$^{212}Pb/^{212}Bi$-EDTMP -Synthesis and biodistribution of a novel bone seeking alpha-emitting radiopharmaceutical

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Summary

At present, haematological toxicity is dose limiting in radionuclide therapy of bone metastases, and there is a need for radiopharmaceuticals with improved tumour/bone marrow dose ratios. Therefore, α-emitters e.g. ^{212}Bi may be more suitable than β-emitters, because of the short range and high LET values of α-particles. In this study, ^{212}Bi and its mother nuclide ^{212}Pb were produced in an isotope generator by collecting gaseous ^{220}Rn emanating from barium (^{228}Th) stearate. The carrier-free $^{212}Pb/^{212}Bi$ were bound to the chelating bone-seeking compound ethylene-diamine-tetra(methylene-phosphonic acid) (EDTMP) with 90% yield. The biodistribution in Balb/c mice was investigated by injecting 100 μl of a saline PBS buffer 0.020 M in EDTMP and 10 MBq/ml in $^{212}Pb/^{212}Bi$. Mice were killed in groups of three at 0.5, 2, 13 and 24 h post-injection times. Both ^{212}Pb-EDTMP and ^{212}Bi-EDTMP localised strongly in the skeleton, especially in the femur, at all time points measured, with the % of injected dose per gram (%ID/g) as high as 15 for ^{212}Pb and 13 for ^{212}Bi. All other organs investigated showed low uptake of both radionuclides,

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with the exception of the kidneys, for which a ratio femur/kidney of 1.5 for ^{212}Bi 2 h post-injection was observed. By comparison the ratio femur/blood was 20 for ^{212}Bi 2 h post-injection. The experiment indicates a potential for ^{212}Pb/^{212}Bi-EDTMP in targeted radiotherapy of osteoblastic bone lesions.

Key words: α-emitters, ^{212}Pb-EDTMP, ^{212}Bi-EDTMP, radiotherapy.

Introduction

For some years, compounds labelled with α-particle emitters have been investigated as potential tumour therapeutic agents (1,2). Heavy charged particles, such as α-particles, leave densely ionized tracks when traversing matter. This is expressed in their high LET value, which is typically of the order of 100 keV/μm. This value gives an optimal radiobiological effect (3). By comparison, β-particles are low LET radiation, with characteristic values around 1 keV/μm. There are several biological consequences of this difference in LET. Firstly, there is a much higher likelihood of cell inactivation with α-particle radiation, due to higher probability of irrepairable cell damage, expressed as a high RBE value. Another aspect is that the fraction of hypoxic cells in a tumour are more radiation resistant than oxygenated cells when low LET radiation is used for therapy. This difference in radiation resistance does not exist for high LET radiation (3). There is also a relatively high probability that a single α-particle traversal of a cell nucleus causes cell death (4,5).

An important characteristic is the short range of α-particles, about 50–100 μm in tissue. Thus, small clusters of tumour cells and single cells can be irradiated with little damage done on surrounding healthy tissue. This is particularly important when tumour cells are close to radiation sensitive cells, e.g. in tumour-cell infiltrated bone marrow.

There are a limited number of α-emitters suitable for radiotherapy (6). The most promising are ^{211}At and ^{212}Bi. ^{211}At has a physical half life of 7.22 h, but can only be

produced in an accelerator. ^{212}Bi, with a physical half life of 60.6 min, occurs in a natural disintegration series starting with ^{232}Th. Both of these nuclides decay to stable lead isotopes. In contrast to ^{211}At, ^{212}Bi can be produced at relatively low cost by different types of generators (7,8). Since 60.6 min half life may be too short for many radiotherapeutic applications, there is a possibility of creating an *in vivo* ^{212}Bi generator by alternatively using the mother radionuclide, ^{212}Pb. ^{212}Pb decays with a low energy β-particle to ^{212}Bi, with a physical half-life of 10.6 h. The decay chain from ^{212}Pb to stable ^{208}Pb is shown in Fig. 1.

