1 ± 6.6)·10^2. Femur:spleen ratio increased from (8.1 ± 0.3)·10^2 at 1 day to (6.4 2.2)·10^3 at 14 days. The femoral bone: marrow ratio was 6.5 ± 2.1 after day 1 and larger than 15 at day 14. All of the tumor-bearing control animals had to be sacrificed because of tumor-induced paralysis 20–30 days after injection with tumor cells, whereas the rats treated with ≥10 kBq of ^{223}Ra had a significantly increased symptom-free survival ($P < 0.05$). Also 36% (5 of 14) of rats treated with 11 kBq and 40% (2 of 5) of rats treated with 10 kBq were alive beyond the 67-day follow-up period. No signs of bone marrow toxicity or b.w. loss were observed in the groups of treated animals. The significant antitumor effect of ^{223}Ra at doses that are tolerated by the bone marrow is most likely linked to the intense and highly localized radiation dose from α-particles at the bone surfaces. The results of this study indicate that ^{223}Ra should be additionally studied as a potential bone marrow-sparing treatment of cancers involving the skeleton.

INTRODUCTION

A substantial percentage of cancer patients suffer from skeletal metastases, including many patients with advanced lung, prostate, and breast carcinoma (1, 2). Established treatments such as hormone therapy, chemotherapy, and external radiotherapy induce temporary responses, but ultimately most patients relapse (3). Therefore, there is a great need for new therapies to relieve pain and inhibit tumor progression.

Bone-targeting radiopharmaceuticals have been studied clinically as a treatment of cancer that has metastasized to the skeleton (4–7). The compounds used were based on β-particle emitters (8) and lately also a conversion electron emitter (9). ^{89}Sr (Metastron) and ^{153}Sm-EDTMP[3] (Quadramet) have been made commercially available for palliation of pain because of skeletal metastases (7, 10, 11), whereas several others are under clinical investigation (10, 11). Because of the relatively long radiation range, a significant bone marrow exposure is associated with the use of β-emitters. This has restricted bone treatment with β-emitters to pain palliation (10, 11).

A possible alternative could be radionuclides emitting α-particles, which are characterized by short range (<0.1 mm) and highly energetic radiation compared with low LET radiation like β-particles and γ-rays. Thus, α-particles are classified as high-LET radiation, which is generally more lethal to cells (12). α-Emitters could potentially be used as sources in metabolically concentrated radionuclide therapy against skeletal metastases, because the short range of the α-particles could affect a reduction in bone marrow exposure. In a recent study, α-emitting ^{211}At and β-emitting ^{131}I linked to bone-seeking bisphosphonates (13) were compared as bone seeking agents. Dosimetry estimates indicated that the bone surface:bone marrow dose ratio could be increased ~3-fold by substituting the β-emitter with the α-emitter (13).

Other than ^{211}At, only a few α-particle-emitting radioisotopes are considered useful for biomedical applications (14). Among these is ^{212}Bi, which has been evaluated as a bone-seeking agent (15). However, the short physical half-life of ^{212}Bi ($t_{1/2}$ = 60 min) and because the time required for the bismuth phosphonate complex to localize in the target tissue is substantial, the use of this compound results in significant normal tissue exposure during the uptake and elimination phases (15). The β-emitter ^{212}Pb ($t_{1/2}$ = 10.6 h) was evaluated as an *in vivo* generator for ^{212}Bi. However, a substantial redistribution of ^{212}Pb and ^{212}Bi was observed (15) rendering this strategy less useful.

The α-emitting radionuclide ^{223}Ra ($t_{1/2}$ = 11.43 days) has been suggested for use in targeted radiotherapy (16). Because of the bone-seeking properties of alkaline earth elements (Ca, Sr, Ba, and Ra), Ra^{2+} may be useful for metabolically concentrated radionuclide irradiation of osseous sites *e.g.*, bone surfaces and skeletal metastases growth zones. These elements are probably concentrated because of inclusion in the bone mineral calcium hydroxy apatite, where radium and strontium could substitute calcium during mineral formation. In a recent study with ^{223}Ra in mice, biodistribution was measured at 1 h, 6 h, 24 h, 3 days, and 14 days after injection. A rapid uptake and prolonged retention was demonstrated in the skeleton, whereas soft tissue radioactivity cleared relatively rapidly. It was also demonstrated, by measuring ^{211}Bi and ^{223}Ra in bone samples immediately after sacrificing the animals, that only very small amounts (<1%) of daughter radionuclides redistributed from the site of ^{223}Ra decay in bone at day 3 after i.v. injection of ^{223}Ra.[4] This high retention of daughters is probably because of the rapid decay of the radon-219 daughter ($t_{1/2}$ = 3.96 s) preventing translocation, because animal experiments with ^{224}Ra, which radon-220 daughter has a $t_{1/2}$ = 56 s, showed that a large fraction of the daughter products escaped from bone (17, 18).

