Thorium and Actinium Polyphosphonate Compounds as Bone-seeking Alpha Particle-emitting Agents

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Abstract. The present study explores the use of α-particle-emitting, bone-seeking agents as candidates for targeted radiotherapy. Actinium and thorium 1,4,7,10 tetraaza-cyclododecane N, N', N'', N''' 1,4,7,10-tetra(methylene) phosphonic acid (DOTMP) and thorium-diethylene triamine N,N',N'' penta(methylene) phosphonic acid (DTMP) were prepared and their biodistribution evaluated in conventional Balb/C mice at four hours after injection. All three bone-seeking agents showed a high uptake in bone and a low uptake in soft tissues. Among the soft tissue organs, only kidney had a relatively high uptake. The femur/kidney ratios for 227Th-DTMP, 228Ac-DOTMP and 227Th-DOTMP were 14.2, 7.6 and 6.0, respectively. A higher liver uptake of 228Ac-DOTMP was seen than for 227Th-DTMP and 227Th-DOTMP. This suggests that some demetallation of the 228Ac-DOTMP complex had occurred. The results indicate that 225Ac-DOTMP, 227Th-DOTMP and 227Th-DTMP have promising properties as potential therapeutic bone-seeking agents.

Bone-targeting radiopharmaceuticals involving the energetic beta-emitters 32P and 89Sr have been used clinically for several decades to palliate pain in patients with skeletal metastases (1,2). The major dose-limiting factor with these radiopharmaceuticals is toxicity to the bone marrow (3-6), presumably caused by the long range of the beta-particles. Radionuclides that emit particles of a shorter range are therefore of interest.

The clinical use of the low-energy beta emitters 153Sm (7,8) and 186Re (9,10) and the conversion electron emitter 117mSn (11,12) has been explored. The results indicate that there is still room for improving the pain relieving and therapeutic potential of bone-targeting radiopharmaceuticals (4-6,13).
to target calcified tissue (22–24). The uptake of polyphosphonates is high in regions with an elevated bone turnover (25), which makes them appealing for use as carriers of radionuclides in the targeting of bone-forming tumors or calcified metastases in the skeleton. The goal of the current work was to investigate if relevant in vivo stability and bone-targeting could be obtained with Th- and Ac-polyphosphonates.

Materials and Methods

Reagents and equipment. The $^{232}$Th-nitrate (J.T. Baker, Phillipsburg, NJ, USA) used in this study had been stored for more than 20 years. AG50W-x 12 and Chelex-100 ion-exchange resins were both obtained from Bio-Rad, Hercules, CA, USA. Amberchrom XAD-7HP resin was purchased from Rohm & Haas (Frankfurt, Germany). Diethylenetriamine $N,N',N''$ penta(methylene)phosphonic acid (DTMP) was obtained from Fluka, Buchs, Switzerland, $1,4,7,10$ tetraazacyclododecane $N,N',N'',N'''$ 1,4,7,10-tetra(methylene) phosphonic acid (DOTMP) was purchased from Macrocyclics, Richardson, TX, USA. All water used was purified through a Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of $^{227}$Th. $^{227}$Th was selectively retained from a $^{227}$Ac decay mixture in 7 M HNO$_3$ solution by anion exchange chromatography (26). A column of 2 mm internal diameter, length 30 mm containing 70 mg of AG-1 x 8 resin (200–400 mesh, nitrate form) was used.

After $^{227}$Ac, $^{223}$Ra and daughters had eluted from the column, $^{227}$Th was extracted from the column with 12 M HCl. The eluate containing $^{227}$Th was evaporated to dryness and the residue dissolved in 0.1 M HNO$_3$.

Preparation of $^{225}$Ac. $^{225}$Ra ($t_{1/2}$=5.75 years), which served as generator material for $^{225}$Ac, was isolated by solvent extraction from $^{225}$Th (27). By this procedure thorium is selectively extracted into the organic phase while radium remains in the aqueous phase. Briefly, $^{225}$Th-nitrate was dissolved in 20 ml of 0.1 M HNO$_3$ and extracted by shaking the aqueous phase three times with 70 ml of a 2 M solution of di-(2-ethylhexyl) orthophosphoric acid (HDEHP) in heptane. The aqueous phase was subsequently washed with 3 x 30 ml heptane. After this, the aqueous solution was concentrated to 10 ml by evaporation and the concentrate applied to a column of 4 mm internal diameter and a length of 70 mm filled with Amberlite XAD-7HP resin for removing residual organic compounds. For further purification, the solution containing $^{225}$Ra and $^{224}$Ra was applied to a 3 x 40 mm column containing 0.2 g of AG50W-X12 cation exchange resin (200–400 mesh, H$^+$-form). The column was washed with 10 ml of 1 M HNO$_3$ followed by stripping $^{224}$Ra, $^{224}$Ra, $^{212}$Pb and $^{212}$Bi with 5 ml of 3 M HNO$_3$. The last elute was left for one month in order to allow $^{224}$Ra to decay.