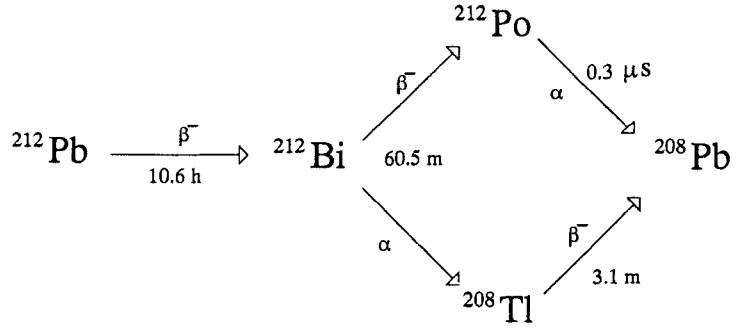

Figure 1. The figure shows the branched decay scheme from ^{212}Pb to stable ^{208}Pb.

Experience with tumour targeting vehicles labelled with ^{212}Pb and/or ^{212}Bi in biological systems is relatively sparce. Most experiments so far have dealt with the properties of different monoclonal antibodies labelled with ^{212}Bi by means of different chelating agents. Promising therapeutic effects have been obtained with intraperitoneal injection in a murine T-cell lymphoma model (9) and in a virus-induced murine leukemia model (1,10). The two latter experiments also showed that stable in vivo complexes with bismuth can be obtained with chelating agents.

In another study ^{212}Pb was co-precipitated with $Fe(OH)_2$ and administered into the peritoneal cavity in a murine ovarian cancer model. Ascites production was strongly reduced and survival prolonged (11). Thus, the results so far indicate that α-particles from

^{212}Bi may be useful in treatment of cancers which are restricted to the surface of a cavity, to the liquid filling it, or free-floating tumour cells in blood and bone marrow. Systemic delivery of ^{212}Bi-labelled MoAbs to solid cancers does not appear to be a realistic option. Even the 10.6 h half-life of ^{212}Pb seems too short to obtain sufficient uptake in solid tumours.

However, several compounds of low molecular weight concentrate in certain types of solid malignant tissue much more rapidly than MoAbs do. Therefore, a third strategy for the use of ^{212}Bi is to bind it or its predecessor ^{212}Pb to small chelating compounds possessing high tumour affinity themselves. The absence of published experiments following this strategy is astonishing.

A high percentage of patients with lung, breast and prostate cancer develop bone metastases (12). The morbidity in this group of patients is substantial. ^{32}P as orthophosphate and ^{89}Sr as $SrCl_2$ have been administered intravenously as palliative agents, but their application is restricted by bone marrow toxicity. For ^{89}Sr an absorbed dose ratio for metastases : red marrow of 10 is reported (13).

These high energy β-emitters are therefore replaced by medium energy β-emitters e.g. ^{153}Sm, in attempts to improve the tumor/bone marrow dose ratio (14). Due to the properties of α-particles, it seems likely that further improvements can be achieved with an α-emitter.

Carrier-free radioisotopes of Pb and Bi are not efficiently enriched in bone (15,16), and therefore need a bone seeking carrier vehicle. As carriers, multidentate aminophosphonate ligands have given promising results. The ^{153}Sm-aminophosphonate complexes have been shown to exhibit a high skeletal localisation, especially in regions with high bone turn-over and osteoblastic lesions (17).

Most promising so far has been ^{153}Sm-EDTMP, with a complex constant of logK=22.39 (18). EDTMP is a compound with high bone affinity and at the same time a strong complexing agent for numerous bi- or tri-valent cations (17,18). The ^{153}Sm-EDTMP complex is already in limited clinical use for palliative treatment of metastatic bone lesions. Phase I/II trials have been positive, but with escalating dose, bone marrow suppression was

limiting (14,19). This compound has also recently been used by one of the authors in the treatment of a patient with an advanced therapy-resistant osteosarcoma (20).

Hence, $^{212}Pb/^{212}Bi$-EDTMP was an obvious candidate for a new bone seeking α-emitting therapeutic agent. The aim of this study was to investigate its *in vivo* stability, skeletal localisation, and biodistribution.

Materials and methods

Production of ^{212}Pb

Carrier free ^{212}Pb with a half life of 10.6 hours was produced in an isotope generator as a daughter product of ^{228}Th ($t_{1/2}$= 1.9 y). The details of our generator will be published separately (8). The ^{228}Th source was purchased commercially as dry nitrate (Harwell, UK) and co-precipitated with barium stearate following the procedure of Hursh and Lovaas (21). Gaseous ^{220}Rn ($t_{1/2}$= 56 s) emanates rapidly from the barium stearate (22), and is collected in polypropylene bottles filled with air at atmospheric pressure. The decay product of ^{220}Rn, ^{212}Pb, deposits on the walls of the bottle, from where it can be easily extracted with more than 70 % yield with distilled water. If a complexing agent is present in the solution, more than 90 % can be extracted. The aquatic solution of ^{212}Pb and its daughter nuclide ^{212}Bi can then be used in the prodution of the $^{212}Pb/^{212}Bi$ EDTMP complex.

