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PHARMACOLOGICAL PROPERTIES OF ORALLY AVAILABLE, AMPHIPATHIC POLYAMINOCARBOXYLIC ACID CHELATORS FOR ACTINIDE DECORPORATION

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Abstract

Commonly used water-soluble polyaminocarboxylic acid (PACA) chelators, such as (EDTA) and DTPA, require intravenous or subcutaneous administration due to their poor bioavailability. The bioavailability of PACAs can be improved by the addition of differing lengths of alkyl side chains that alter amphipathic properties. Orally administered amphipathic triethylenetetramine pentaacetic acid (TT) compounds are efficacious for decorporation of Pu and Am. The synthesis, efficacy, binding affinities, and some initial pharmacokinetics properties of amphipathic TT chelators are reviewed. ¹⁴C-Labeled C₁₂TT and C₂₂TT chelators are reasonably well absorbed from the intestine and have a substantial biliary/fecal excretion pathway, unlike DTPA, which is mostly excreted in the urine. Whole body retention times are increased as a function of increasing lipophilicity. Neutron-induced autoradiography studies demonstrate that the oral administration of the chelators can substantially inhibit the redistribution of ²³⁹Pu in skeletal tissues. In summary, amphipathic TT-based chelators have favorable bioavailability, have a significant biliary excretion pathway, have demonstrated efficacy for americium and plutonium and are thus good candidates for further development. Furthermore, some of the pharmacological properties can be manipulated by changing the lengths of the alkyl side chains and this may have some advantage for decorporation of certain metals and radionuclides.

INTRODUCTION

Polyaminocarboxylic acid (PACA) chelators, such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA), are used for the removal of some metals and radionuclides that have been incorporated into the body. For example, EDTA is used for the chelation of lead (Mycyk et al. 2004), and Zn- and Ca-salts of DTPA have been used for the chelation of humans exposed to americium and plutonium (Wood et al. 2000; James et al. 2007; Durbin 2008). The Zn- and Ca-salts of DTPA were approved by the USFDA in 2004 for decorporation of plutonium, americium and curium (USFDA 2004). Approved routes of DTPA administration include intravenous and inhalation administration. There are reports of DTPA having some oral efficacy (Stradling et al. 1993; Taylor et al. 2007), but because the intestinal absorption is so low, larger quantities must be administered.

There is considerable interest in developing orally effective chelators for the actinides. We developed a series of amphipathic PACA chelators that have demonstrated efficacy for the removal of established deposits of plutonium and americium in the body (Bruenger et al.

1992; Miller et al. 1992; Miller et al. 1993; Miller et al. 2006). They also appear to have some efficacy for decorporation of iron (Liu et al. 2006; Liu et al. 2008). These chelators are based on triethylenetetraminehexaacetic acid (TTHA) and are rendered partially lipophilic by the addition of linear alkyl chains, denoted here as C_n TT, where “n” is the length of the linear alkyl chain.

In this report, we review some of the data on efficacy of these oral chelators on americium and plutonium decorporation, present some data on the prevention of plutonium redeposition in bone, describe several synthesis and purification methods, summarize the binding affinities, and present some initial pharmacokinetics results on the absorption and excretion of these chelators.

Synthesis and purification of amphipathic TT-based chelators

Two synthesis methods for the TTHA-based chelators with different lengths of alkyl chains (C_n TT) have been described. Bruenger et al. (1992) reported that C_n TT could be obtained by carboxymethylation of monoalkylated triethylenetetramine followed by hydrolysis of the ester (Fig. 1). Sonoda et al. (2001) have described an alternative one-pot strategy (Fig. 1). The Bruenger method produces higher yields while the Sonoda method is more rapid. However, considerable additional purification is required with the Sonoda method compared with the Bruenger method. The final structures of the alkyl chain substituted TTHA-based chelators are illustrated in Fig 1. The C_n TT chelators can be prepared as the free acids or as the zinc/sodium or calcium/sodium salts.

Binding affinities DTPA, TTHA and C_{12} TT

The relative binding affinities (stability constants) of DTPA, TTHA, and C_{12} TT for some alkaline earth metals, transition metals, rare earth metals, and actinides were obtained from literature sources (Sonoda et al. 2001; Anderegg et al. 2005) and are presented in Table 1. The stability constants for the calcium and zinc salts of C_{12} TT are similar to those reported for DTPA and TTHA. Interestingly, the stability constants reported for C_{12} TT for calcium and strontium are greater than those reported for DTPA and TTHA, but generally less than DTPA and TTHA for the lanthanides and actinides. However, there may be limitations to how stability constants for C_{12} TT are measured *in vitro* due to its lipophilic character and the *in vitro* studies may not reflect the whole interactions and binding of the metal/radionuclide deposits in the body.