^{223}Ra has several favorable features, which could be exploited in radionuclide therapy. Firstly, it can be produced relatively inexpensively, readily, and in large amounts: sources of ^{227}Ac ($t_{1/2}$ = 21.7 years) could potentially be used as a long-term operating generator for ^{223}Ra

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[3] The abbreviations used are: EDTMP, ethylene *diamine N,N′*-tetra(methylene) phosphonic acid; b.w., body weight; % ID/g, percentage of injected dose per gram tissue; % ID·g/g, percentage of injected dose per gram tissue × gram body weight; LET, linear energy transfer.

[4] G. Henriksen, D. R. Fisher, J. C. Roeske, Ø. S. Bruland, and R. H. Larsen. Targeting of osseous sites with α emitting ^{223}Ra: comparison with the β emitter ^{89}Sr in mice, submitted for publication.

(19).^{227}Ac can in turn be produced by neutron irradiation of relatively commonly available ^{226}Ra. Secondly, ^{223}Ra has a physical half-life of 11.4 days, which provides sufficient time for preparation, distribution, including long distance shipment, and administration of the α-emitting radionuclide. Thirdly, ^{223}Ra decays through a chain of progeny (Fig. 1) with the emission of approximately 28 MeV of energy. The fraction of energy borne by particulate radiation emitted as α-particles is ~96%.

In the present paper a significant antitumor effect of ^{223}Ra on experimental skeletal metastases is reported. The model used was based on the injection of human cancer cells to the systemic circulation of athymic nude rats. MT-1 cells, which is an estrogen and progesterone receptor-negative human breast cancer cell line, were used in the study. This model consistently exhibit bone and bone marrow metastases after intracardial injection, as well as tumors to the spinal cord and some soft tissues. Animals developed symptoms like hind leg paralysis or inactivity combined with a kyfotic (hunchback) posture after a mean lag time of 22.1 days. In addition, skeletal tumors and also lesions in soft tissue organs were frequently observed. Microscopical examination of rats injected with MT-1 cells revealed large masses of tumor cells replacing the normal bone marrow and eroding the bony part of the spine. Tumor cell deposits were also found in the spinal cord, tibia, adrenal gland, lungs, and brain (20). To confirm that the biodistribution data of ^{223}Ra in rat was similar to data obtained from a biodistribution study in mice,[4] a limited study was performed with rats at 1 day and 14 days after injection of ^{223}Ra.

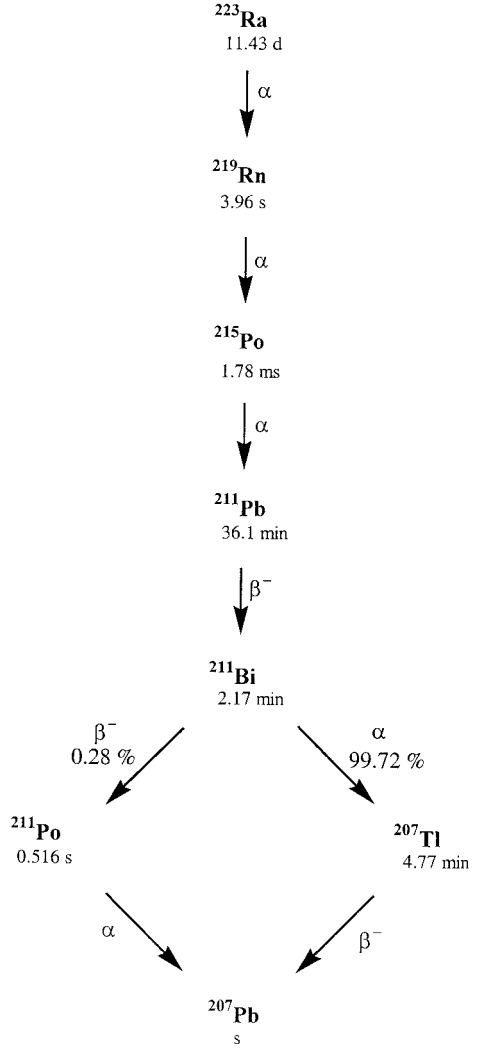

Fig. 1. Decay of ^{223}Ra.

