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Antitumor Activity of Novel Bone-seeking, α-emitting ^{224}Ra-solution in a Breast Cancer Skeletal Metastases Model

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Abstract. *Background/Aim: Bone metastases are associated with increased morbidity and poor prognosis in a variety of cancers. The present study investigated the effects of targeted radionuclide therapy with α-emitting, bone-seeking radium-224 (^{224}Ra) on osteolytic bone metastasis of MDA-MB-231(SA)-GFP human breast cancer cells injected intracardially into nude mice. Materials and Methods: Vehicle, ethylenediamine tetra (methylene phosphonic acid) (EDTMP) and ^{224}Ra-solution (45, 91 or 179 kBq/kg) with EDTMP were intravenously administered to mice two days after cell injection. The bone-seeking EDTMP was added to the ^{224}Ra-solution to improve bone targeting of ^{212}Pb, which is a progeny of ^{224}Ra. Results: Radium-224 solution treatment decreased in a dose-dependent manner the areas of osteolytic lesions in the hind limbs and the number of tumor foci in the whole skeleton, and extended survival. Paraplegia was not observed in 179 kBq/kg ^{224}Ra-solution group. Conclusion: Radium-224-solution containing chelated ^{212}Pb is a promising candidate for the treatment of breast cancer patients with bone metastases.*

Bone is the most frequent site of metastasis among patients with advanced breast and prostate cancer (1). Bone metastases lead to pain and skeletal-related events (pathological fractures, spinal cord compression, hypercalcemia of malignancy), which cause significant morbidity and reduced quality of life (1). The development of bone metastases is associated with shortened life expectancy for patients (1). Available treatments such as ablation, bisphosphonates, radiotherapy, chemotherapy, monoclonal antibody therapy often show limited benefits (1). Prevention and effective treatment of bone metastases can have a significant positive impact on the outcomes of patients with advanced cancers.

Targeted radionuclide therapies based on β-emitting radioisotopes have long been used to alleviate pain in patients with bone metastases from breast and prostate cancer (1). However, β-emitting radionuclides can induce myelosuppression (1). Radium-223 (^{223}Ra) dichloride (Xofigo®, Bayer HealthCare Pharmaceuticals Inc.) is the first-in-class commercially available α-emitter approved by the FDA and EMA in 2013 for the treatment of bone metastases in patients with metastatic castrate-resistant prostate cancer. This treatment improves overall survival and delays symptomatic skeletal events (2). Radium-223, as a calcium mimetic, has a natural bone-seeking capability and preferentially binds to areas of increased bone turnover in bone metastases targeting osteoblastic metastatic lesions (2). The efficacy of ^{223}Ra has been demonstrated in animal models indicating significant antitumor effect in experimental skeletal metastases in nude rats (3), increased survival in a mouse model of breast (4) and antitumor effect in prostate cancer growth in mouse bone (5). Toxic effects on adjacent tissues and particularly the bone marrow are minimal due to a short path length (<100 μm) of the α-particles.

Similarly to ^{223}Ra, ^{224}Ra is a natural bone-seeking α-emitting radionuclide, and its bone-seeking properties in humans are well known (6). The attractive aspect of ^{224}Ra is the shorter half-life (3.63 days) than that of ^{223}Ra (11.43 days). Radium-224 solutions have been used earlier in the treatment of noncancerous bone diseases such as ankylosing spondylitis (6-8). There are limited reports on the development of radiopharmaceuticals or devices with ^{224}Ra for cancer treatment (9, 10). Preclinical results in mice have demonstrated that ^{224}Ra loaded wires (7-65 kBq) inserted into solid tumors can effectively destroy tumors (9, 10).

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One potential problem with ^{224}Ra solutions is the generation of the progeny β-emitting lead-212 (^{212}Pb) with the significant half-life of 10.6 h (Figure 1). Lead-212 together with the α-emitting progeny bismuth-212 (^{212}Bi) could contribute to unwanted non-target tissue and cell exposure (11, 12). However, ^{212}Pb and ^{212}Bi have been successfully used with different carriers in preclinical and clinical studies (12-15). The bone-seeking ethylenediamine tetra (methylene phosphonic acid) (EDTMP) was suggested to direct ^{212}Pb to bone (11, 16). Recently, Larsen demonstrated that the addition of EDTMP to ^{224}Ra solution reduced the uptake of ^{212}Pb in soft tissues compared with ^{224}Ra without EDTMP (16). In the current study EDTMP was added to reduce soft tissue exposure to ^{212}Pb and to exploit its daughter α-emitting ^{212}Bi as part of the therapy. This was achieved by *in situ* generation of the bone-seeking ^{212}Pb-EDTMP complex in the ^{224}Ra-solution, thus, increasing bone uptake and soft tissue elimination of ^{212}Pb (16). At secular equilibrium, the number of ^{212}Pb atoms is about 14% of the ^{224}Ra atoms (16). It is therefore advantageous to complex ^{212}Pb before injection of ^{224}Ra solution which may be stored for a while as it would be the case with centralized production and long-distance shipment of ^{224}Ra-solutions.

