Radium-223: From Radiochemical Development to Clinical Applications in Targeted Cancer Therapy

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Abstract: The radiobiological and radiochemical properties of radium-223 (^{223}Ra, $T_{1/2}$ = 11.4 d) render this alpha-emitting radionuclide promising for targeted cancer therapy. Together with its short-lived daughters, each ^{223}Ra decay produces four alpha-particle emissions which enhance therapy effectiveness at the cellular level. In this paper, we review the recently published data reported for pre-clinical and clinical use of ^{223}Ra in cancer treatment. We have evaluated two distinct chemical forms of ^{223}Ra *in vivo*: 1) cationic ^{223}Ra as dissolved $RaCl_2$, and 2) liposome-encapsulated ^{223}Ra. Cationic ^{223}Ra seeks metabolically active osteoblastic bone and tumor lesions with high uptake and strong binding affinity based on its similarities to calcium. Based on these properties, we have advanced the clinical use of ^{223}Ra for treating bone metastases from breast and prostate cancer. The results show impressive anti-tumor activity and improved overall survival in hormone-refractory prostate cancer patients with bone metastases. In other studies, we have evaluated the biodistribution and tumor uptake of liposomally encapsulated ^{223}Ra in mice with human osteosarcoma xenografts, and in dogs with spontaneous osteosarcoma and associated soft tissue metastases. Results indicate excellent biodistributions in both species. In dogs, we found considerable uptake of liposomal ^{223}Ra in cancer metastases in multiple organs, resulting in favorable tumor-to-normal soft tissue ratios. Collectively, these findings show an outstanding potential for ^{223}Ra as a therapeutic agent.

Keywords: Cancer therapy, radionuclide, alpha-emitter.

INTRODUCTION

Alpha-emitting radionuclides can be prepared by several means. For example, ^{211}At is produced by alpha beam activation of bismuth in a cyclotron. Actinium-225 and its daughter ^{213}Bi are produced from ^{229}Th found in fissile ^{233}U, while ^{223}Ra and ^{227}Th are produced from ^{227}Ac generated by neutron irradiation of ^{226}Ra [1]. At the moment there exists modest source material for ^{225}Ac production and the cyclotron capacity is currently limiting the possibility of preparing ^{211}At at a commercial clinical scale [2]. Source material for ^{223}Ra and ^{227}Th already exists in large amounts and can be further increased using well established techniques [3]. This availability has allowed the completion of several phase II clinical studies with ^{223}Ra against skeletal metastases from prostate cancer and ^{223}Ra is expected to enter into clinical phase III in 2008. Thus, on the basis of commercial availability, ^{223}Ra seems to be the most likely alpha-emitter candidate for large scale medical use at the moment.

MEDICAL USES OF RADIUM

The element radium (from Latin *radius*, ray) has played a unique role in the development of radiochemistry and medical uses of ionizing radiation in diagnosis and treatment [4]. In the early 1900s, low-activity sources of ^{226}Ra were used in brachytherapy [5] while stronger sources were used in external teletherapy of cancer [6] until replaced by ^{137}Cs and ^{60}Co.

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In Table **1**, the properties and decay chains of the different naturally occurring radium nuclides are presented. Because of long half-lives ^{228}Ra and ^{226}Ra are not suitable for internal radionuclide therapy. Radium-223 and ^{224}Ra both have suitable half-lives for radionuclide therapy and both produce cascades of alphas from the decaying daughter nuclides, amounting to four alphas per series. Notable differences between ^{223}Ra and ^{224}Ra, including their respective gaseous radon daughters half-lives, dictate their usefulness for medical applications. The ^{223}Ra series has ^{219}Rn with a half-life of 4 seconds while the ^{224}Ra series has a half-life of 56 seconds, which potentially could affect a higher degree of translocalization of daughter products in the ^{224}Ra series. Also, the lead and bismuth daughters have significantly longer half-lives in the ^{224}Ra series versus the ^{223}Ra series, which may potentially cause some translocalization problem for these decay steps with the ^{224}Ra series.