Preparation of EDTMP

EDTMP was synthesised according to a Mannich-type reaction as outlined by Moedritzer and Irani (23).

A concentrated HCl solution was carefully added to stoichiometric amounts of ethylenediamine and phosphorous acid and then heated to reflux temperature (aprox. 110-125 °C). Thereafter a 100 % excess of aqueous formaldehyde solution was added dropwise over the course of 1 hour. For optimum yield the reaction was carried out in aprox. 2 - 3 moles of hydrochloric acid per mole of ethylenediamine. After refluxing for an additional period of 1 - 2 hours, the resulting EDTMP was filtered from the solution and purified by three recrystallizations in distilled water.

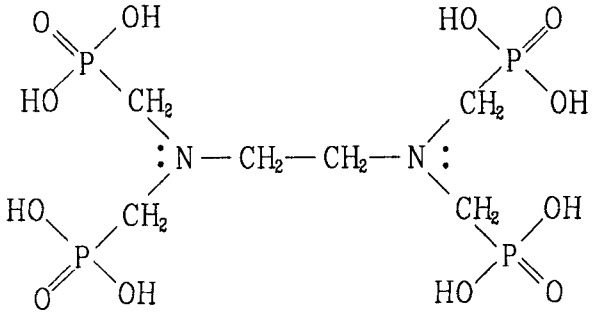

Figure 2. Ethylene-Diamine-Tetra(Methylene-Phosphonic acid) (EDTMP)

Preparation of $^{212}Pb/^{212}Bi$-EDTMP

To a solution of EDTMP with pH adjusted to 10 with NaOH was added the desired amount of $^{212}Pb/^{212}Bi$ activity, and the mixture heated at 60 °C for 5 min. The product was then purified from unbound activity by separating the complexed $^{212}Pb/^{212}Bi$ from uncomplexed $^{212}Pb/^{212}Bi$ on a 3 x 30 mm Chelex 100, 100-200 Mesh (Bio-Rad) chelating column. The unbound $^{212}Pb/^{212}Bi$ was then retained on the column, while the anionic complexes were eluted in void with distilled water. To the EDTMP with complexed $^{212}Pb/^{212}Bi$ was then added a physiological sodium phosphate saline buffer. The final solution was 0.020 M in EDTMP and 10 MBq/ml in $^{212}Pb/^{212}Bi$. At the time of injection, ^{212}Bi was in radioactive equilibrium with ^{212}Pb.

Biodistribution experiments

Unanesthetized Balb/c mice, weighing 20 g, were injected (into the tail vein) with 70-100 microliters of the $^{212}Pb/^{212}Bi$-EDTMP solution. Shortly after the injection tetany was observed in some of the animals, probably due to hypocalcemia caused by an overdose of EDTMP. All the affected animals recovered within a few minutes. Mice were killed in groups of three by cervical dislocation at 0.5 h, 2 h, 13 h and 24 h post-injection times. Blood samples were drawn from the heart and weighed immediately after killing. In some of the animals, urine was collected from the bladder. The animals were then dissected, the tissue samples were weighed and the radioactivity content measured.

Radioactivity measurement

Since a clear discrimination between ^{212}Pb and ^{212}Bi was essential, all radioactivity measurements were performed on a calibrated high purity 50 % Ge γ-ray detector (Canberra) coupled to a multichannel analyzer (EG & Ortec). The ^{212}Pb activity was determined by measurement of its 238.6 keV (43.6 %) γ-ray. To quantify the ^{212}Bi content, the intensity of the 583.1 keV (32.5 %) γ-ray of its ^{208}Tl daughter nuclide ($t_{1/2}$= 3.07 min) was measured at transient equilibrium with the parent. All radioactivity measurements of the samples were compared to diluted standards, and thereby automaticaly corrected for decay and differences in detector efficiency.

Results

Complexation yield

The yield of the carrier free $^{212}Pb/^{212}Bi$ EDTMP complex after purification was 90 % of the total activity in the original solution. No detectable complex dissociation was observed over a 24 h period at pH 7.4.