In this work, we studied the effects of a single injection of ^{224}Ra solution with ^{212}Pb-EDTMP in a mouse model mimicking breast cancer skeletal metastasis. This solution containing ^{224}Ra in equilibrium with ^{212}Pb-EDTMP is hereafter denoted as the ^{224}Ra-solution.

Materials and Methods

Cell culture. Human MDA-MB-231(SA) breast cancer cells were kindly provided by Dr. T. Guise (Indiana, USA) (17). These cells were transfected with green fluorescent protein (GFP, pTurboGFP-N vector, Evrogen JSC, clone 6, Moscow, Russia). MDA-MB-231(SA)-GFP cells were authenticated in June 2014 using short tandem repeat analysis (GenePrint10 system, Promega, Madison, WI, USA) at the Finland Institute for Molecular Medicine (FIMM, Helsinki, Finland). The authenticated frozen stock was used in the study. MDA-MB-231(SA)-GFP cells were grown in DMEM media supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin in an incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37˚C.

Preparation of the ^{224}Ra solution. Radium-224 was extracted from a generator based on ^{228}Th (Eckert & Ziegler, Braunschweig, Germany) immobilized on a DIPEX® (Eichrom Technologies LLC, Lisle, IL, USA) actinide resin (18). It was purified and found with no traces of ^{228}Th (18). The details of the ^{224}Ra generator setup have been described elsewhere. Radium-224 (half-life 3.66 days) was in equilibrium with daughter radionuclides including ^{212}Pb (half-life 10.6 h). Radium-224 was combined with EDTMP to complex free ^{212}Pb in the solution to make ^{212}Pb a better bone seeker and to avoid its uptake in hematopoietic cells etc. (16). A concentration of 5 mg/ml EDTMP was used.

Radioactivity measurements. Since ^{224}Ra decay has a modest γ-emission in an energy region with more abundant γ from daughter ^{212}Pb, ^{224}Ra activity was determined indirectly from the counts in the 70-80 keV or 60-110 keV windows. The details of the radioactivity measurements have been described elsewhere (18). A Capintec CRC-25R dose calibrator and Cobra II Autogamma counter (Packard Instrument, Downers Grove, II, USA) were used for radioactivity measurements.

Animals. Female nude mice (Hsd:Athymic nude-*Foxn1^{nu}*, Harlan Laboratories B.V., currently Envigo, Horst, the Netherlands) were used. The mice were 4-5 weeks of age weighing approximately 20 g at the beginning of the study. These animal studies were approved by the National Committee for Animal experiments of Finland. All experiments were performed in accordance with relevant guidelines and regulations. Mice were kept under pathogen-free and controlled conditions and fed with a soy-free diet (Harlan Teklad Global Rodent diets, irradiated 2916, Harlan Laboratories, B.V., currently Envigo, Horst, the Netherlands) and tap water ad libitum. The mice were randomized based on body weight into five groups (12 animals per group) and marked with ear marks.

On day 0, MDA-MB-231(SA)-GFP cells (10^5 in 0.1 ml PBS) were inoculated into the left cardiac ventricle of mice. Analgesia (0.1 mg/kg buprenorphine administered subcutaneously) was given at least one hour before intracardiac inoculation, which was performed under isoflurane anesthesia. Viability of cells was determined before and after inoculations.

On day 2, mice received either intravenous injections (5 μl per g body weight) of (1) vehicle (isotonic sodium chloride, pH 5-7), (2) bone-seeking EDTMP (ethylenediamine tetra (methylene phosphonic acid)), 5 mg/ml in sodium chloride, (3) 45 kBq, 91 kBq, or 179 kBq ^{224}Ra-solutions (supplied with 5 mg/ml EDTMP).

Mice were given analgesia (0.02 mg/ml buprenorphine in the drinking water) during the last study days when needed.

Radiography and fluorescence imaging were performed at the end of the study (at sacrifice) to detect metastatic lesions.

Cachexia, paraplegia and survival. Body weight was monitored two or three times a week. Mice were killed when cachexia or paraplegia was observed. Mice were considered cachectic when lost over 20% of body weight from the maximum weight. Animals with loss of function in any limbs were considered paraplegic. Mice were killed by cervical dislocation under anesthesia. Maximum study length was 60 days but individual mice were sacrificed earlier when sacrifice criteria were met. Survival is reported as time from study day 0 to sacrifice day.

X-ray radiography. The development of osteolytic lesions was monitored in an anteroposterior position with the Faxitron Specimen Radiographic System MX-20 D12 (Faxitron Corporation, Wheeling, IL, USA) using the Faxitron Dicom 3.0 software. One radiograph (both hind limbs) per animal was taken on each X-ray occasion (31 kV, 10 seconds, 2x magnification). The number of lesions was counted and the lesion area were measured in hind limbs from the images with the MetaMorph image analysis software (Molecular Devices, LLC, Sunnyvale, CA, USA).