^{224}Ra

Radium-224 ($t_{1/2}$ = 3.66 days) has a suitable half-life for internal radionuclide therapy. It can be prepared from ^{228}Th ($t_{1/2}$ = 1.9 y), using this nuclide as a long term operating generator. Thorium-228 can be produced in large quantities indirectly from neutron irradiation of ^{226}Ra, i.e., the initially produced ^{227}Ac has a high cross-section for neutron capture yielding ^{228}Th. Until recently ^{224}Ra has been used in Germany to treat ankylosing spondylitis. Good pain relief and disease stabilization have been reported [7, 8]. Solutions with cationic Ra in the form of dissolved $RaCl_2$ were used. Long term follow up of patients over several decades indi-

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Table 1. Summary of Half-Lives, Daughter Nuclides and Decay Properties for ^{223}Ra, ^{224}Ra, ^{226}Ra and ^{228}Ra

^{223}Ra (11.43 d, α)	^{224}Ra (3.66 d, α)	^{226}Ra (1600 y, α)	^{228}Ra (5.75 y, β)
^{219}Rn (3.96 s, α)	^{220}Rn (56 s, α)	^{222}Rn (3.8 d, α)	^{228}Ac (6.13 h, β)
^{215}Po (1.78 ms, α)	^{216}Po (0.15 s, α)	^{218}Po (3.05 min, α)	^{228}Th (1.9 y, α)
^{211}Pb (36.1 min, β)	^{212}Pb (10.6 h, β)	^{214}Pb (26.8 min, β)	^{224}Ra (3.66 d, α)
^{211}Bi (2.14 min, α)	^{212}Bi (60.6 min, β)	^{214}Bi (19.8 min, β)	^{220}Rn (56 s, α)
^{207}Tl (4.77 min, β)	^{212}Po(0.4 μs, α)	^{214}Po(162 μs, α)	^{216}Po (0.15 s, α)
^{207}Pb(stable)	^{208}Pb(stable)	^{210}Pb (22 y, β)	^{212}Pb (10.6 h, β)
		^{210}Bi (5.0 min, β)	^{212}Bi (60.6 min, β)
		^{210}Po(138 d, α)	^{212}Po(0.4 μs, α)
		^{206}Pb(stable)	^{208}Pb(stable)

From Chart of the Nuclides, November 1981, Kernforscungszentrum Karlsruhe, Germany.

cated increased risk for induction of bone tumors particularly in juveniles. However, in adults, no overall increased risk of tumor development or reduced life expectancy were observed, compared to controls, indicating that the use of bone-seeking alpha-emitters could be performed with an acceptable safety profile in grown-up individuals [9, 10].

^{223}Ra

The first report on use of cationic ^{223}Ra in humans was published about 40 years ago and was related to a biokinetic study with tracer amounts of radioactive alkaline earth metals for the purpose of acquiring data for radiation safety assessments [11]. The first study with therapeutic relevant quantities of dissolved ^{223}Ra salt was initiated in 2001. As with dissolved $^{89}SrCl_2$ (Metastron™), radium cations are incorporated within the bone matrix of metabolically active bone, probably by inclusion in the calcium hydroxyapatite crystals.

PREPARATION OF BONE-SEEKING ^{223}Ra AND PRECLINICAL STUDIES

Source material, ^{227}Ac, was prepared from irradiated samples of ^{226}Ra. By cation and anion exchange methods ^{227}Ac was prepared in good purity from ^{226}Ra [3]. After allowing time for ingrowth, ^{223}Ra was separated from ^{227}Ac and ^{227}Th trapped in Ac-resin [12]. The ^{223}Ra eluted from the resin had high purity with no detectable amounts of the longer lived generator nuclides ^{227}Ac and ^{227}Th as measured by gamma spectroscopy on fresh and on decayed (> 6 month old) samples. The ^{223}Ra was dissolved in physiologically compatible sodium chloride/sodium citrate buffer and sterile filtered. The cationic integrity of ^{223}Ra was determined using barium sulphate as a precipitation agent, yielding routinely better than 99 % precipitation.

Biodistribution analysis in rodents showed that cationic ^{223}Ra was an avid bone-seeker with little soft tissue uptake, and that the daughter radionuclides were well retained with minimal translocalization after uptake of the parent radionuclide in bone [13, 14]. Dosimetric modelling based on biodistribution data indicated that alpha-emitting bone-seekers, including ^{223}Ra, may have some advantages over beta-emitters, at least in terms of bone-surface to bone-marrow radiation dose ratios [13, 15]. A study addressing acute and sub-acute toxicity in mice from injected ^{223}Ra as dissolved salt, indicated that the bone marrow and the bone associated cells were severely affected by large dosages of ^{223}Ra, but that animals could survive doses far above those considered clinical relevant in humans in terms of kBq/kg [16].