MATERIALS AND METHODS

Radionuclide Production. ^{223}Ra was produced using a ^{227}Ac-based generator system as described elsewhere (19). Briefly, ^{227}Ac and ^{227}Th were immobilized on the actinide-selective extraction chromatographic material Dipex-2 (EiChrom, Darien, IL), which is based on *P,P′*-di(2-ethylhexyl)methanediphosphonic acid. This method allows highly effective retention of actinium and thorium at conditions where ^{223}Ra elutes (19). After elution of ^{223}Ra from the generator in 1 M HNO_3, ^{223}Ra was concentrated on a column of 3 mm inside diameter and length of 20 mm, containing 0.1 g of AG 50W-X12 cation exchange resin (Bio-Rad, Hercules, CA) at a flow rate of 2 ml/cm^2·min Thereafter, the column was washed with 10 ml 1 M HNO_3. ^{223}Ra was stripped from the column with 2–3 ml 8 M HNO_3.

The eluate containing ^{223}Ra was evaporated to dryness, and thereafter 5 mM sodium citrate (pH 7.4) was added followed by filtration through sterile 0.22-μm nylon filters (Nalgene, Rochester, NY). The final solution used for injection had a ^{223}Ra activity concentration of 25–150 kBq/ml in 5 mM sodium citrate.

Biodistribution Experiment. Nude rats (Han *rnu/rnu*: Rowett) with a b.w. of 120–150 g ($n = 4$) were used in the biodistribution experiment. This was slightly heavier than the animals used for therapy. ^{223}Ra was administered by injection into the tail vein using 200 μl of a 5 mM sodium citrate solution containing 10 kBq of ^{223}Ra. Animals were sacrificed and dissected either at 24 h or at 14 days after injection.

The tissue uptake of ^{223}Ra was measured with its progeny at radioactive equilibrium. Hence, samples were stored for a time interval corresponding to at least five half-lives of ^{211}Pb before the measurement was performed. Two different procedures were used: (*a*) tissue samples were measured by using a NaI(Tl) well-type detector (Harshaw Chemie BV, De Meern, Holland) combined with a Scaler Timer ST7 digital unit (NE Technology Ltd., Reading, United Kingdom); and (*b*) to improve counting statistics for samples with lower quantities of ^{223}Ra, some tissues were recounted using the more sensitive but also more laborious and waste producing liquid scintillation counting method. For this procedure, soft tissue samples were dissolved by adding 1–3 ml of Soluene 350 (Packard, BioScience BV, Groningen, Holland) per 100 mg tissue, and bone samples were dissolved in $HClO_4$:H_2O_2 1:2 (v/v). All of the tissue samples were kept at 50°C until they were completely dissolved. When required, nontransparent soft tissue samples were bleached by adding H_2O_2. Finally, Instagel Plus II scintillation mixture (Packard) was added, and the samples were then stored in the dark to allow decay of luminescence.

Samples of ^{223}Ra in equilibrium with its daughter nuclides were used as counting references.

Detection validation studies with the two methods showed a good agreement between the two radioactivity counting procedures.

Activity ratio between two tissues was calculated as mean ± SD by using the ratios for each individual animal as the input values.

Therapy Study. Tumors were established in nude rats (Han: *rnu/rnu* Rowett) by intraventricular injection of ~1 × 10^6 MT-1 breast cancer cells into the left side of the heart (20).

The effect of ^{223}Ra in prolonging symptom-free survival of rats bearing experimental breast cancer skeletal metastases was studied for two treatment regimens. In treatment regimen A, animals were treated 7 days after tumor cell inoculation. At this time animal b.w.s varied between 80 and 110 grams, and animals were randomized in groups so the average weight within a group was ~100 g at the time of treatment. Each animal in the treatment groups received either 6 or 11 kBq of ^{223}Ra administered by tail vein injection, whereas animals in the control group were injected with an equivalent volume of 5 mM sodium citrate solution.

For treatment regimen B, in an effort to inhibit bone erosion, which could possible remove ^{223}Ra from the tumor-affected skeletal area, the bone resorption inhibitor, 3-amino-1-hydroxypropylidene bisphosphonate, di-sodium salt (Aredia, Novartis AG, Switzerland), a bisphosphonate used against skeletal complications of cancer (21), was added as a supplemental treatment to ^{223}Ra. Animals in the treatment groups were treated on day 7 after tumor cell inoculation, which is in accordance with previous studies using this model

(Table 1), by i.v. injection of 5, 10, or 30 kBq of ^{223}Ra. The following day, 1.5 mg/kg b.w. of Aredia, which is the standard dose for humans, was administered by i.v. injection. Animals in the control group received 5 mM sodium citrate solution on day 7 and 1.5 mg/kg b.w. of Aredia on day 8.