Fluorescence imaging of GFP. Tumor burden was monitored by imaging the fluorescence emitted by MDA-MB-231(SA)-GFP cells using an LT 9 GFP-imaging system LT-MACIMSYSPLUSC

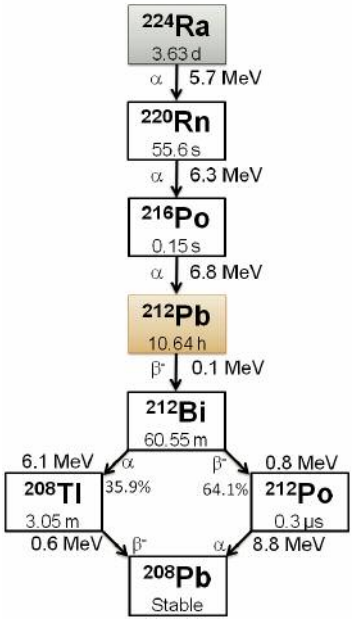

Figure 1. *The decay chain of ^{224}Ra, including details on each nuclides' half-life, main mode of decay and mean energies.*

(Lightools Research, Encinitas, CA, USA). Excitation wavelength was 470 with 40 nm band-pass and emission wavelength cut-off filter was at 515 nm.

Statistical analysis. The data were analyzed using a statistical software R (version 3.1.2, www.r-project.org) or SigmaPlot version 12.5 (Systat Software, Inc., Chicago, IL, USA). For the numeric end-point parameters, normality and homogeneity of group variances were checked. If these criteria were fulfilled as such or after some data transformation (logarithmic, square root, reciprocal), the data were analyzed using one-way ANOVA followed by Tukey's HSD test. If the criteria were not fulfilled, the results were analyzed using Kruskal-Wallis test followed by Mann-Whitney *U*-test. The weight curves were analyzed using a mixed model and model contrasts. The model had effects for treatment and their interaction as well as subject-wise random effect. Value at day -4 was used as baseline covariate in the models. Survival curves were estimated using the Kaplan-Meier estimator and compared using the log-rank test by pairwise comparisons. Paraplegia and cachexia analysis were carried out using Fisher's exact test by pairwise comparisons. The obtained *p*-values were adjusted for multiple comparisons. The p threshold for significance was 0.05.

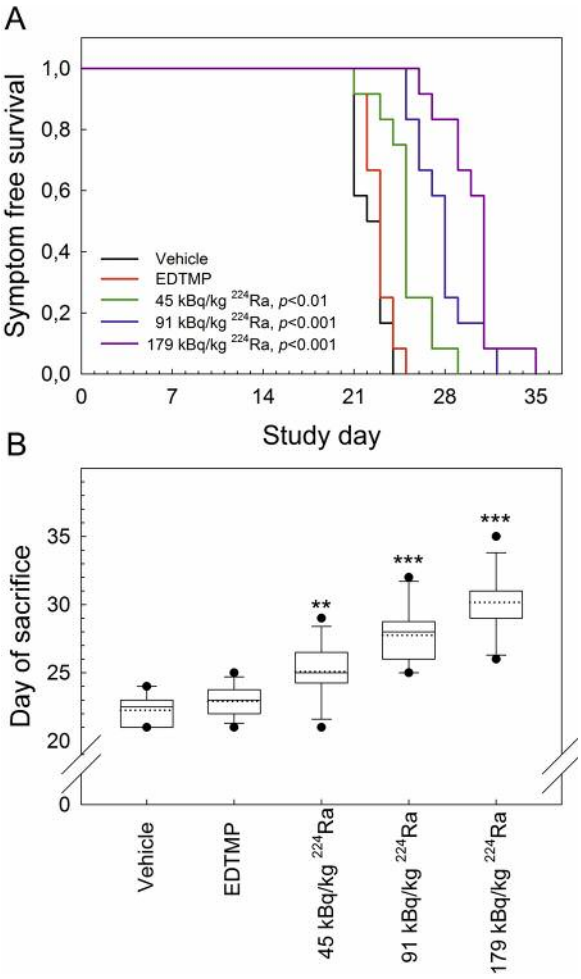

Figure 2. *Effect of ^{224}Ra-solution in prolonging symptom-free survival (A) and the day of sacrifice (B) of nude mice. Metastases were established in mice by injection of 10^5 MDA-MB-231(SA)-GFP breast cancer cells in the left cardiac ventricula. Animals were treated two days after cell inoculation. The treatment groups received 45, 91 or 179 kBq/kg of ^{224}Ra-solution administered by tail vein injection, whereas animals in the control groups were injected with vehicle (sodium chloride) or bone-seeking EDTMP (25 mg/kg). (A) Symptom-free survival data were estimated by the Kaplan-Meier analysis followed by the log-rank test against the EDTMP group. (B) Bottom of the boxes represent the 25th percentile, dashed lines represent means, solid lines represent medians, top of the boxes represent the 75th percentile, and whiskers represent the 5th and 95th percentiles. Asterisk (*) indicates statistically different values when comparing the groups to the EDTMP group (***p<0.001, **p<0.01).*

Results

Radium-224-solution prolonged symptom-free survival in mice with breast cancer metastases. A single injection of ^{224}Ra-solution extended significantly symptom-free survival in a dose-dependent manner (Figure 2A). The control groups receiving either the sodium chloride vehicle or EDTMP in sodium chloride solution had no survivors beyond day 26. While 45, 91 and 179 kBq/kg ^{224}Ra-solution groups had 25% (3 of 12), 67% (8 of 12) and 92% (11 of 12) survivors at day 26, respectively (Figure 2A). Mean survival (time to sacrifice) was 22 days and 23 days in the vehicle and EDTMP groups, respectively (Figure 2B). The median survival of mice was around 14% (3 days), 27% (6 days) and