In a comparative study of ^{223}Ra and the beta-emitter ^{89}Sr it was shown that ^{223}Ra and ^{89}Sr had similar bone uptake, and that estimates of dose deposition in bone marrow suggested a clear advantage of alpha-particle emitters for sparing bone marrow [13]. A therapeutic study of ^{223}Ra in a nude rat skeletal metastases model showed a significant antitumor activity. This model was resistant to high doses of cisplatin, doxorubicin and an immunotoxin, as well as to both pamidronate (Aredia™) and ^{131}I-labeled bisphosphonate treatment, suggesting that ^{223}Ra is therapeutically more effective and could be beneficial in the treatment-resistant skeletal metastases [14]. Thus, animal data and dosimetric studies indicated that bone-targeted alpha-emitters can deliver therapeutically relevant radiation doses to bone surfaces and skeletal metastases at activity levels that are acceptable in terms of bone marrow radiation exposure [13-16], suggesting a basis for clinical investigation.

CLINICAL STUDIES WITH ^{223}Ra

A clinical development program for $^{223}RaCl_2$ (Alpharadin™) was initiated based on the results presented above and on approval obtained from the relevant institutional review boards and regulatory authorities.

Phase IA

In a phase I study of single-dosage administration of increasing levels of ^{223}Ra (46, 93, 163, 213, or 250 kBq/kg) in 25 patients with bone metastases from breast and prostate cancer [17] dose-limiting hematological toxicity was not reached. Mild and reversible myelosuppression was observed in some cases, with only grade 1 toxicity for thrombocytes at

Table 2. Summary of Effective Energy and Dose Constants for ^{223}Ra and Progeny

Nuclide (half-life)	Effective energy* (MeV)	Dose constant Δ (Gy kg Bq^{-1} s^{-1})
^{223}Ra (11.43 d)	5.85	9.37×10^{-13}
	5.65**	9.05×10^{-13}
^{219}Rn (3.96 s)	6.81	1.09×10^{-12}
	6.75**	1.08×10^{-12}
^{215}Po (1.78 ms)	7.53	1.21×10^{-12}
	7.53**	1.21×10^{-12}
^{211}Pb (36.1 min)	0.512	8.20×10^{-14}
^{211}Bi (2.14 min)	6.73	1.08×10^{-12}
	6.67**	1.07×10^{-12}
^{207}Tl (4.77 min)	0.498	7.98×10^{-14}

Schematic summary of decay data extracted from the MIRD data-base (http://www.nndc.bnl.gov/mird). Database version of July 2, 2007.

*Includes alpha, beta, photon, X-ray, and electron energies.

**Includes only alpha-particle energies. Branching of less than 1 % is not considered.

the two highest dose levels. Quality of life was evaluated at baseline and at 1, 4, and 8 weeks after injection, and pain relief was indicated for all time points in more than 50 % of the patients [17]. Notably, a decline in total serum alkaline phosphatase greater than 50 %, a marker for abnormal bone metabolism in metastatic prostate cancer, was observed among patients with elevated pre-treatment values. Radium-223 showed rapid blood clearance with only 12 % of its initial value at 10 minutes after injection, and a further reduction to 6 % at one hour and to less than 1 % at 24 hours after infusion. Gamma-camera scintigraphy indicated that ^{223}Ra accumulated in skeletal lesions similar to patterns observed in diagnostic bone scans with ^{99m}Tc-MDP [17]. Radium-223 cleared mainly by the intestinal route, in accordance with findings in older studies with dogs and primates receiving injections with dissolved radium salts. This could potentially be a problem since intestines are considered to be among the radiation sensitive tissues. If the activity is located in the intestinal content, intestinal clearance may be less of a problem with alpha-emitters since the range of less than 100 μm for alphas would cause only a superficial exposure of the inner intestinal surfaces. And indeed, a biodistribution study with two dogs 24 hours after i.v. infusion of a ^{223}Ra solution revealed that the sources were mainly located in the intestinal contents and not in the intestinal wall [17].