Biodistribution in mice

Tables 1 and 2 show the % of injected dose per gram of organ at 0.5 h, 2 h, 13 h and 24 h post-injection times for ^{212}Pb and ^{212}Bi respectively. The data are also presented in figs. 3-6. The ratios % ID/g cortical bone / % ID/g organs are given in Table 3 for ^{212}Pb and Table 4 for ^{212}Bi.

A considerable affinity for bone was observed for both radionuclides at all time points measured. Femur, skull and sternum were all investigated, and the highest values were found in the femur. All the other organs investigated showed low uptake of both ^{212}Pb and ^{212}Bi, with the exception of kidneys, which showed high uptake at every time point measured. The renal clearance of ^{212}Pb and ^{212}Bi was high 2 h after injection, but had decreased substantially 13 h after injection.

The highest uptake in femur was seen 2 h after injection. For ^{212}Pb it was 14.5 % of ID/g and for ^{212}Bi it was 12.5 % ID/g (Tables 1 and 2). The kidney uptake of ^{212}Bi and

^{212}Pb at this time point were 8.46 % ID/g and 2.40 % ID/g respectively. This gives a ratio femur/kidney of 1.52 for ^{212}Bi (Table 4). The ratios femur/blood and femur/liver for ^{212}Bi at this time point were 16.3 and 11.8 respectively (Table 4).

At the p=0.05 level there was no difference in % ID/g of ^{212}Bi in femur when all the measurement times were compared. Neither was there any significant difference in % ID/g of ^{212}Bi in the kidneys. There was however significantly more (p=0.02) ^{212}Pb 13 h post-injection than there was 0.5 h post-injection in femur, and significantly more ^{212}Pb in kidneys both 13 h (p=0.03) and 24 h (p=0.05) post-injection compared to 0.5 h post-injection.

When the activity levels of ^{212}Pb and ^{212}Bi were compared, it was found significantly more (p=0.04) ^{212}Pb than ^{212}Bi in femur 13 h postinjection, but this significant difference was not observed for the other time points measured. At 2 h and 24 h, no conclusion is possible due to higher uncertainties (p=0.45 and p=0.76, respectively). It was significantly less ^{212}Pb than ^{212}Bi in kidneys 0.5 h (p=0.002), 2 h (p=0.003) and 13 h (p=0.001) post-injection but not 24 h post-injection (p=0.08).

Discussion

As expected from biodistribution data of ^{153}Sm-EDTMP (14,17,24), ^{212}Pb/^{212}Bi-EDTMP showed high renal clearance during the first minutes after injection. The rapid and high uptake of ^{212}Pb/^{212}Bi-EDTMP in bone was also comparable to ^{153}Sm-EDTMP. The radioisotopes remained bound to the bone over a time-frame longer than two ^{212}Pb half-lives. In contrast to ^{153}Sm-EDTMP (17) the uptake of ^{212}Pb/^{212}Bi in the kidneys was high, and they were the only organs showing marked difference between ^{212}Pb and ^{212}Bi, with ^{212}Bi as the highest.

Several explanations for the high kidney uptake of ^{212}Pb and ^{212}Bi may be possible. The high values even after 13 h and 24 h showed that both radionuclides had a high affinity to this organ when injected as EDTMP-complexes.

Table 1.

Distribution of ^{212}Pb in Balb/c mice in terms of % ID/g after injection of 70-100 μl of a 0.020 M solution of EDTMP with carrier free $^{212}Pb/^{212}Bi$. Unless otherwise stated each time point is the mean of three animals. Calculated standard deviation is given in parenthesis after the mean value.

Organ	0.5 h	2.0 h	13 h	24 h
Blood	0.8(2)	0.8 **	0.6 (b)	0.53(13)
Spleen	0.39(14)	0.38(13)	0.28(4)	0.37(12)
Liver	0.7(2)	1.0(4)	0.71(10)	0.8(3)
Kidney	2.3(5)	2.4(9)	3.5(4)	3.6(7)
Heart	0.27 (b)	0.12(4)	0.08(3)	0.17 *
Lung	0.9(3)	0.5 (b)	0.35(8)	0.27(14)
Brain	0.19 *	n.s.	0.09 *	n.s.
Stomach	1.2 (b)	1.0(9)	0.29(15)	0.22 **
Muscle	0.23(8)	0.5(4)	0.3 (b)	0.2 *
Femur	10.0(8)	15(3)	13.0(1.2)	10(6)
Skull	3.7(3.9)	8(1)	9(4)	7(2)
Sternum	3.5(1.5)	3.0(3)	2.8(5)	2.8(6)
Bladder	--	5.0 (b)	n.s.	n.s.
Urine	--	$2 \cdot 10^2$ (b)	2.9 (a)	n.s.
Intestine	--	0.62 (a)	0.50 (a)	0.30 (b)
Faeces	--	19 (a)	1.5 (a)	--

* Only one measurement significantly different from 0.