Animals were sacrificed when symptoms caused by tumor growth appeared, *i.e.,* when they developed hind-leg paralysis or became inactive with a kyfotic (hunchback) posture. Symptom-free animals were followed for at least 50 days after the treatment. The significance of treatment response was related to symptom-free survival and evaluated using the Wilcoxon rank sum test.

Toxicity Study. In a separate experiment, conventional Balb/c mice were injected with 1 MBq/kg b.w. of ^{223}Ra and observed for short and longer term (90 days) to investigate if possible life-threatening bone marrow toxicity would occur and to demonstrate that the use of α-emitter could spare the bone marrow even for high average skeletal doses and extremely high bone surface doses.[4]

All of the procedures and experiments involving animals were approved by the National Animal Research Authority and carried out according to the European Convention for the Protection of Vertebrates Used for Scientific Purposes.

RESULTS

A high and selective uptake of ^{223}Ra in bone was observed in the biodistribution study in nude rats (Fig. 2). The bone uptake was as follows: femur, 6.2 ± 0.2 and 3.0 ± 0.3; spine, 3.5 ± 0.1 and 2.6 ± 0.1; rib, 2.5 ± 1.1 and 1.7 ± 0.3% ID/g at the 24-h and 14-day point, respectively. For soft tissues the following was found: blood, $(2.2 \pm 0.5) \times 10^{-3}$ and $(6.1 \pm 1.6) \times 10^{-3}$; kidney, $(1.1 \pm 0.3) \times 10^{-2}$ and $(4.5 \pm 0.2) \times 10^{-3}$; liver, $(1.0 \pm 0.3) \times 10^{-3}$ and $(4.1 \pm 0.3) \times 10^{-3}$; spleen, $(1.3 \pm 0.7) \times 10^{-2}$ and $(1.3 \pm 1.5) \times 10^{-3}$; large intestine, $(2.9 \pm 0.1) \times 10^{-2}$ and $(4.9 \pm 1.1) \times 10^{-3}$ % ID/g at the 24-h and 14-day time points, respectively.

Table 1 *Comparison of the effect of different treatment modalities in the MT-1 experimental breast cancer metastases model*[a]

Therapy agent	Long-term surviving rates[b]		Reference
	Fraction	%	
Cisplatin[c]	0/4	0	20
Doxorubicin[d]	0/5	0	25
425.3-PE conjugate[e]	1/5	20	25
BM-7-PE conjugate[f]	0/5	0	25
Aredia	0/5	0	Current study
^{131}I-bisphosphonate 200 MBq/kg[g]	0/5	0	26
^{131}I-bisphosphonate 300 MBq/kg[g]	0/5	0	26
^{131}I-bisphosphonate 400 MBq/kg[g]	0/5	0	26
^{223}Ra 60 kBq/kg[h]	1/5	20	Current study
^{223}Ra 110 kBq/kg[h]	5/14	36	Current study
^{223}Ra 50 kBq/kg[i]	1/5	20	Current study
^{223}Ra 100 kBq/kg[i]	2/5	40	Current study
^{223}Ra 300 kBq/kg[i]	2/5	40	Current study

[a] Tumors were established in nude rats by intraventricular injection of $\sim 1 \times 10^6$ MT-1 breast cancer cells into the left side of the heart. Therapy agents were administered by i.v. injection.

[b] Survival >50 days after tumor cell inoculation.

[c] Animals were treated with cisplatin (4 mg/kg) on day 7 and day 14 after tumor cell inoculation.

[d] Animals were treated with doxorubicin (4 mg/kg) on day 7 and day 14 after tumor cell inoculation.

[e] Animals were treated with antiepidermal growth factor antibody 425.3-*Pseudomonas* exotoxin A conjugate (20 μg/animal) on day 7 after tumor cell inoculation. Follow-up period: ≥ 83 days.

[f] Animals were treated with anti-MUC-1 mucin antibody BM-7-*Pseudomonas* exotoxin A conjugate (20 μg/animal) on day 7 after tumor cell inoculation.

[g] ^{131}I-labeled bisphosphonate with similar bone uptake as ^{223}Ra. Animals were treated 7 days after tumor cell inoculation.

[h] On day 7 after tumor cell inoculation, the treatment group received ^{223}Ra administered by tail vein injection, whereas animals in the control group were injected with 5 mM sodium citrate solution. Follow-up period was 67 days.