Phase IB

A small phase IB feasibility study involving six patients with advanced prostate cancer was then performed [18] to evaluate the safety profile of repeated ^{223}Ra injections. Six prostate cancer patients were administered up to 250 kBq kg^{-1} body weight, either as a fractionated regimen of two injections of 125 kBq kg^{-1} bodyweight with a six-week interval (three patients) or 50 kBq kg^{-1} body weight given five times with a three-week interval (three patients). The patients in the 50 kBq kg^{-1} × 5 group did not experience any additional toxic effects related to repeated treatment compared with the single-injection in the phase IA study. The hematological profiles were less determined by the fractionation schedule compared to a single dosage totaling the same as the five fractions combined. Because of non-skeletal disease progression, only one of the patients in the 125 kBq kg^{-1} × 2 group actually got the second dosage. Of the two patients not given the 125 kBq kg^{-1} follow-up dosages, one died due to progression of liver metastases, and the other was deemed unfit for further treatment due to recurrence of a previous heart condition. Mild and reversible myelosuppression occurred, with nadir two to three weeks after injection, and complete recovery during the follow-up period. The thrombocytes revealed only grade 1 toxicity, whereas neutropenia of maximum grade 3 occurred in one of the patients. Few other adverse events were seen [18]. The main experience from this small phase IB study was that repeated administration of ^{223}Ra was well tolerated, and that the time span between injections should be scheduled according to the dosages given, so that the blood cell count could normalize before a new injection.

Phase II

Data from a phase II randomized trial, where late stage prostate cancer patients received external beam radiation plus either saline injections (four times with 4-week intervals) or four times repeated ^{223}Ra (50 kBq/kg given at 4-week intervals), have recently been published [19]. Adjuvant ^{223}Ra treatment resulted in a statistically significant decrease in bone alkaline phosphatase from baseline compared to placebo, showing a strong decrease in patients with elevated pre-treatment levels [19]. The median relative change during treatment for the external radiation plus ^{223}Ra group (33 patients) was – 65.6 % *vs*. + 9.3 % in the external beam radiation plus saline group (31 patients). This observation showed that the areas mostly affected by ^{223}Ra were the regions with an elevated bone metabolism [17]. In the external radiation

plus ^{223}Ra group, 15 of 31 patients had a prostate-specific antigen (PSA) decrease of more than 50 % from baseline compared to only five of 28 patients in the group receiving external radiation plus saline. The median time to PSA progression was 26 weeks in the ^{223}Ra group and eight weeks in the placebo group [19].

A favorable adverse-event profile was confirmed with minimal bone marrow toxicity for patients who received ^{223}Ra [19]. The myelosuppression observed after ^{223}Ra treatment was minimal and seemed to be different from that observed with the beta-emitting nuclides [20, 21]. With ^{223}Ra, the neutrophils decreased more than thrombocytes, whereas for beta-emitters, thrombocytopenia are commonly dose limiting. With alpha-emitters, the endosteal bone surface received high radiation doses, whereas considerable fractions of the bone-marrow were spared.

Survival analyses from this phase II trial showed a significant overall survival benefit [19]. The hazard ratio for overall survival, adjusted for baseline covariates was 2.12 ($p = 0.020$, Cox regression). The clinical follow-up data from the phase II trial also showed that more than twice as many patients receiving Alpharadin™ were alive (10 of 33) two years following start of treatment compared to those that received placebo (four of 31) [22]. These findings suggested that ^{223}Ra, alone or in combined treatment strategies, should be included in future therapeutic studies to further delay disease progression and improve survival in patients with skeletal metastases from hormone-refractory prostate cancer.

DOSE ESTIMATES FOR ^{223}Ra

One of the challenges when using alpha-emitting radionuclides in cancer therapy is that the high cytotoxicity of alpha particles limits the activity of radionuclides that may be administered. For instance, using ^{223}Ra as a bone-seeker to target skeletal metastases from prostate or breast cancer, typically less than 5 MBq may be administered per treatment. In contrast, a routine bone scan is usually performed with 750 MBq of ^{99m}Tc-MDP. Imaging is possible with ^{223}Ra by virtue of its photon emissions (81 keV at 15 %, and 84 keV at 25.6 %, 154 keV at 6 %, and 269 keV at 13.6 %, respectively) that are emitted during its decay. Daughter product photons (^{211}Bi with a 351 keV gamma at 13 % abundance) may be followed separately by imaging.