** Only two measurements significantly different from 0.

n.s. None of the measurements were significantly different from 0.

(a) Only one tissue sample

(b) Only two tissue samples

Table 2.

Distribution of ^{212}Bi in Balb/c mice in terms of % ID/g after injection of 70-100 μl of a 0.020 M solution of EDTMP with carrier free $^{212}Pb/^{212}Bi$. Unless otherwise stated each time point is the mean of three animals. Calculated standard deviation is given in parenthesis after the mean value.

Organ	0.5 h	2.0 h	13 h	24 h
Blood	0.9(3)	0.7 **	n.s.	0.7(3)
Spleen	0.6(3)	0.4 **	0.28(8)	0.5(3)
Liver	1.0(3)	1.1(3)	0.64(10)	0.8(2)
Kidney	7.6(1.2)	8.5(1.3)	7.0(6)	7(2)
Heart	0.30 (b)	0.3 **	0.11(2)	n.s.
Lung	0.9(2)	0.76 (b)	0.39(10)	0.5 *
Brain	0.44 *	n.s.	n.s.	n.s.
Stomach	1.3 (b)	1.2(1.1)	0.28(14)	0.34 *
Muscle	0.38 **	0.49 (b)	0.3 (b)	0.1 *
Femur	10.0(4)	13(3)	10.3(1.0)	8(4)
Skull	4.1(4.3)	7.1(7)	8(5)	5.7(1.2)
Sternum	4(2)	2.7(4)	2.3(7)	2.5(4)
Bladder	--	4.7 (b)	n.s.	n.s.
Urine	--	$2 \cdot 10^2$ (b)	23 (a)	n.s.
Intestine	--	0.94 (a)	0.56 (a)	0.63 **
Faeces	--	23 (a)	6.4 (a)	--

* Only one measurement significantly different from 0.

** Only two measurements significantly different from 0.

n.s. None of the measurements were significantly different from 0.

(a) Only one tissue sample

(b) Only two tissue samples

Table 3.

Distribution of ^{212}Pb as the ratio of % ID/g for femur / % ID/g for organ. Unless otherwise stated each time point is the mean of three animals. Calculated standard deviation is given in parenthesis after the mean value.

Organ	0.5 h	2.0 h	13 h	24 h
Blood	13(4)	$2{\cdot}10^1$ (b)	$2{\cdot}10^1$ (b)	18(6)
Spleen	28(7)	$4{\cdot}10^1$ ($3{\cdot}10^1$)	47(9)	25(7)
Liver	16(4)	17(8)	19(3)	12(3)
Kidneys	4.5(7)	7(3)	3.7(6)	2.6(1.0)
Heart	36 (b)	$1.4{\cdot}10^2$ ($8{\cdot}10^1$)	$1.7{\cdot}10^2$($7{\cdot}10^1$)	49 *
Lung	13(5)	$3{\cdot}10^1$ (b)	38(7)	36(6)
Brain	51 *	n.s.	$1.4{\cdot}10^2$ *	n.s.
Stomach	11 (b)	$3{\cdot}10^1$ ($2{\cdot}10^1$)	$6{\cdot}10^1$ ($4{\cdot}10^1$)	49 **
Muscle	46(11)	$5{\cdot}10^1$ ($5{\cdot}10^1$)	$8{\cdot}10^1$ (b)	$6{\cdot}10^1$ *
Femur	1	1	1	1
Skull	5(3)	1.8(5)	1.6(8)	1.4(5)
Sternum	3(2)	4.94(14)	4.7(6)	3.4(1.2)
Bladder	--	5.6 (b)	n.s.	n.s.
Urine	--	0.3 (b)	4.1 (a)	n.s.
Intestine	--	29 (a)	29 (a)	45 (b)
Faeces	--	0.94 (a)	7.9 (a)	--

* Only one significant measurement.

** Only two significant measurements.

n.s. No significant measurement.

(a) Only one tissue sample

(b) Only two tissue samples

Table 4.