[i] The treatment groups received ^{223}Ra administered by tail vein injection on day 7 after tumor cell inoculation. The following day, 1.5 mg/kg body weight of 3-amino-1-hydroxypropylidene bisphosphonate, di-sodium salt (Aredia) was administered by i.v. injection. Animals in the control group were injected with 5 mM sodium citrate solution on day 7 and 1.5 mg/kg body weight of Aredia on day 8. Follow-up period was 67 days.

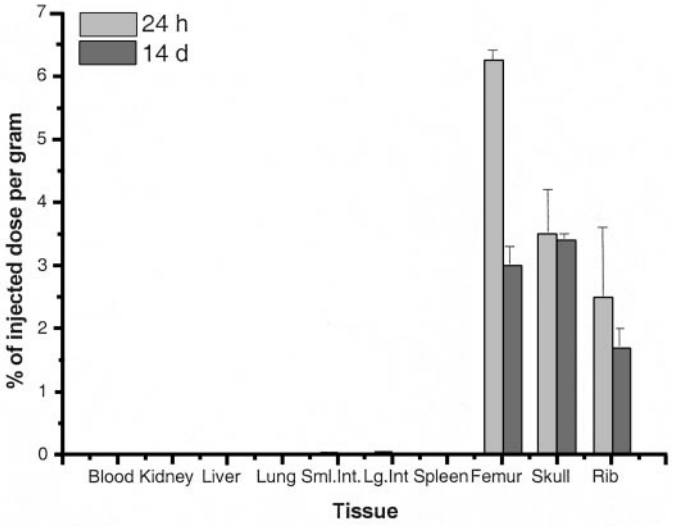

Fig. 2. Uptake of ^{223}Ra in selected tissues of rats; *bars*, ± SD.

To determine the uptake of ^{223}Ra in the bone marrow, marrow samples were extracted from the femurs of rats. For animals sacrificed and dissected 24 h after injection, the marrow was found to contain 1.0 ± 0.3% ID/g of ^{223}Ra, which is a significant amount. At 14 days, the marrow content of ^{223}Ra was below the detection limit, corresponding to <0.2% ID/g. This indicates a low retention of ^{223}Ra in the marrow compared with the bone matrix.

Normalized for b.w. the femur content of ^{223}Ra was 800 ± 56% ID·g/g (mean ± SD) 1 day after the injection and 413 ± 23% ID·g/g at 14 days. The femur:kidney ratio increased from $(5.9 \pm 2.0) \times 10^2$ at 1 day to $(7.2 \pm 3.0) \times 10^2$ at 14 days, whereas the femur:liver ratio increased from $(6.2 \pm 0.2) \times 10^2$ to $(9.1 \pm 6.6) \times 10^2$. Femur:spleen ratio increased from $(8.1 \pm 0.3) \times 10^2$ to $(6.4 \pm 2.2) \times 10^3$. The femoral bone:marrow ratio was 6.5 ± 2.1 after day 1 and >15 at day 14.

Therapy Study. Dose survival plots for rats treated with ^{223}Ra under therapy regimen A are presented in Fig. 3. The increase in survival for animals injected with 11 kBq relative to control was significant at the 1% level. Also, when taken together, the increase in survival of the animals in the 6 and 11 kBq groups was significant compared with the control group ($P < 0.05$). Furthermore, the 11 kBq group had 36% (5 of 14) symptom-free survivors beyond 50 days, whereas the animals injected with 6 kBq had 20% (1 of 5) symptom-free survivors, but survival improvement was not significant, because paralysis occurrences were not statistical significantly delayed compared with the control group.

In Fig. 4 the survival of groups treated under regimen B are presented. The control group receiving Aredia had no survivors beyond day 21 and, hence, showed no therapeutic response at the dose level administered. The 10 and the 30 kBq groups each had 40% (2 of 5) survivors beyond 50 days. The 10 kBq and 30 kBq groups had a survival that was significantly improved ($P < 0.05$) compared with the control. However, at the 5 kBq dose level the survival was not significantly different from the control group. To compare larger cohorts of animals an additional graph was made by combining the animals receiving ^{223}Ra (with or without Aredia) in one cohort, and these were compared with a cohort made of all of the control animals (with or without Aredia treatment) in Fig. 5. The data indicated a significant therapeutic response ($P < 0.005$) in the ^{223}Ra cohort compared with the controls.