$^{225}$Ac were separated from $^{224}$Ra on a fresh 3 x 40-mm column of AG50W-X12. $^{224}$Ra was eluted in 5 ml 3 M HNO$_3$ before eluting $^{225}$Ac with 5 ml of 6 M HNO$_3$. In preparation for chelation chemistry, the solution containing $^{224}$Ac was evaporated to dryness and $^{224}$Ac dissolved in 0.1 M HNO$_3$. The $^{224}$Ac produced in this manner contained less than 0.5 Bq $^{224}$Ra / kBq $^{224}$Ac, as measured by $\gamma$-spectroscopy on samples stored for a time corresponding to >10 half lives of $^{224}$Ac.

Preparation of thorium and actinium polyphosphonate chelates. A 50 mM aqueous solution of the desired chelating agent was made and the pH adjusted to 5-5.5 by adding 3 M ammonium acetate. Twenty to thirty $\mu$l of this solution was mixed with 50-100 $\mu$l of a 0.1 M HNO$_3$ solution containing the radionuclide. The pH was adjusted to 5-5.5 using 3 M ammonium acetate and the reaction mixture was kept at 60°C for one hour. After this, the solution was applied to a 2 x 20-mm column containing 40 mg of Chelex-100 cation-exchange resin, (100-200 mesh, ammonium form) for isolation of radionuclide complexes.

More than 80% of the $^{227}$Th eluted from the column with both the DTMP and DOTMP chelators. The corresponding value obtained with $^{225}$Ac-DOTMP was in the order of 60%.

$^{225}$Ac-DOTMP, $^{227}$Th-DTMP and $^{227}$Th-DOTMP solutions for injection were prepared by diluting the complex in a 0.1 M solution of 2-[(N-morpholino)ethanesulfonic acid (MES), sodium salt, followed by filtration through sterile 0.22-µm nylon filters (Whatman, Maidstone, UK). The final concentration of DOTMP and DTMP was 5 mM.

Preparation of $^{227}$Th-acetate/MES solution. A solution containing $^{227}$Th as a weakly complexed cation was prepared in the following manner: 0.1 M HNO$_3$ solution containing $^{227}$Th was added to 3 M ammonium acetate to obtain a pH of 5.5. This solution was diluted to the desired activity concentration and to a pH of 7.4 by using 0.1 M MES buffer. Finally, the solution was filtered through sterile 0.22-µm nylon filters.

Biodistribution experiments. The biodistribution of $^{227}$Th-acetate, $^{227}$Th-DTMP, $^{227}$Th-DOTMP and $^{225}$Ac-DOTMP were studied in conventional mice. All procedures and experiments involving animals in this study 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.

Young Balb/C mice with an average body weight of 14 g were used in the biodistribution experiments. The preparations were administered by tail vein injection of 100 $\mu$l solution to each animal containing approximately 5 kBq of $^{227}$Th or $^{225}$Ac. Groups of three animals were sacrificed by cervical dislocation after 4 h and the tissue distribution of radionuclides was determined.

$^{227}$Th was measured by its 236 keV $\gamma$-ray (12.3 % probability) employing a HPGe detector (Canberra, Meriden, CT, USA) coupled to a multichannel analyzer (EG&G ORTEC, Oak Ridge, TN, USA) and also by liquid scintillation counting using a Beckmann LS 6500 (Beckmann, Fullerton, CA, USA) after dissolution of tissue samples. Before the liquid scintillation counting, soft tissue samples were dissolved by adding 1-3 ml of Soluene 350 (Packard, BioScience BV, Groningen, The Netherlands) per 100 mg tissue and bone samples were dissolved in HClO$_4$:H$_2$O$_2$:1:2 (v/v). All tissue samples were kept at 50°C until they were completely dissolved. If required, soft tissue samples were bleached by adding H$_2$O$_2$. Finally, Instagel Plus II scintillation cocktail (Packard) was added and the samples were then stored in the dark to allow decay of luminescence.