Distribution of ^{212}Bi as the ratio of % ID/g for femur / % ID/g for organ. Unless otherwise stated each time point is the mean of three animals. Calculated standard deviation is given in parenthesis after the mean value.

Organ	0.5 h	2.0 h	13 h	24 h
Blood	12(5)	$2 \cdot 10^1$ **	n.s.	13(4)
Spleen	20(8)	$8 \cdot 10^1$ **	40(14)	18(5)
Liver	10(3)	12(4)	16(3)	11(2)
Kidney	1.3(2)	1.5(6)	1.5(3)	1.2(2)
Heart	34 (b)	$1 \cdot 10^2$ **	$9 \cdot 10^1(2 \cdot 10^1)$	n.s.
Lung	10(2)	24 (b)	27(5)	$3 \cdot 10^1$ *
Brain	23 *	n.s.	n.s.	n.s.
Stomach	10 (b)	19(15)	$4 \cdot 10^1(3 \cdot 10^1)$	58 *
Muscle	28 **	33 (b)	$7 \cdot 10^1$ (b)	$1 \cdot 10^2$ *
Femur	1	1	1	1
Skull	4(3)	1.8(3)	1.5(8)	1.4(4)
Sternum	3(2)	4.7(1.4)	4.6(1.1)	3.3(1.0)
Bladder	--	3.7 (b)	n.s.	n.s.
Urine	--	0.2 (b)	0.40 (a)	n.s.
Intestine	--	17 (a)	20 (a)	16 **
Faeces	--	0.69 (a)	1.4 (a)	--

* Only one significant measurement.

** Only two significant measurements.

n.s. No significant measurement.

(a) Only one tissue sample

(b) Only two tissue samples

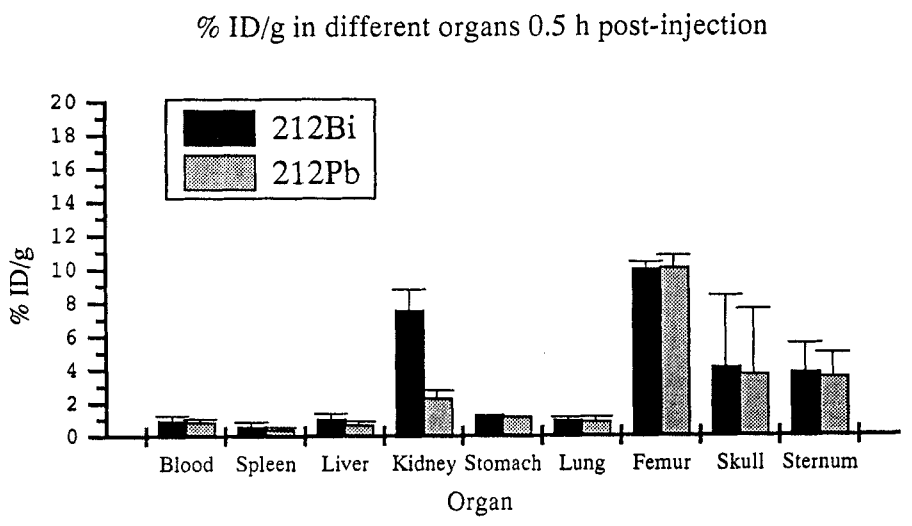

Figure 3. The % ID/g of ^{212}Bi-EDTMP and ^{212}Pb-EDTMP in different organs at 0.5 h post-injection.

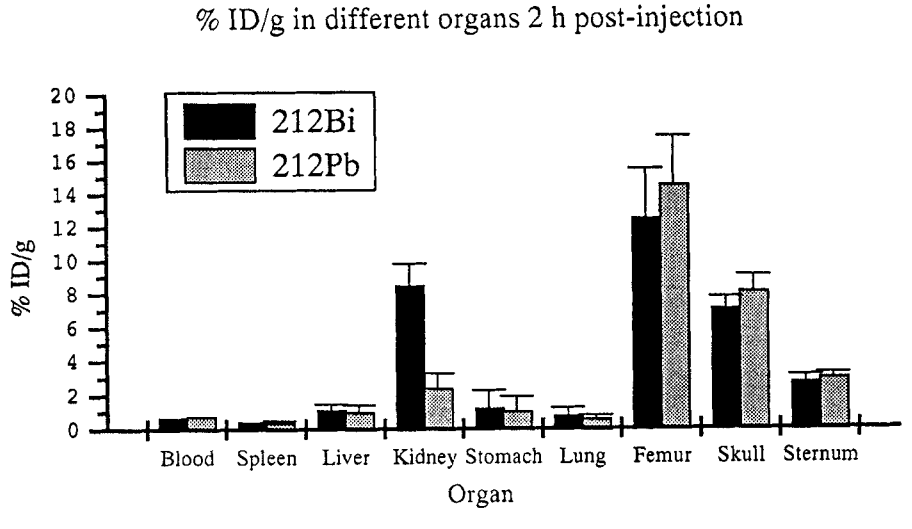

Figure 4. The % ID/g of ^{212}Bi-EDTMP and ^{212}Pb-EDTMP in different organs 2 h post-injection.