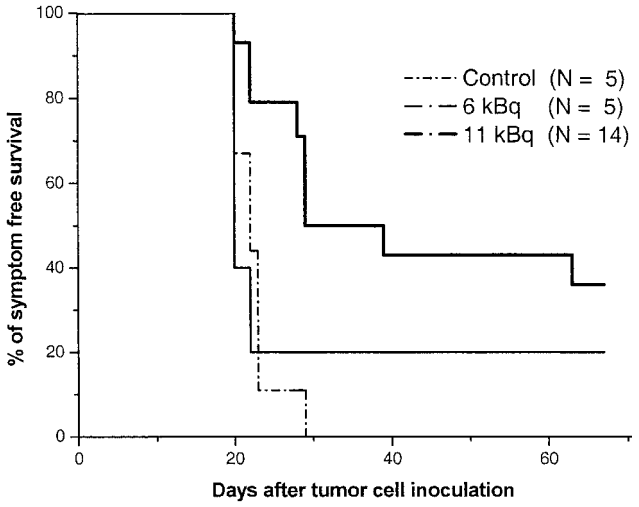

Fig. 3. Effect of ^{223}Ra in prolonging symptom-free survival of rats. Tumors were established in nude rats by injection of $\sim 1 \times 10^6$ MT-1 breast cancer cells in the left cardiac ventricula. Animals were treated 7 days after tumor cell inoculation. The treatment groups received 6 or 11 kBq of ^{223}Ra administered by tail vein injection, whereas animals in the control group were injected with 5 mM sodium citrate solution.

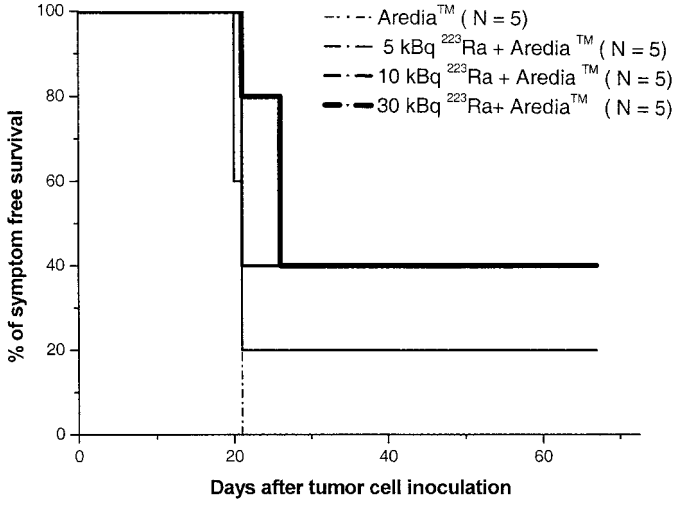

Fig. 4. Effect of ^{223}Ra in prolonging symptom-free survival of rats. Tumors were established in nude rats by injection of $\sim 1 \times 10^6$ MT-1 breast cancer cells in the left cardiac ventricula. Animals in the treatment groups were treated on day 7 after tumor cell inoculation with 5, 10, or 30 kBq of ^{223}Ra. The following day, 1.5 mg/kg b.w. of 3-amino-1-hydroxypropylidene bisphosphonate, di-sodium salt was administered by i.v. injection. Animals in the control group received 5 mM sodium citrate on day 7 and 1.5 mg/kg b.w. of Aredia on day 8.

DISCUSSION

In the treatment of skeletal metastases with bone-seeking radionuclides, it seems advantageous to deliver a therapeutically relevant radiation dose to the bony surfaces while sparing the bone marrow. With low LET radiation emitters, myelotoxicity is observed frequently at doses needed for pain palliation restricting their potential as antitumor treatment (10, 11). High LET α-particles, on the other hand, could possibly have a future role in the treatment of metastatic cancers because their short range in tissues match well to treatment requirements of multiple small tumor foci. Because their maximum range is typically <100 μm, crossfire into bone marrow from sources located on the bone surfaces should be significantly reduced compared with β- and conversion electron-emitting bone seekers. Also, because of the high LET quality of α-particles, their cytotoxicity is nearly independent of dose rate, cell cycle phase, and oxygen concentration (12). α-Particles could strongly inhibit tumor development if they can be successfully targeted to tumors.

To compare the bone-seeking properties of $^{223}Ra^{2+}$ with those of other bone-seeking radioactive compounds, data from uptake in rodents were compiled and normalized so that animals of different size could be compared (Table 2). As can be seen $^{223}Ra^{2+}$ was among the bone seekers with the highest uptake and had a considerably higher bone uptake than the commercially available bone seekers $^{89}Sr^{2+}$ and ^{153}Sm-EDTMP. It should be noticed that the magnitude of bone uptake of $^{223}Ra^{2+}$ in rats and mice was almost identical when b.w. normalization was performed.