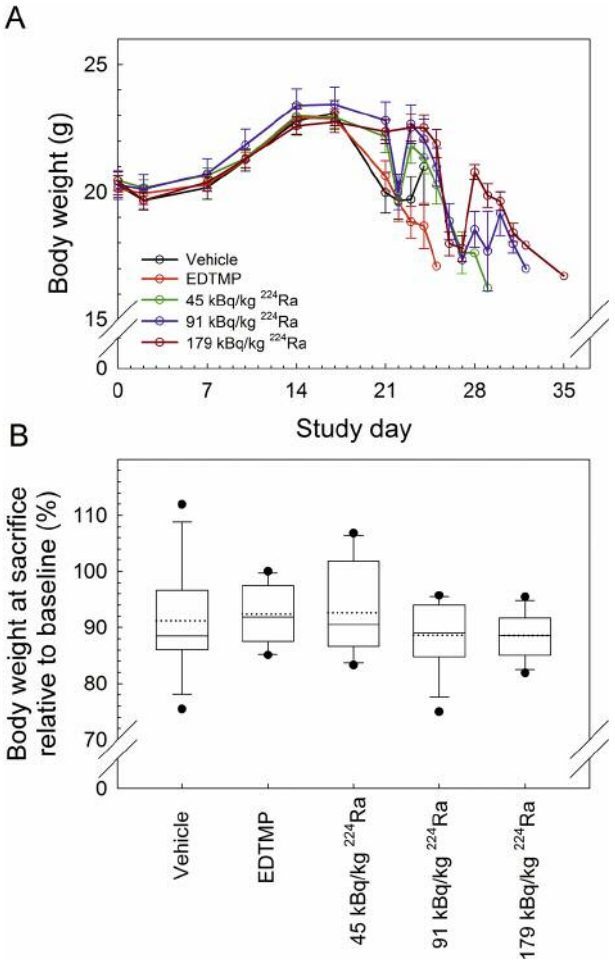

Figure 3. *Effect of ^{224}Ra-solution on mouse body weight changes (A) and mouse body weight relative to baseline at the time of sacrifice (B). (B) Bottom of the boxes represent the 25th percentile, dashed lines represent means, solid lines represent medians, top of the boxes represent the 75th percentile, and whiskers represent the 5th and 95th percentiles. The data (B) were analyzed using the Kruskal-Wallis test.*

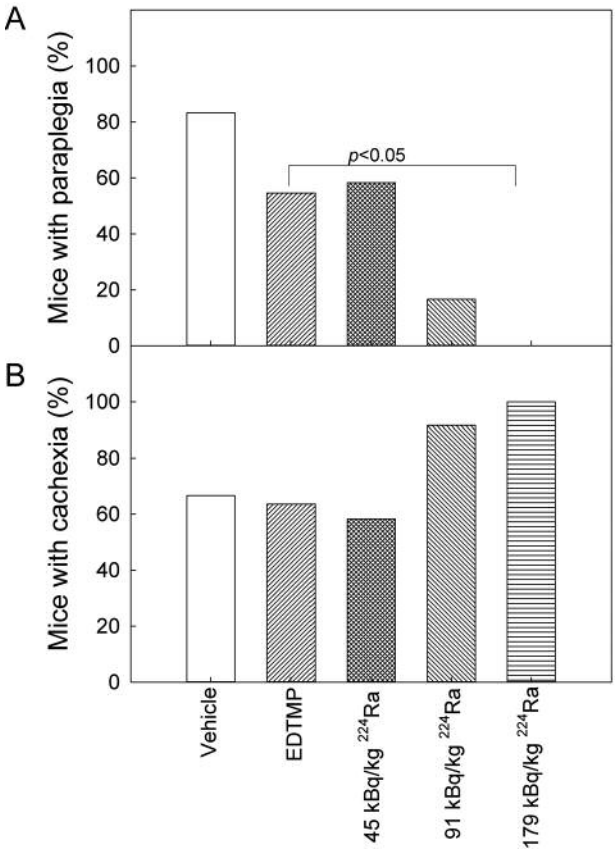

Figure 4. *Percentage of mice with paraplegia (A) and cachexia (B) at the time of sacrifice. The data were analyzed using the Fisher's exact test.*

41% (9 days) longer in 45, 91 and 179 kBq/kg ^{224}Ra groups compared to the sodium chloride vehicle group (Figure 2B). The increase in survival for the mice treated with ^{224}Ra solution relative to the two control groups was significant for all ^{224}Ra groups (Figure 2B).

The body weight of mice increased during the first two weeks and later started to decline in all groups (Figure 3A). These results showed that there was no significant difference in the body weight of mice between treatment and control groups at the beginning of the study, during the experiment and at sacrifice day (p>0.05, Figure 3).