DTPA and TTHA have strong binding affinities for Fe^{3+} (Anderegg et al. 2005) and while iron binding affinities for the amphipathic TT-based chelators have not been determined, their ability to chelate iron *in vivo* has been demonstrated (Liu et al. 2005). Because Pu^{4+} shares many properties with Fe^{3+} , including coordination chemistry, intracellular pathways, and deposition patterns in tissues (White et al. 1988; Durbin 2006), these common characteristics predict that DTPA and TTHA would have similarly strong binding affinities for plutonium.

Decorporation of americium and plutonium

The ability of the C_n TT chelators, given orally, to reduce body stores of entrenched deposits of ^{241}Am and ^{239}Pu was demonstrated in a series of manuscripts (Bruenger et al. 1992; Miller et al. 1992; Miller et al. 1993; Miller et al. 2006). The protocol that was employed was to administer the ^{241}Am and/or ^{239}Pu to the animal and then wait 7-14 days before chelation treatments. This permitted the radionuclides to become firmly deposited in tissue depots and may more closely replicate many exposure scenarios where treatments might not begin immediately after exposures. The chelators were added to the daily food ration.

In the initial studies, the more lipophilic C₂₂TT was found to be more effective than equivalent molar doses of the less lipophilic C₁₂TT for the removal of ²⁴¹Am (Bruenger et al. 1992). A later study demonstrated dose- and time-related efficacy of orally administered C₂₂TT for the decorporation of ²⁴¹Am and ²³⁹Pu (Miller et al. 1993). The efficacy of all doses of C₂₂TT for all tissues was most pronounced during the first month of treatment, with diminishing effects up to 3 months of treatment. The greatest reductions in ²⁴¹Am and ²³⁹Pu contents were observed in the liver, particularly when compared with the skeleton (Fig. 2).

A more recent study evaluated equal molar doses of the chelators with differing lengths of alkyl chains (C₈, C₁₂, C₁₆, and C₂₂) on decorporation efficiency (Miller et al. 2006). Efficiency generally increased with increasing chain length. This could have been due to either greater intestinal absorption and/or greater retention with increasing lipophilicity. All of the chelators (C₈TT, C₁₂TT, C₁₆TT, and C₂₂TT) were very effective in reducing liver stores of ²⁴¹Am and ²³⁹Pu, but were less able to remove these radionuclides from established bone deposits. C₂₂TT was slightly, but not significantly, more efficacious in removing these deposits from bone than was optimal injected doses of DTPA.

C₂₂TT slows the redistribution of ²³⁹Pu in bone

Americium and plutonium are largely deposited and retained in the skeleton and liver (Suslova et al. 2002), although lung retention may be significant following an inhalation exposure. In humans and primates, about 60% of the plutonium that initially enters the blood stream will deposit in the liver and about 30% in the skeleton (Durbin 2007). With time, there is a gradual movement of plutonium from the liver to the skeleton (Leggett et al. 2005) and this may be accelerated with liver disease (Suslova et al. 2006). In skeletal tissues, americium and plutonium are initially deposited on bone surfaces, but become buried into the bone volume as new bone is formed over these deposits (Polig et al. 2000). There is no evidence that these deposits buried in bone in the living animal can be effectively removed by chelation treatment, rather it appears that the nuclides must be captured as they are released from bone during bone remodeling. A measure of chelator efficacy is, therefore, the ability of the chelator to capture the released radionuclide and prevent its redeposition back onto bone surfaces (Miller et al. 1993; 2006). This was determined from images of bone prepared by neutron-induced autoradiography (NIAR).

The relative amounts of plutonium that had been reincorporated back into newly formed bone were quantified using neutron-induced autoradiography (NIAR) images of bones from rats that had been chelated with C₂₂TT. The images were prepared as previously described (Miller et al. 2006). The images were analyzed using an image analysis system (BioQuant) and the number of fission tracks were normalized to the new bone area. Daily oral treatment with C₂₂TT, added to the diet, was able to reduce the amount of plutonium that was redeposited in skeletal tissues by about 75%.

Preliminary studies on intestinal absorption of C₁₂TT and C₂₂TT

For the initial pharmacokinetic studies, ¹⁴C-labeled C₁₂TT and C₂₂TT were prepared according to the method described by Sonoda et al. (2001) and illustrated in Fig. 1. Bromo [1-¹⁴C] acetic acid (GE Health Care) was used followed by exhaustive carboxymethylation with large excess of regular bromoacetic acid (Sigma, St. Louis, Mo). The residue was purified with ion-exchange chromatography (Dowex 50 W-X8, H⁺ form). Appropriate fractions were combined and concentrated, and the radioactivity was determined by scintillation counting (Beckman Coulter, LS6500).

Male Fischer rats (Harlan, Madison, WI) at about 90 days of age were used. Three rats in each group were fasted overnight. They were then given by gastric gavage an aqueous

suspension of 50 μMol C_{12}TT or C_{22}TT /kg body weight containing 74 kBq of ^{14}C - C_{12}TT or ^{14}C - C_{22}TT . Blood samples obtained from the jugular vein were collected at 1, 2, 4, 6 and 8 hours later. The serum was solubilized and the radioactivity was measured by scintillation counting. This protocol was approved by the University of Utah Institutional Animal Care and Use Committee.