In a preclinical mouse study, it was found that cationic ^{223}Ra had a similar biodistribution as cationic ^{89}Sr, with rapid and highly selective uptake in metabolic active bone versus soft tissues [13].

Dose distribution modelling indicated that the bone surface to bone marrow ratio was better for the alpha-emitting ^{223}Ra compared to beta-emitting ^{89}Sr. Another important finding was that the daughter products from the ^{223}Ra decay were retained almost quantitatively in the bone and did not translocate into the bone marrow compartment. To deliver an average of 1 Gy to the bone of mice would require injections of 0.04 kBq of ^{223}Ra and 1.22 kBq of ^{89}Sr per gram of bodyweight, respectively [13]. If one assumed a relative biological effectiveness of 5 for alpha emissions relative to 1 for beta emissions, to obtain 1 Sv (equivalent dose) to the bone would require injections of 8 Bq of ^{223}Ra *vs*. 1.22 kBq of ^{89}Sr per g of bodyweight.

During the phase I study in breast and prostate cancer, initial attempts were made to image the distribution of ^{223}Ra. Those images suggested a similar bone uptake of ^{223}Ra similar to that of ^{99m}Tc-MDP with a particularly high accumulation in the skeletal metastases. The investigators confirmed that ^{223}Ra in contrast to most bone-seeking radiopharmaceuticals mainly cleared *via* the intestinal route [17]. Based on the assumptions for elemental radium inherent in the ICRP-67 recycling model with some modifications for an overall greater initial skeletal uptake in the cancer patient, and a quality factor of 5, we estimated the equivalent doses for various tissues in man from ^{223}Ra injections [18]. These estimates indicated that for a 50 kBq per kg of bodyweight dosage, the bone surfaces would receive 13.05 Sv while the red bone marrow would receive inhomogeneous dose-depositions, i.e., close to 0 % at sites distant from the bone surfaces and almost 100 % of the bone surfaces equivalent dose for bone marrow cells nearby the bone surfaces. The average bone-surface to red bone marrow dose ratio was estimated to be 10.3 [18]. As for the soft tissues, liver, the large intestines, and colon, these critical organs would receive equivalent doses of 0.635, 0.367 and 0.254 Sv, respectively.

Tumor dosimetry has not yet been presented for ^{223}Ra. If one assumes as found in mice [13], that an activity ratio of 1: 30 causes the same absorbed radiation dose for ^{223}Ra as for ^{89}Sr in human skeleton and bone metastases some crude tumor dose assessments can be made. Silberstein and Williams [23] estimated the tumor absorbed dose to be 8.1 cGy/MBq for ^{89}Sr. This would then correspond to 243 cGy/MBq and 12.15 Sv/MBq for ^{223}Ra in tumors. By extension, a 70 kg person receiving 50 kBq/kg ^{223}Ra would therefore receive a tumor dose equivalent of 42.5 Sv. This simplified dose assessment does not take into account tumor size or radionuclide microdistribution variability. There is therefore a need for more accurate information on dosimetry of ^{223}Ra in cancer patients.

The company Algeta ASA is currently conducting a study with Alpharadin™ in humans which is expected to gain more detailed data on the distribution and dosimetry of ^{223}Ra in human patients.

LIPOSOMAL ^{223}Ra

To extend the use of the ^{223}Ra to solid tumors and soft tissue metastases, ^{223}Ra would have to be incorporated in a carrier compound with tumor-seeking properties. A few studies have suggested that liposomes may have a potential as carrier for radionuclides [24-26]. Pegylated liposomes were introduced clinically as a mean to reduce the cardiotoxicity of chemotherapeutics, especially doxorubicin [27]. One formula of liposomal doxorubicin (Caelyx™/Doxil™) is today commercially available for the treatment of cancer. Some tumor selectivity is obtained by exploiting the capillary leakage property of neovasculature of malignant tissue. A study was therefore initiated to evaluate the therapeutic potential of liposome-encapsulated alpha-emitters. A method was established allowing high-yield preparation of lipsome-

encapsulated radionuclides like ^{223}Ra, ^{225}Ac, ^{212}Bi, ^{212}Pb using ionophore-mediated active loading [25]. It was also shown that the liposomes would retain ^{212}Bi after intraliposomal decay of ^{212}Pb [24].