$^{225}$Ac was measured by the HPGe detector by its 911.2 keV $\gamma$-ray (26.6 % probability) and by a NaI(Tl) well-type detector (Harshaw Chemie BV, De Meern, Holland) combined with a Scaler Timer ST7 (NE Technology Ltd, Reading, UK) digital unit.

Samples of the radionuclide preparations were used as references in the measurement procedures.
Results

Actinium and thorium chelated to polyphosphonates were shown to give high bone to soft tissue ratios indicating adequate stability of the coordination compounds for in vivo targeting (Figure 1). The bone samples from femur, skull and ribs all had much higher uptake than the soft tissue organs studied, reflecting a strong and selective bone affinity of the compounds (Figure 1). Among the soft tissue organs, only kidneys had a relatively high uptake 4 h after injection, which is consistent with the rapid excretion of the polyphosphonate chelates from the soft tissues. The femur/kidney ratios for \(^{227}\text{Th}-\text{DTMP}, ^{228}\text{Ac}-\text{DOTMP}\) and \(^{227}\text{Th}-\text{DOTMP}\) were 14.2, 7.6 and 6.0, respectively. Furthermore, the femur/liver ratio for \(^{228}\text{Ac}-\text{DOTMP}\) was 16.4. The liver uptake of \(^{228}\text{Ac}-\text{DOTMP}\) was higher than the corresponding data for \(^{227}\text{Th}-\text{DTMP}\) and \(^{227}\text{Th}-\text{DOTMP}\) (Figure 1). This suggests that some demetallation of the complex had occurred.

For comparison, tissue distribution data for \(^{227}\text{Th}\) in acetate/MES solution are presented in Table I. It can be seen that the bone uptake of cationic thorium was comparable to that obtained with the bone-seeking polyphosphonate coordination compounds in this study. However, the affinity of the thorium cation for the soft tissue organs, especially liver and spleen, renders this chemical form unsuitable as a bone-targeting agent.

Discussion

\(^{225}\text{Ac}\) and \(^{227}\text{Th}\) are attractive \(\alpha\)-particle emitters for use in targeted radiotherapy for several reasons:

1) The relatively long physical half-lives of 10 days and 18.7 days are advantageous by allowing sufficient time for preparation, shipping and administration of radiopharmaceuticals.

2) They can be produced from radionuclide generators securing continuous supply based on long-lived source material.

3) More than 90% of the total energy emitted in the decay cascade stems from \(\alpha\)-particles. Thus, it may be possible to deliver an intense and highly localized irradiation dose to bony surfaces if the nuclide can be targeted to bone.

In the current study it was found that the bone uptake of actinium-DOTMP, thorium-DOTMP and thorium-DTMP in mice was high and selective compared to the uptakes in the soft tissues. Biodistribution of actinium in mice has recently been published (28). These data revealed that this element, as a weakly complexed cation, does not show sufficiently selective bone uptake relative to soft tissue uptake to be suitable as a bone-targeting endoradiotherapeutic agent.

Figure 2 illustrates the localisation index of the chelates versus the respective free radionuclide. Actinium-DOTMP and thorium-DOTMP/DOTMP in mice was high and selective compared to the uptakes in the soft tissues. Biodistribution of actinium in mice has recently been published (28). These data revealed that this element, as a weakly complexed cation, does not show sufficiently selective bone uptake relative to soft tissue uptake to be suitable as a bone-targeting endoradiotherapeutic agent.

The biodistribution of \(^{225}\text{Ac}\) has been reported for various concentrations of ethylene diamine \(N,N'\) tetra methylene phosphonic acid (EDTMP) (29). However, at the concentration of EDTMP required to prevent a high soft tissue uptake, the femur uptake was below 10% of I.D./g (29). The results from the present study with \(^{228}\text{Ac}-\text{DOTMP}\) show that conditions can be found which result in a low soft tissue uptake with concurrent high uptake of actinium in bone. This shows that DOTMP is a more effective bone-targeting carrier for actinium than EDTMP.