Radium-224-solution prevented tumor-related paraplegia. Paraplegia occurred in 83% of the animals in the vehicle group and was the main cause for sacrifice in this group (Figure 4A). Radium-224-solution treatment decreased the incidence of paraplegia in a dose-dependent manner (Figure 4A). Paraplegia was not observed in 179 kBq/kg ^{224}Ra-solution group (Figure 4A). The main cause for sacrifice of mice in this group was cachexia, which was significantly higher than in the control groups (Figure 4B).

Radium-224-solution inhibited bone metastasis in mice. Osteolytic lesions in the hind limbs of mice were detected and analyzed by X-ray radiography (Figure 5). Tumor growth in the whole body was detected and analyzed by fluorescence imaging of GFP (Figures 6 and 7). In the present study, all mice (100%) in all groups developed bone metastasis after inoculation of MDA-MB-231(SA)-GFP cells into the left ventricle of the heart, which is a well-established technique to develop bone metastases (17). There was no significant difference between the two control groups. (Figures 5-7). After ^{224}Ra-solution treatment the formation of osteolytic lesions was inhibited in a dose-dependent manner (Figure 5B). High statistical significance (p<0.001) was observed for highest activities of ^{224}Ra (91 and 179 kBq/kg) when compared to control groups. Animals in 179

kBq/kg ^{224}Ra-solution group had around five times smaller area of osteolytic lesions and a 1.5-fold less osteolytic lesions at sacrifice when compared to EDTMP control group (Figures 5B and C).

MDA-MB-231(SA)-GFP cells formed tumors in the bone sites such as in the scapula, wrist, ribs, spine, hind limbs and iliac bone (bright green spots in the images in Figure 6 indicated by white arrows as examples in the vehicle group images A and B). Total tumor burden at sacrifice was not decreased by ^{224}Ra-solution treatment (Figure 7A) but it must be noted that the animals treated with ^{224}Ra-solution had more time to develop soft tissue metastases as these animals lived longer than the controls. However, one difference in the location of tumors was observed. Less tumor burden was seen at bone sites in mice treated with ^{224}Ra-solution, especially in the 179 kBq/kg ^{224}Ra-solution group (Figure 6) but tumor burden was still noted in the head area and eyes (Figure 6, red arrows). The number of tumor foci at bone sites was decreased strongly by ^{224}Ra-solution treatment by 2, 2.5 and 10 times, respectively (Figure 7B).

Discussion

The current work represents the first study of an injectable solution of the α-emitter ^{224}Ra in a cancer model. We chose to add EDTMP to improve the properties of the ^{212}Pb progeny. Previously, ^{224}Ra without EDTMP was used in treatment of ankylosing spondylitis (6-8). Long-term toxicity and carcinogenicity data in humans exist from this usage (6, 19-24) indicating a quite acceptable safety profile compared with other cancer therapeutics, and could possibly be further improved upon by adding EDTMP for soft tissue protection and by using a more moderate dosing intensity. Our data demonstrate for the first time that a single treatment with ^{224}Ra-solution resulted in a significant prolongation of symptom-free survival, reduction of paraplegia induction and decreased number and areas of bone metastases. Radium-224 decays with a physical half-life of 3.64 days *via* several progenies to ^{208}Pb by emitting four α-particles within a relatively short time span (Figure 1). The antitumor effect is most likely linked to the delivery of an intense and highly localized radiation zone from α-particles targeting the bone surfaces. The treatment with ^{224}Ra-solution seemed to be well tolerated because no signs of body weight loss could be seen in the groups of treated animals (Figure 3).

In the present study the tetraphosphonate chelator, EDTMP, with a high affinity for areas of active bone turnover (25), had no significant effect by itself on survival of mice, reduction of paraplegia and cachexia or formation of bone metastasis (Figures 2-7). Lead-212 ($t_{1/2}$=10.6 h), which is the longest living progeny in the ^{224}Ra series, yields indirectly one α-particle (*via* ^{212}Bi). By adding EDTMP to the ^{224}Ra solution, soft tissue exposure to ^{212}Pb present in the ^{224}Ra-solution was reduced (16).