Preparation: Radium-223 solution as described above was used for loading into liposomes. Both commercial liposomes and liposomes prepared in the laboratory were evaluated. Caelyx™, which comprises 2 mg/ml doxorubicin encapsulated in liposomes averaging 80 nm in diameter, was subjected to buffer exchange to a 20 mM HEPES and 300 mM sucrose buffer adjusted with NaOH to pH 7-8, using a centrifuge concentration cartridge (UFW2BTK, 30 KNMWL, Millipore, Bedford, IL, USA). Caelyx™ was used at a concentration two to four times the initial concentration. To a 2 ml glass vial was added 15 µg of Ca-ionophore (Calcimycin, Sigma, St. Louis, MO, USA) dissolved in either DMSO or chloroform. The solvent was evaporated with a stream of N_2 gas, and thereafter ^{223}Ra solution and the liposomes were added and heated to 60 °C, then gently incubated for 20 min. A volume of about 50 µl of 10 mM EDTA was added and the solution further incubated for 5 min. at room temperature before elution through a Sephadex G-25 PD-10 column (GE Health/Pharmacia, Lund, Sweden). Product yields were typically 60-90 %. When liposomes were tested for stability in serum at 37 °C for 24 h, more than 95 % of the radionuclide was retained with the liposomes. It was demonstrated that liposome-encapsulated radium as well as actinium could be prepared from pre-formed liposomes by ionophore-mediated loading by the methods described above, and that ^{223}Ra was retained by the liposomes after serum incubation at 37 °C [25].

To evaluate the therapeutic potential of alpha-emitting liposomes, *in vivo,* behaviour and stability were studied for liposome-encapsulated ^{223}Ra. Well-characterized liposomes (Caelyx™/Doxil™) were used as carriers for ^{223}Ra. Biodistribution and blood clearance were evaluated in mice with or without xenografts, and were tested later in dogs [28, 29].

In vivo studies: Liposomal ^{223}Ra was administered intravenously. A pre-treatment of Caelyx™ was given before the injection of liposomal ^{223}Ra to reduce the reticulo-endothelial-system uptake. A pilot study was conducted to establish the best pre-treatment/treatment-schedule to achieve optimal blood-to-liver and blood-to-spleen ratios in mice. An extensive biodistribution study was later performed in Balb/C mice.

Results: Improved blood-to-liver and blood-to-spleen ratios of liposomal radium were achieved in animals pre-treated with Caelyx™ four days in advance. Blood clearance in mice was relatively slow with $t_{1/2}$ ~28 h (Balb/C mice). In dogs it was even slower with $t_{1/2}$ ~ 40 h. In mice the liver uptake appeared to be relatively low in contrast to the spleen, where there was a significant uptake. The results showed that *in vivo* the ^{223}Ra largely was retained in the liposomes, in accordance to the *in vitro* stability shown with the in Caelyx™/Doxil™ liposomes as well as in empty pegylated liposomes loaded with ^{223}Ra [25, 28]. The observed biodistribution and blood half-life of liposomal-encapsulated ^{223}Ra agreed with values reported for Caelyx™/Doxil™ in rodents. Because of the considerable half-life of ^{223}Ra, bone uptake (of released cationic ^{223}Ra) increased when liposomes were gradually catabolized at later time-points, as measured by biodistribution at 6 days and 14 days after injection.