Table I. Biodistribution of \(^{227}\text{Th}\).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% of I.D./g</th>
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<tbody>
<tr>
<td>Blood</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>56.5 ± 9.4</td>
</tr>
<tr>
<td>Femur</td>
<td>23.4 ± 6.9</td>
</tr>
<tr>
<td>Skull</td>
<td>18.9 ± 7.6</td>
</tr>
<tr>
<td>Rib</td>
<td>14.6 ± 4.4</td>
</tr>
<tr>
<td>Lung</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Heart</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>14.2 ± 2.8</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.6 ± 1.0</td>
</tr>
</tbody>
</table>

Data are presented as percent of injected dose per gram tissue in female Balb-C mice, weight 11-16 g at 4 h after injection. The data are presented as mean ± s.d., N=2.

\(^{227}\text{Th}\): Thorium-227 was injected as an ammonium acetate/N-morpholine ethane sulphonic acid solution.

Figure 1. Uptake of \(^{227}\text{Th}-\text{DTMP}, ^{227}\text{Th}-\text{DOTMP}\) and \(^{228}\text{Ac}-\text{DOTMP}\) in normal tissues from mice 4 h after injection, \(N=3\). The error bars represent the standard deviation.
An alternative approach to achieve bone-targeting with $^{225}\text{Ac}$ could be to use the bone-seeking $\beta$-emitter $^{225}\text{Ra}$ ($t_{1/2}=14.8$ d), which decays to $^{225}\text{Ac}$. Because of the significant half-life of $^{225}\text{Ra}$, the major transformation from $^{225}\text{Ra}$ to $^{225}\text{Ac}$ would take place after incorporation into the skeleton (and skeletal metastases) and elimination from soft tissues have occurred. The initial beta particle dose from $^{225}\text{Ra}$ would be low since only low levels of radioactivity are required to deliver therapeutically relevant alpha-particle doses.

An interesting mother nuclide/daughter nuclide situation exists also when a bone-targeting compound is based on $^{227}\text{Th}$ as this nuclide decays to $^{223}\text{Ra}$, another $\alpha$-particle emitter with high bone affinity. If the $^{227}\text{Th}$--labeled bone-seeker was free from $^{223}\text{Ra}$ at the time of administration, the total radioactivity in bone should increase as the decay of the parent proceeds. Furthermore, the results from the current study suggest that, with $^{227}\text{Th}$-DTMP/DOTMP, the maximum dose rate to bone occurs at a time when most of the $^{227}\text{Th}$ has cleared from blood and soft tissues, hence further increasing the bone to soft tissue dose ratios.

In a recently reported biodistribution study with $^{223}\text{Ra}$ in mice, it was shown that at early times after injection only a very low fraction of daughter nuclides from $^{223}\text{Ra}$ located in bone were redistributed (18). Also, the redistribution decreased with time from about 2% at 6 h to less than 1% at 3 days. Evaluation of the long-term retention of actinium and thorium radionuclides and their daughters was not made in this study. It is known from the literature that the skeletal retention half-times of $\text{Ac}^{3+}$ and $\text{Th}^{4+}$ are very long (20, 21) compared to the half-life of $^{225}\text{Ac}$ and $^{227}\text{Th}$. Based on the half-life similarities with $^{223}\text{Ra}$, it can be expected that $^{227}\text{Th}$- and $^{225}\text{Ac}$-labeled bone-seekers would have sufficient time for incorporation into the bone matrix for their daughters to be stably retained as well.

In conclusion, a high and selective uptake in bone of actinium-DOTMP, thorium-DOTMP and thorium-DTMP was demonstrated, indicating that polyphosphonate complexes of $^{225}\text{Ac}$ and $^{227}\text{Th}$ could have a relevant in vivo stability and be useful to deliver alpha-particle radiation to primary bone cancer and skeletal metastases from soft tissue cancers.

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References


Figure 2. The figure presents location index, i.e., the ratio of tissue uptake of thorium-DTMP vs. free thorium, thorium-DOTMP vs. free thorium and actinium-DOTMP vs. free actinium 4 h after injection. Data for the $^{227}\text{Th}$-DTMP, $^{228}\text{Ac}$-DOTMP and $^{227}\text{Th}$-DOTMP chelates and $^{227}\text{Th}$-acetate (dissolved) was obtained from this work. Data for actinium-acetate was taken from the work of Davis et al. (28) ($^{225}\text{Ac}$-acetate, female Balb-C mice).

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