Peak blood levels were found to occur from 4-6 hours after gastric intubation and about 40% of the chelator appears to be absorbed (data not shown). However, ongoing studies suggest that bioavailability may be improved if the more lipophilic chelators are given with a food or with an oil-based carrier.

Preliminary studies on the excretion kinetics of C_{12}TT and C_{22}TT

Three rats in each group were given by intravenous injection 37 kBq of ^{14}C - C_{12}TT or ^{14}C - C_{22}TT . The rats were then placed in metabolism cages and urine and feces collected over 4 days. The excreta were solubilized and the radioactivity measured by scintillation counting.

The initial results indicate that both C_{12}TT and C_{22}TT compounds have a greater fecal excretion than urinary excretion (data not shown). This is unlike DPTA or other hydrophilic chelators. DTPA, for example, is reported to have greater than 90% excreted in the urine (Harmuth-Hoene et al. 1966).

Furthermore, the more lipophilic C_{22}TT compound has a greater than 50% longer retention time in the body than the less lipophilic C_{12}TT compound. For example, the peak urinary excretion of C_{12}TT occurs within the first 24 hours whereas the peak urinary excretion of C_{22}TT occurs from 24 to about 48 hours. Most of the fecal excretion of C_{12}TT occurs within the first 48-72 hours whereas this is from 96-144 hours for C_{22}TT .

Summary

Polyaminocarboxylic acid chelators were rendered orally available by the addition of a linear alkyl chain. This also changes some of their pharmacokinetic properties. These chelators have demonstrated efficacy, when given orally, for decorporation of americium and plutonium. Initial studies demonstrate reasonable oral bioavailability which may be increased with improved formulations. Initial studies also demonstrate a substantial biliary/fecal excretion route, unlike conventional hydrophilic chelators such as Zn- or Ca-DTPA. It might be possible to manipulate the contributions to the kidney/urinary and biliary/fecal excretion pathways by altering the lengths of the attached alkyl chains, thus changing the lipophilic and amphipathic character of the molecules. For example, because plutonium and americium are mainly sequestered in the liver, a biliary excretion pathway may help deplete liver stores of the radionuclide. Total retention time was also increased as a function of alkyl chain length and this may offer some therapeutic advantages. Overt signs of toxicity have not been observed, but more detailed toxicology studies will be required. These PACA-based, orally administered amphipathic chelators show considerable promise for the decorporation of americium, plutonium and perhaps other radionuclides and metals.

Acknowledgments

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Fig. 1.

The two synthesis methods for the C₁₂TT and C₂₂TT chelators as described by Bruenger et al. (1992) and Sonoda et al. (2001).

Fig. 2. The effects of different chain length C_nTT chelators on the removal of ²³⁹Pu from the lumbar vertebra and liver of rats. Chelation treatments were started at 14 days after exposure to plutonium and continued for 30 days. C_nTT compounds were added to the daily food ration and DTPA was given by subcutaneous injection. Data are redrawn from Miller et al. (2006).

Fig. 3. The relative number of ^{239}Pu fission tracks quantified in NIARs at sites where new bone had formed after the administration of plutonium, but during the period of C_{22}TT treatments. The C_{22}TT resulted in a marked reduction in the number of ^{239}Pu fission tracks normalized to bone area compared with controls.

Fig. 4. NIAR images illustrating the fission track density in trabecular bone that was formed after ^{239}Pu administration, thus the plutonium in the bone is from translocation or redeposition from other sites. A) Control. B) C₂₂TT treated. Final magnification = 200x.

Table 1Stability Constants of C₁₂TT, DTPA, TTHA-Metal Complexes (log).

	C ₁₂ TT ^a	DTPA ^b	TTHA ^b
<u>Alkali earth metals</u>			
Ca ²⁺	11.3	10.7	9.89
Sr ²⁺	12.4	9.7	9.26 ^d
<u>Transition metals</u>			
Cu ²⁺	16.9	21.5	20.5
Ni ²⁺	18.2	20.1	19.1
Zn ²⁺	19.0	18.6	18.1
<u>Rare earth metals (lanthanides)</u>			
La ³⁺	14.4	19.5	23.4
Sm ³⁺	17.5	22.3	23.7
Er ³⁺	22.1	22.7	23.2
<u>Actinides</u>			
Th ⁴⁺	24.5	28.8	32.0
Am ³⁺	about 20 ^c	23.0	27.6

^aSonoda et al., J. Coord. Chem. 54:153, 2001. Ionic strength (I) = 0.1, T = 25 °C.^bAnderegg et al., Pure Appl. Chem. 77:1445, 2005. I = 0.1, T = 20 or 25°C, except as noted.^cEstimated.^dT = 30°C.