Comparative biodistributions of cationic and liposomal ^{223}Ra were evaluated in mice and dogs. In addition to much slower blood clearance of liposomal ^{223}Ra compared to cationic ^{223}Ra, distinct differences in tissue distribution were observed for each. With liposomal ^{223}Ra higher activity was observed in blood and soft tissues compared to cationic ^{223}Ra. Spleen had the highest uptake using liposomal ^{223}Ra while bone had the highest uptake using cationic ^{223}Ra. Tumor uptake of liposome-encapsulated ^{223}Ra was evaluated after intravenous injection in nude mice with osteosarcoma xenografts and dogs, spontaneously affected by osteosarcoma. In dogs, the primary tumor was removed when diagnosed, but multiple soft tissue metastases containing various degrees of calcification had developed when treated with liposomal ^{223}Ra. Adequate uptake of ^{223}Ra encapsulated-liposomes through capillary leakage into solid tumors required sufficient circulation time for the compound in the blood. In the xenograft mouse model, a prolonged retention of liposomal ^{223}Ra in the tumor versus soft tissue was observed, with maximum retention of ^{223}Ra after four to five days. In spleen, the uptake was higher than in other soft tissues, particularly at early time points, and at later time points the uptake was relatively high in bone. In dogs, the uptake was considerably higher in both calcified and non-calcified tumor metastases of different organs than in normal tissue. Splenic uptake was lower in dogs than in mice, while the uptake in most tumors was higher in dogs than in mice xenografts when activity (Bq/g) in the tissues relative to a given dosage (kBq/kg of body weight) were compared in the two species [29].

OTHER CHEMICAL FORMS OF ^{223}Ra

Radioimmuntherapy with ^{223}Ra could be another possible use of this radionuclide. However, the chemical properties of Ra may be challenging to overcome when searching for suitable chelators for conjugating ^{223}Ra to antibodies. Several compounds, including DTPA, Kryptofix 2.2.2, DOTA, DOTMP and the calixarene, 4-tert-butyl calyx[4]-tetraacetic acid, were evaluated *in vitro*. Generally, the ^{223}Ra com plexes of the different compounds were too unstable for serious applications in radioimmunotherapy. The least instability was observed with the calixarene compared to the other compounds, but even for this complex the dissociation of the complex were deemed to rapid to support *in vivo* evaluation [30]. Thus to develop ^{223}Ra for radioimmunotherapy, there is a need to search for novel conjugation agents to stably link the radionuclide to an antibody, e.g., nanoparticle or nanotube encapsulation.

CONCLUSION

Radium-223 is today the only alpha-emitter used in large scale clinical trials. More than 200 patients with skeletal metastases have to date received treatment with this radionuclide in the form of a solution containing cationic Ra (Alpharadin™). GMP production facilities have been established and with current technologies it can relatively easily be produced in quantities required for worldwide commercial

pharmaceutical usage. The clinical results so far show an excellent safety profile, impressive anti-tumor activity and improved overall survival in hormone-refractory prostate cancer patients with bone metastases. In other studies, the biodistribution and tumor uptake of liposome-encapsulated ^{223}Ra in mice with human osteosarcoma xenografts, and in dogs with spontaneous osteosarcoma and associated soft tissue metastases were evaluated. Results indicate promising biodistributions in both species. In dogs, considerable uptake of liposomal ^{223}Ra in cancer metastases was observed, resulting in favorable tumor-to-normal soft tissue ratios. Collectively, these findings show an outstanding potential for ^{223}Ra as a therapeutic agent.

REFERENCES

[1] Feinendegen, L.E.; McClure, J.J. Workshop: Alpha emitter for medical therapy, Denver Colorado, May 30-31, **1996**, DOE/NE-0113, US Department of Energy.

[2] McClure, J.J.; Feinendegen, L.E. Alpha emitter for medical therapy. Second bi-annual workshop. Toronto, Canada, June 4-5, **1998**, DOE/NE-0116, US Department of Energy.

[3] Gmelin's handbook, Radium, Vol. *2*, Springer Verlag: Berlin/Heidelberg/New York, **1977**.

[4] Mazeron, J.J.; Gerbaulet, A. *Radiother. Oncol.,* **1998**, *49*(3), 205.

[5] Sorbe, B.; Kjellgren, O.; Stensson, S. *Acta Oncol.,* **1990**, *29*(1), 29

[6] Carling, E.R.; Allclein, F.M. *Proc. R. Soc. Med*., **1935,** *28*(8), 1145.

[7] Zhang, Z.; Siegert, J.; Maywaldt, U.; Kirch, W. *Med. Klin., (Munich),* **2007**, *102*(7), 540.

[8] Tiepolt, C.; Grüning, T., Franke W.G. *Nucl. Med. Commun.,* **2002**, *23*(1), 61.

[9] Wick, R.W.; Nekolla, E.A.; Gøssner, W.; Kellerer, A.M. *Radiat. Res.,* **1999**, *152*, S8.