The therapeutic effect of ^{223}Ra in rats bearing experimental skeletal metastases was studied in the current work. Nude rats injected intracardially with cells from the MT-1 breast cancer cell line could also

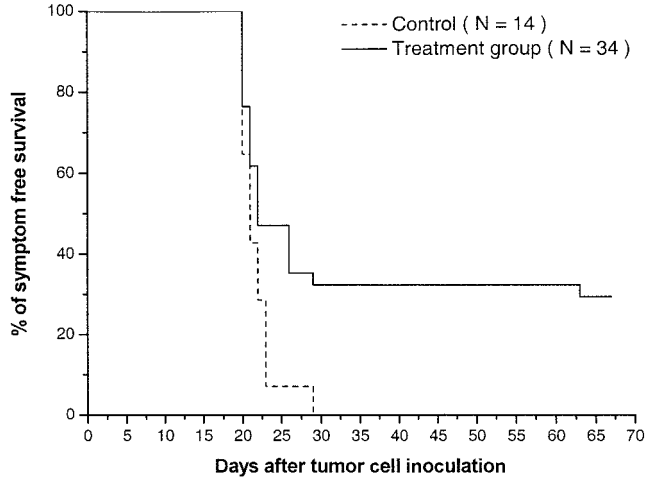

Fig. 5. Survival of ^{223}Ra-treated animals *versus* controls. The ^{223}Ra cohort combines all of the ^{223}Ra-treated animals irrespective of dosage, and includes animals with and without Aredia treatment. The control cohort includes all of the control animals with and without Aredia treatment, and also includes 5 extra controls (without Aredia) not presented in Figs. 3 and 4.

Table 2 *Bone uptake[a] of various bone-seeking radioactive compounds in rodents*

Type of compound[b]	Femur uptake/animal species/time point	Reference
Bisphosphonates		
^{131}I-BDP3	2.5×10^2/rats/24 h	27
^{131}I-IBPB	4.2×10^2/mice/24 h	13
^{131}I-HB	8.5×10^2/mice/24 h	26
^{211}At-ABPB	4.9×10^2/mice/24 h	13
Polyphosphonates		
^{153}Sm-EDTMP	5.8×10^2/rats/24 h	28
^{212}Pb-DOTMP	1.6×10^2/mice/2 h	15
^{212}Bi-DOTMP	3.6×10^2/mice/2 h	15
Carboxylic acids		
^{117m}Sn (IV)DTPA	5.4×10^2/mice/24 h[c]	29
Alkaline earth cations		
$^{89}Sr^{2+}$	3.6×10^2/mice/24 h	Footnote 4
$^{223}Ra^{2+}$	8.0×10^2/rats/24 h	Current study
$^{223}Ra^{2+}$	8.2×10^2/mice/24 h	Footnote 4
$^{223}Ra^{2+}$	7.3×10^2/mice/1 h	Footnote 4

[a] Data were normalized to the percentage of injected gram dose per gram (% g dose/g) by multiplying values given as percentage of injected dose per gram (%ID/g) with the b.w. to allow a comparison between animals of different sizes. All of the preparations were administered by i.v. injections. Data represent mean values.

[b] Abbreviations: ^{211}At-ABPB:3-[^{211}At]astatobenzamide-*N*-3-hydroxypropylidene-3,3-bisphosphonate; ^{212}Bi-DOTMP: ^{212}Bi-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-1,4,7,10-tetramethylene phosphonate, ^{131}I-BDP3: *p*-hydroxy-*m*-[^{131}I]iodobenzylidene-1-amino-1,1-bisphosphonate; ^{131}I-IBPB: 3-[^{131}I]iodobenzamide-*N*-3-hydroxypropylidene-3,3-bisphosphonate; ^{131}I-HB: 1-hydroxy(*m*-^{131}I-iodophenyl)-ethylidene-1,1-bisphosphonate; ^{212}Pb-DOTMP: ^{212}Pb-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-1,4,7,10-tetramethylene phosphonate; ^{153}Sm-EDTMP: ^{153}Sm-ethylene diamine-*N*,*N*′-tetramethylene phosphonate; and ^{117m}Sn-DTPA: ^{117m}Sn-diethylene triamine-*N*,*N*′,*N*″-pentaacetic acid.

[c] Data refers to uptake in unspecified bone.

develop tumors in the brain, adrenal gland, and lungs (20), but the earliest symptoms are usually caused by skeletal tumor growth. Hence, the model was therefore considered relevant to study cancer therapy with ^{223}Ra. The results from this study show that animals injected with about ≥10 kBq of ^{223}Ra had significantly increased symptom-free survival compared with control, indicating that metabolically concentrated α-particle emitters irradiating the bone surfaces and calcified tumor sites could slow down progression of skeletal metastases.