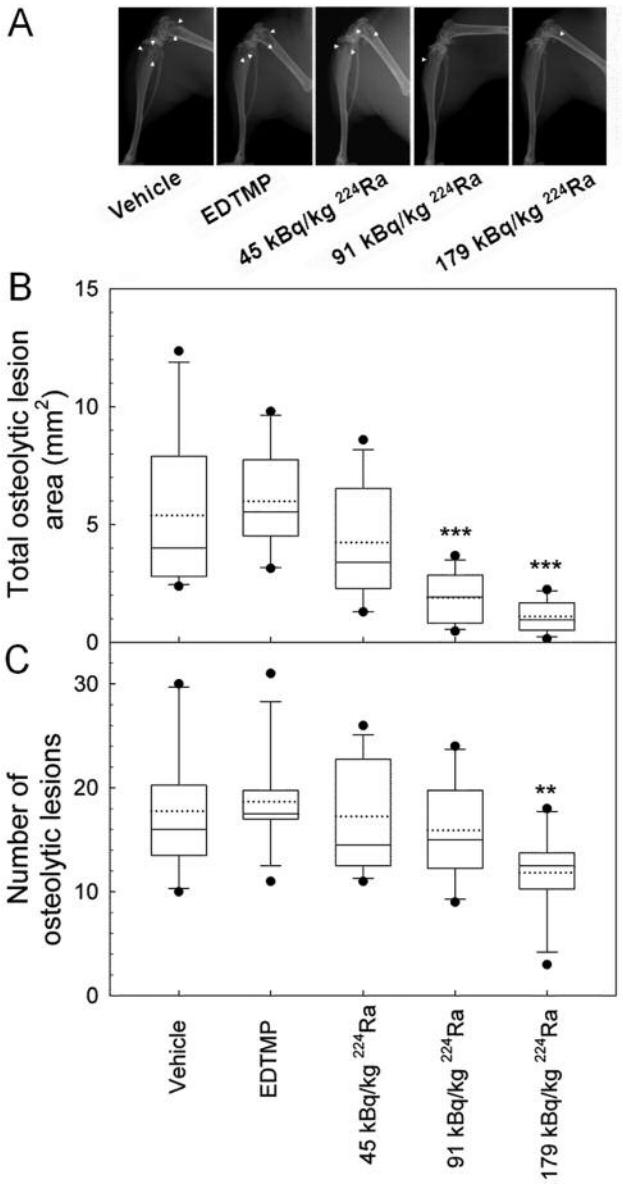

Figure 5. *Representative radiographs (A) of hind limbs of mice bearing tumors after intracardiac inoculation of MDA-MB-231(SA)-GFP cells. Osteolytic bone metastases are indicated by arrows. The images were taken at day of sacrifice. Total osteolytic lesion area (B) and total number of osteolytic lesions (C) per mouse at the time of sacrifice. Development of bone metastases on hind limbs was monitored by X-ray radiography. The results are shown as the sum of areas of bone lesions (B) or the count of individual bone lesions (C) in right and left tibia and femur per animals. Bottom of the boxes (B) represent the 25th percentile, dashed lines represent means, solid lines represent medians, top of the boxes represent the 75th percentile, and whiskers represent the 5th and 95th percentiles. Prior to statistical analysis, the data (B) were transformed using logarithmic transform. Statistical analysis was performed using ANOVA (A) or Kruskal-Wallis test (C). As statistical differences were observed for b and c, the pairwise comparison was performed using Tukey's HSD test (B) or Mann-Whitney U-test (C). Asterisk (*) indicates statistically different values when comparing the animals in EDTMP group (***p<0.001, **p<0.01).*