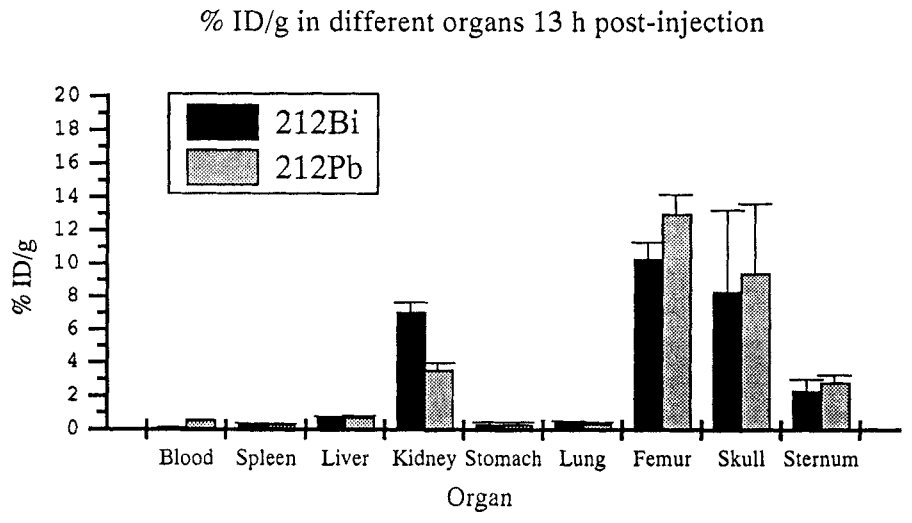

Figure 5. The % ID/g of ^{212}Bi-EDTMP and ^{212}Pb-EDTMP in different organs 13 h post-injection.

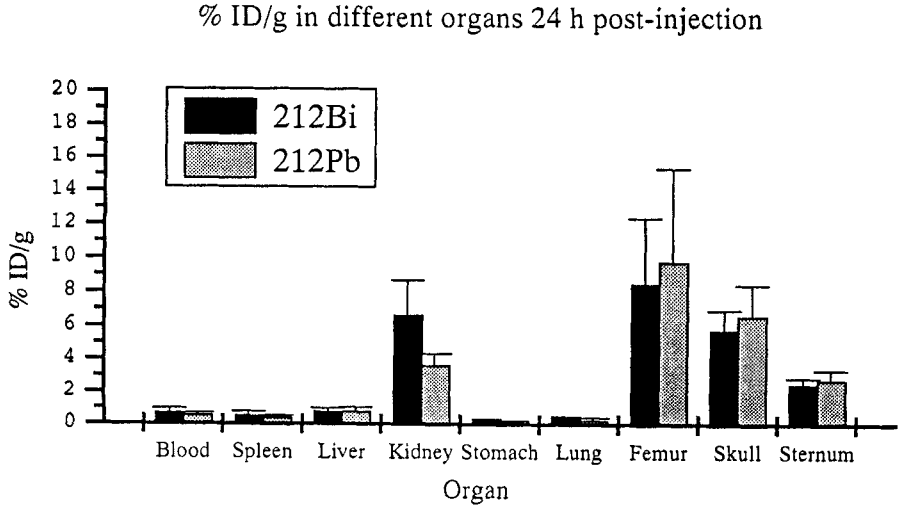

Figure 6. The % ID/g of ^{212}Bi-EDTMP and ^{212}Pb-EDTMP in different organs 24 h post-injection.