For comparison, the results of the current study and previous studies exploring various therapeutic modalities in the MT-1 model are presented in Table 1. The data indicate that ^{223}Ra has a significant therapeutic potential for use against metastatic bone disease compared with chemotherapeutic agents, immunotoxins and bisphosphonate labeled with the therapeutic β-emitter ^{131}I evaluated in the MT-1 model. Because ^{223}Ra is concentrated in osseous sites including the skeletal surfaces, the antitumor effect of ^{223}Ra may be expected to be independent of the cell characteristics, which may impede the effect of chemotherapeutic agents and immunotoxins. In contrast to immunotoxins, the antitumor effect of ^{223}Ra is not dependent on a high and uniform expression of the antigen on all of the tumor cells and a relatively low cross-reactivity with normal cells. In addition, because ^{223}Ra is a high LET radiation α-emitter, a significant antitumor effect should also be expected on nonproliferating (resting) tumor cells adjacent to the bone surface.

The highest activity of ^{223}Ra that was administered in the tumor treatment study corresponded to ~0.3 MBq/kg b.w. or 30 kBq/animal. The therapeutic gain of increasing the dosage beyond the 10–11 kBq/animal level was not significant, indicating that a therapeutic plateau was reached with ^{223}Ra. There could be several reasons for this. One reason could be that the complex growth pattern of the tumor model used. Because the uptake of radioactive bone seekers, like radium and strontium, would be in the growth zones of skeletal metastases, *i.e.,* the mineralized interface between the bone and the tumor, and not in the tumor cell itself, the tumor could in some instances be out of reach for the short ranging α-particles, *e.g.*, metastases could develop from inside of marrow cavity instead of growing adjacent to the bone surface.

The bone metabolism is a complex process involving osteoblasts and osteoclasts, which are cells affecting bone formation and bone resorption. Irradiation could affect the activity of these cells in bone, and if osteoclast-mediated demineralization of bone was the effect, this could cause resorption of radionuclide from the bone. Therefore, additional treatment with the osteoclast inhibitor Aredia was included as a substudy in this work. However, the treatment did not seem to improve therapeutic response at the 30-kBq *versus* the 10-kBq dose level.

In general the therapeutically effective activity of ^{223}Ra seemed to be well tolerated, because no signs of bone marrow toxicity or b.w. loss could be seen in the groups of treated animals. In the experiment with mice, an activity level corresponding to 1 MBq/kg was administered without signs of severe bone marrow toxicity or b.w. loss within 3 months after the injection of ^{223}Ra. This indicates that therapeutically relevant doses of ^{223}Ra could be tolerated.

^{223}Ra decays via a multistep chain releasing four α-particles. Another radium isotope with multistep decay chain, *i.e.*, ^{224}Ra, has been used medically in the treatment of noncancerous bone diseases, *e.g.*, ankylosing spondylitis (22, 23). A new study of ^{224}Ra in patients with ankylosing spondylitis was reported recently (24) indicating that significant pain relief could be obtained in the majority of patients. Compared with ^{223}Ra the half lives of ^{224}Ra and the nuclides in the decay chain after ^{224}Ra transformation could be less favorable for the purpose of bone targeting. As has been demonstrated in animal experiments, a significant fraction of the daughter isotopes of ^{224}Ra escape from bone (17, 18), most probably because of the noble gas daughter radionuclide, ^{220}Rn ($t_{1/2}$ = 55.6 s), which diffused away from the site of ^{224}Ra decay. ^{223}Ra, on the other hand, has a half life of 11.43 days, which is about three times that of ^{224}Ra. This allows a deeper incorporation into the bone matrix before decay occurs, and less radiation to soft tissues during the uptake and elimination phase because a lower fraction of the injected radium atoms would decay in the first few days. Also, perhaps even more important, the radon daughter ^{219}Rn has a short half-life (3.9 s), which is likely to diminish translocation of the radon daughter. Data from rodent indicate a high retention of ^{223}Ra daughter nuclides in bone as measured by comparing the activity levels of ^{211}Bi with ^{223}Ra in bone samples. On the basis of the favorable properties demonstrated in preclinical studies, ^{223}Ra has been included recently in a clinical trial concerning patients suffering from skeletal metastases from breast and prostate cancer.

In conclusion, the present work shows that i.v. injection of ^{223}Ra may slow down tumor progression in the skeleton. The antitumor effect is most likely linked to the delivery of an intense and highly localized radiation zone from α-particles targeting the bony surfaces. This may eliminate early skeletal metastases, whereas bone marrow cells distant from the bone surfaces are spared. A clinical trial has been initiated recently to evaluate ^{223}Ra treatment in humans.

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