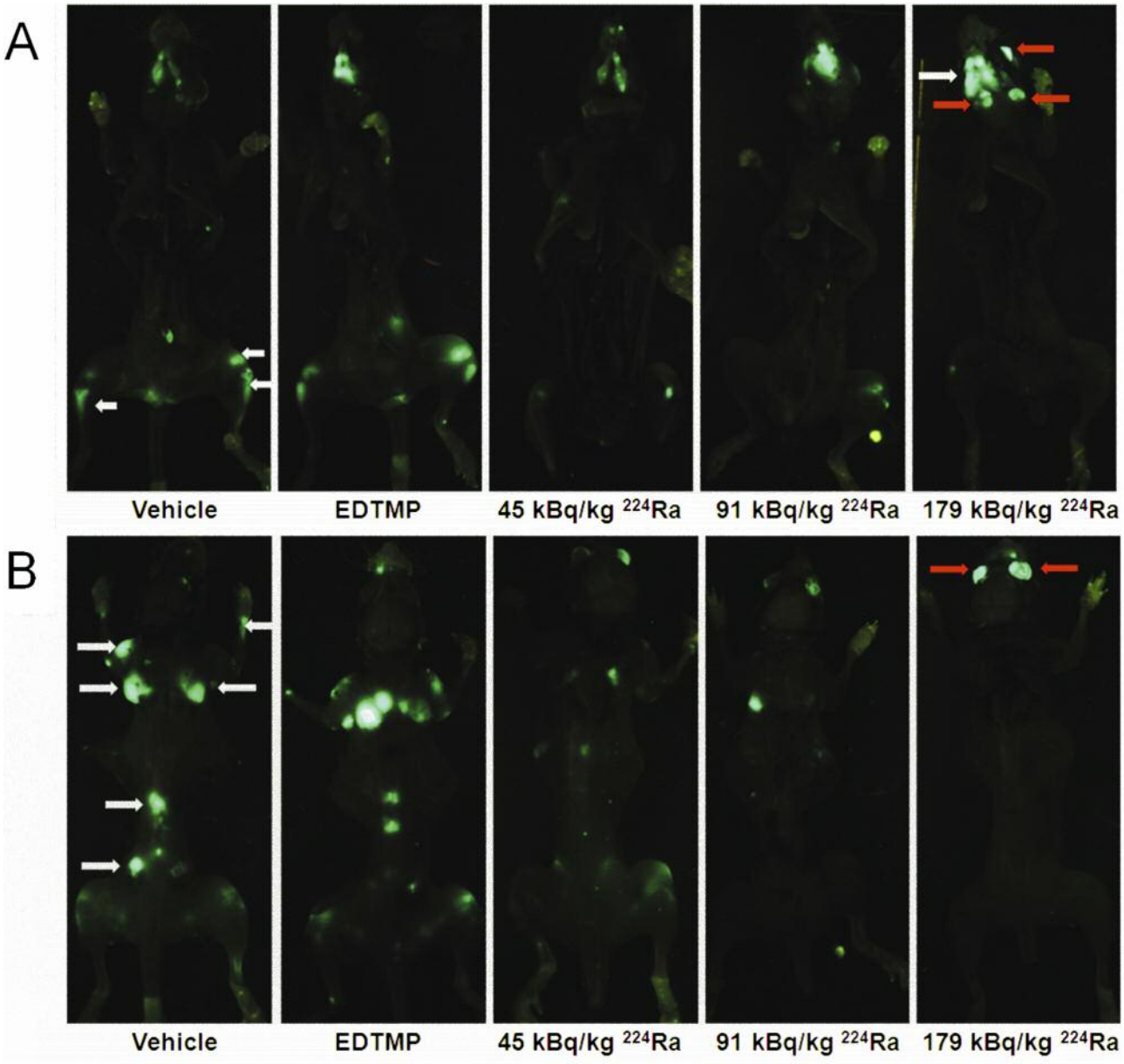

Figure 6. *Representative whole-body fluorescence images of GFP of mice bearing tumors after intracardiac inoculation of MDA-MB-231(SA)-GFP cells. (A) Ventral view, (B) dorsal view. The skeleton has been exposed and therefore, visceral organs, heart and lung have been removed. Example metastases are indicated by white (bone metastases) and red (eye metastases) arrows. These images were taken at the day of sacrifice.*

Spinal cord compression from spinal metastasis is a common complication in breast and prostate cancers and, if left untreated, permanent paraplegia or quadriplegia will occur (26). Patients with paraplegia have significantly decreased quality of life and shortened survival. Therefore, it is important to find effective treatments for preventing or improving the outcome of metastatic spinal cord compression. The intracardially injected MDA-MB-231(SA) cells form osteolytic lesions and induce paraplegia of the hind legs, both of which are characteristic of osteolytic metastasis (17, 27). The treatment with ^{224}Ra-solution (179 kBq/kg) prevented paraplegia totally (Figure 4A). This happened because ^{224}Ra had a direct effect on the tumor foci in the spinal bones, strongly slowing down tumor growth and decreasing foci number (Figure 6).

In the study by Suominen *et al.* (4) ^{223}Ra was given at a dosage of 300 kBq/kg in mice of similar age receiving the

same number of cells injected intracardially as in our current study. In one of their cohorts the animals received treatment on day two. It should be noted that since ^{223}Ra ($t_{1/2}$=11.4 days) and the ^{223}Ra series also generate four α-particles with similar total energy as for the ^{224}Ra series, 300 kBq/kg dose of ^{223}Ra would be approximately five times the amount of radium cations as the 189 kBq/kg ^{224}Ra dosing due to the longer half-life. Despite this large difference in radium dosing, the two treatments had similar efficacy in this model with life prolongation of about 41% (^{224}Ra, 179 kBq/kg) and 45% (^{223}Ra, 300 kBq/kg), respectively, indicating that the ^{224}Ra-solution had high biological activity.

Advantage of developing ^{224}Ra based therapeutics lies in the well documented data on long term and short-term toxicity from its previous use in non-cancerous human subjects. From 1946 to 1975 in Germany repeated intravenous injections of up to 2 MBq of ^{224}Ra per injection twice a week for months were given to patients with ankylosing spondylitis (7). Injected amounts were up to 140 MBq ^{224}Ra (7). Such high cumulative doses of ^{224}Ra led to increased incidences of myeloid leukemia and malignancies of bones, kidneys and thyroid, and ^{224}Ra was abandoned since 1990 (6, 22). An elevated risk for malignant bone tumors, however, has not been proven after low-dose treatment with 10 MBq ^{224}Ra (6). In Germany, ^{224}Ra-Cl (^{224}Spondyl*AT*®) was approved for the intravenous administration in ankylosing spondylitis patients in 2000 (28). Patients now received the total activity of only 10 MBq, with 1 MBq (low-dose) per injection (28). The excess absolute risk associated with this treatment was estimated to be 0.2% for malignant bone tumors and 0.4% for leukemia (6). The ^{224}Ra-Cl product was discontinued in 2005 (28). Since then, ^{224}Ra has not been used in clinical settings. Moderate doses of ^{224}Ra with complexed ^{212}Pb may be an excellent option in cancer metastasis treatment as the complexion of ^{212}Pb may reduce risk associated with soft tissue exposure to co-injected ^{212}Pb.