[10] Nekolla, E.A.; Kellerer, A.M.; Kuse-Isingshulte, M.; Eder, E.; Spiess, H. *Radiat. Res.,* **1999**, *152*, S3.

[11] Harrison, G.E.; Carr, T.E.F.; Sutton, A. *Nature,* **1966**, *209*, 526.

[12] Henriksen, G.; Hoff, P.; Alstad, J.; Larsen, R.H. *Radiochim. Acta,* **2001**, *89*, 661.

[13] Henriksen, G.; Fisher, D.R.; Roeske, J.C.; Bruland, Ø.S.; Larsen, R.H. *J. Nucl. Med*., **2003**, *44*, 252.

[14] Henriksen, G.; Breistøl, K.; Bruland, Ø.S.; Fodstad, Ø.; Larsen, R.H. *Cancer Res*., **2002**, *62*, 3120.

[15] Larsen, R.H.; Murud, K.M.; Akabani, G.; Hoff, P.; Bruland, Ø.S.; Zalutsky, M.R. *J. Nucl. Med*., **1999**, *40*, 1197.

[16] Larsen, R.H.; Saxtorph, H.; Skydsgaard, M.; Borrebæk, J.; Jonasdottir, T.J.; Bruland, Ø.S.; Klastrup, S.; Harling, R.; Ramdahl, T. *In Vivo,* **2006**, *20*, 325.

[17] Nilsson, S.; Larsen, R.H.; Fosså, S.D.; Balteskard, L.; Borch, K.W.; Westlin, J.E.; Salberg, G.; Bruland, Ø.S. *Clin. Cancer Res.,* **2005**, *11*(12), 4451.

[18] Bruland, Ø.S.; Nilsson, S.; Fisher, D.R.; Larsen, R.H. *Clin. Cancer Res.,* **2006**, *12*(20 suppl.), 6250s.

[19] Nilsson, S.; Franzen, L.; Parker, C.; Tyrrell, C.; Blom, R.; Tennvall, J.; Lennernas, B.; Petersson, U.; Johannessen, D.C.; Sokal, M.; Pigott, K.; Yachnin, J.; Garkavij, M.; Strang, P.; Harmenberg, J.; Bolstad, B.; Bruland, Ø.S. *Lancet Oncol.,* **2007**, *8*, 587.

[20] Finlay, I.G.; Mason, M.D.; Shelley, M. *Lancet Oncol.,* **2005**, *6*(6), 392.

[21] Lewington, V.J. *J. Nucl. Med.,* **2005**, *46*, 38s.

[22] Alpharadin treatment more than doubles survival rate in prostate cancer patients. Ramdahl, T.; Swallow, M.; Galilee, H. Ed., WWW.algeta.com. Press release, September 25th, **2007**.

[23] Silberstein, E.B.; Williams C. *J. Nucl. Med.,* **1985**, *26*(4), 345.

[24] Henriksen, G.; Schoultz, B.W.; Hoff, P.; Larsen, R.H. *Radiochim. Acta,* **2003**, *91*, 109.

[25] Henriksen, G.; Schoultz, B.W.; Michaelsen, T.E.; Bruland, Ø.S.; Larsen, R.H. *Nucl. Med. Biol.,* **2005**, *31*, 441.

[26] Harrington, K.J.; Mohammadtahi, S.; Uster, P.S.; Glass, D.; Peters, A.M.; Stewart, J.S. *Clin. Cancer Res.*, **2001**, *7*(2), 243.

[27] Gabizon, A.A.; Shmeda, H.; Zalipsky, S. J. *Liposome Res.,* **2006**, *16 (3)*, 175.

[28] Jonasdottir, T.J.; Fisher, D.R.; Borrebæk; J.; Bruland, Ø.S.; Larsen, R.H. *Anticancer Res.,* **2006**, *26*, 2841.

[29] Jonasdottir, T.J.; Borrebæk; J.; Bruland, Ø.S.; Salberg, G.; Moe, L.; Larsen, R.H. Alpha-emitter symposium Aachen, Germany, April 29-30, **2007**.

[30] Henriksen, G.; Hoff, P.; Larsen, R.H. *Appl. Radiat. Isot.,* **2002**, *56*, 667.

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