The Bi/Pb ratio showed that some of the ^{212}Bi in the kidney must have another origin other than the decay of ^{212}Pb already localised in this organ. This extra ^{212}Bi contribution was probably due to rupture of the complex in the β-decay of ^{212}Pb to ^{212}Bi. This is consistent with observations of Mirzadeh et al. (25), who reported that for ^{212}Pb complexed to DOTA, 36 % of the nuclear transitions from ^{212}Pb to ^{212}Bi induced rupture of the ^{212}Bi-DOTA complex, due to electronic excitations after conversion electron emission. This interpretation was supported by the fact that at 0.5 h, there were almost equal amounts of ^{212}Pb and ^{212}Bi in femur, but at 13 h post-injection there was significantly less ^{212}Bi compared to ^{212}Pb. At 13 h post-injection all the ^{212}Bi-EDTMP initially injected had decayed. This implies that all ^{212}Bi activity must have been produced *in vivo* from the ^{212}Pb mother nuclide, while at 0.5 h post-injection the major contribution was from the ^{212}Bi-EDTMP injected.

The high uptake of ^{212}Pb and of ^{212}Bi at 0.5 h can not be explained that way, but must be due to the chemical *in vivo* behavior of the EDTMP complexes. There were no indications for a lower *in vivo* chemical stability of ^{212}Pb-EDTMP compared to ^{212}Bi-EDTMP, although this could have been expected because of the different valence of Pb(II) and Bi(III).

A biodistribution study in rats of carrier free ^{205}Bi showed that bismuth mainly located in the kidneys (15). The reported ratio kidney/bone was of the order of 100, 2 h post-injection, in contrast to the kidney/femur ratio of only 0.7 found in the present study 2 h post-injection. Thus, it was clear that most of the ^{212}Bi produced *in vivo* from ^{212}Pb-EDTMP remained bound to the bone. The somewhat higher levels of $^{212}Bi/^{212}Pb$ in blood and other organs compared to ^{153}Sm-EDTMP could be due to the fact that $^{212}Bi/^{212}Pb$ were carrier free.

A study with intravenously injected carrier added ^{203}Pb as $PbCl_2$ (16) showed a distribution pattern in soft tissue similar to this study, but with only 1-2 % of injected dose per gram observed in different bone structures. This is also clearly different from the bone localisation observed by us.

Distribution experiments in mice and rats with ^{14}C labelled 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (APD) showed that the ratios bone/liver and bone/kidney decreased as the injected amount of APD increased (26,27). Since the injected amount of EDTMP in this study was approx. 40 mg/kg, there is a possibility that more favourable ratios can be obtained with lower EDTMP concentrations. This will most likely prevent the tetany observed in some of the animals.

Even if the ratios bone/organ were slightly inferior to those observed for ^{153}Sm-EDTMP, they exceeded 10 for all the investigated organs except for kidneys. The bone localisation of $^{212}Bi/^{212}Pb$-EDTMP was fast compared to the physical half-life of the radionuclides. Importantly these ratios will be much more favourable for metastases inducing pathologically enhanced bone turn-over. Ratios tumour bone/normal bone for organic phosphonates are reported as high as 10-20, in bone lesions which are due to metastatic deposits in the skeleton (13) as well as in the "malignant bone" synthesised in osteoblastic osteosarcomas (20). For the latter, the pathological bone formation is a characteristic feature of the tumour cells themselves. Hence, it is possible that an α-emitting bone seeking radiopharmaceutical could be an important achievement in the treatment of this disease.

A decrease of the high initial dose to the renal clearance system may probably be achieved by injecting purified ^{212}Pb-EDTMP. ^{212}Pb will produce the α-emitter ^{212}Bi to 50 % saturation at 1 h, and since the important contribution to the dose is from ^{212}Bi, there will be a dose build-up during the first few hours. Distribution data of ^{153}Sm-EDTMP in rats have shown that 80 % of the total excretion is done in the first 30 min (17), during which time the major part of the excreted EDTMP complexes would be very mildly radiotoxic ^{212}Pb-EDTMP, with the clear consequence that the dose to the renal clearance system is lowered. Since the kidneys seems to be the only critical organ, ways of reducing the kidney uptake should be exploited. Other multidentate aminophosphonate ligands may show more suitable properties than EDTMP as bone seeking agents for $^{212}Pb/^{212}Bi$.

In conclusion, the present study has shown that the bone seeking properties of $^{212}Pb/^{212}Bi$-EDTMP are good, with the kidney as the apparently sole problematic organ. The biodistribution of the compound is substantially different from free Pb and Bi. Further experiments will be conducted to clarify the potential for therapy of bone lesions or osteosarcomas with $^{212}Pb/^{212}Bi$ bound to EDTMP or similar compounds.

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