The presence of disseminated MDA-MB-231(SA)-GFP cells in the bone marrow two days after inoculation of the cells was studied earlier in the same mouse model, and tumor cells were observed in all histological sections from control mice (4). Findings in the present study indicate the effectiveness of ^{224}Ra-solution to reduce bone metastasis but not soft tissue metastases, which were noted in the eyes (Figure 6, visceral organs were removed). It is known that ^{224}Ra has the highest uptake in the skeleton of animals and humans (6, 29). Uptake of cationic Ra into the bone is very fast with only 8% of the injected dose remaining in the circulating blood after 15 min. Eight hours after injection only 1.5% is still found in the blood (8). After injection, ^{224}Ra is incorporated into areas of pathological ossification. Radium-224 is enriched in sites of active bone formation and exerts its analgetic effect by blocking the secretion of pro-

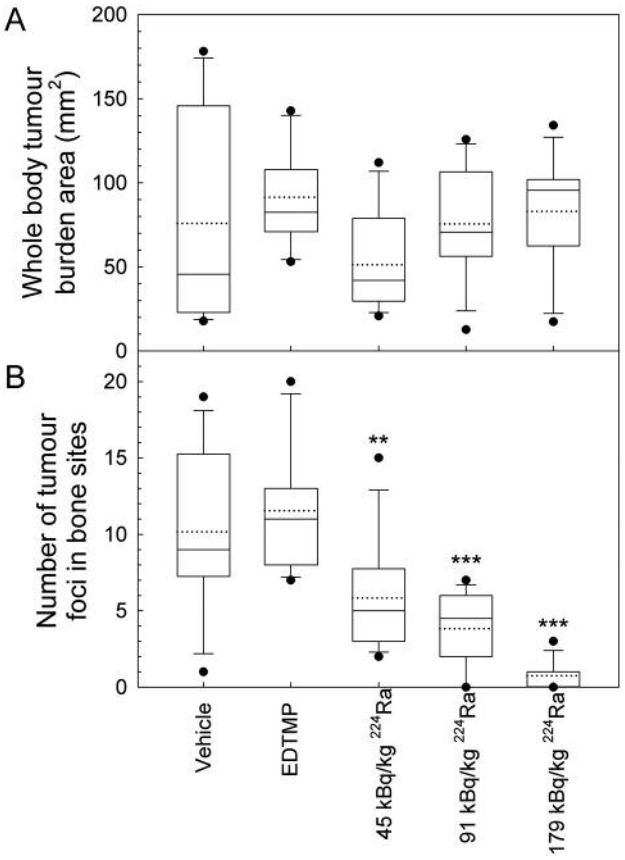

Figure 7. *Whole body (A) and bone sites (B) tumor burden at the time of sacrifice of mice. Development of metastases was monitored by fluorescence imaging of GFP. (A) The results are shown as the average of fluorescence area in two pictures (prone and supine positions) of each animal. (B) Assessed areas include spine and limbs referring to the reprehensive images in Figure 4B. Bottom of the boxes (A, B) represent the 25th percentile, dashed lines represent means, solid lines represent medians, top of the boxes represent the 75th percentile, and whiskers represent the 5th and 95th percentiles. Statistical analysis was performed Kruskal-Wallis test. No statistical differences were observed for whole body tumor burden (A). As statistical differences were observed for b, the pairwise comparison was performed using Mann-Whitney U-test (B). Asterisk (*) indicates statistically different values when comparing to the animals in the EDTMP group (***p<0.001, **p<0.01).*

inflammatory cytokines (8). Osteolytic demineralization shown in the current study is inhibited in addition to the anti-inflammatory and analgetic activity, emphasizing the clinical relevance of ^{224}Ra for the treatment of bone metastases.

The used *in vivo* model of breast cancer bone metastasis MDA-MB-231(SA)-GFP developed not only bone metastases, but also soft tissue metastases (Figure 6) and that happens in patients too (30). As an alternative to EDTMP it is also possible to use a bifunctional chelating agent TCMC (1,4,7,10-tetra-(2-carbamoyl methyl)-cyclododecane) conjugated with a

monoclonal antibody (for example, trastuzumab (31, 32)) that can be added to the ^{224}Ra-solution in equilibrium with ^{212}Pb to scavenge ^{212}Pb (16, 18). Upon administration, the antibody moiety of ^{212}Pb-TCMC-trastuzumab binds with high affinity to the extracellular domain of human epidermal growth factor receptor 2 (HER2) which is overexpressed on the surface of breast cancers (31, 32). After internalization ^{212}Pb delivers a cytotoxic dose of α radiation (*via* ^{212}Bi) to the HER2-expressing tumor cells. This could potentially affect the soft tissue component of metastatic or primary bone cancers as the antibody component could target tumor cells also in circulation and in soft tissues.

There are some limitations to this study. Further studies are needed to evaluate the effects of ^{224}Ra-solution treatment on more established breast cancer bone metastases as opposed to the micrometastatic setting used in the current study. The level of activity in this study could probably have been significantly increased due to moderate toxicity of the bone seeking α emitter ^{223}Ra in mice (33), and ^{224}Ra, with its shorter half-life, is expected to be similar or less toxic per kBq compared with ^{223}Ra. Additionally, recently reported potential prognostic targets for bone metastatic disease in breast cancer, such as circulating MIC1/GDF15 levels and follistatin expression (34-35), can be investigated before and after treatment with ^{224}Ra-solution to correlate with treatment outcome.

Our findings show that a single dose of ^{224}Ra-solution prolonged survival time and lowered the incidence of paralysis and bone metastases in nude mice with breast cancer micrometastases. Radium-224 is a promising candidate for the treatment of breast cancer patients with bone metastases.

Acknowledgements

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