

^{211}At - and ^{131}I -Labeled Bisphosphonates with High In Vivo Stability and Bone Accumulation

Roy H. Larsen, Kristin M. Murud, Gamal Akabani, Per Hoff, Øyvind S. Bruland and Michael R. Zalutsky

Department of Chemistry, University of Oslo, Oslo; Department of Oncology, The Norwegian Radium Hospital, Oslo, Norway; and Department of Radiology, Duke University Medical Center, Durham, North Carolina

Bisphosphonates were synthesized for use as carriers for astatine and iodine radioisotopes to target bone neoplasms. **Methods:** Radiohalogenated activated esters were coupled to the amino group in the side chain of the bisphosphonate. The bisphosphonate 3-amino-1-hydroxypropylidene bisphosphonate was combined with four different acylation agents: N-succinimidyl 3- ^{211}At astatobenzoate, N-succinimidyl 3- ^{131}I iodobenzoate, N-succinimidyl-5- ^{211}At astato-3-pyridinecarboxylate and N-succinimidyl-5- ^{131}I iodo-5-pyridinecarboxylate. The products, 3- ^{131}I iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate (IBPB), 3- ^{211}At astato-benzamide-N-3-hydroxypropylidene-3,3-bisphosphonate (ABPB), 5- ^{131}I iodopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate (IPPB) and 5- ^{211}At astatopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate (APPB), were injected intravenously into Balb/c mice. MIRD and Monte Carlo methods were used on the basis of cumulated activity calculated from biodistribution data to estimate dose to organs and bone segments. **Results:** All ^{131}I - and ^{211}At -labeled analogs were strongly incorporated into osseous tissue and retained there at stable levels, while a rapid clearance from blood was observed. The bone uptake was found to be similar for ^{211}At - and ^{131}I -labeled bisphosphonate when compared in paired label experiments. Bone uptake and bone-to-tissue ratios were better for IBPB compared with IPPB, and ABPB compared with APPB. All four compounds appeared to be highly resistant to in vivo dehalogenation as indicated by low uptake of $^{131}\text{I}/^{211}\text{At}$ in the thyroid gland and stomach. According to dosimetric estimates, the bone surface-to-bone marrow ratio was three times higher with ^{211}At than with ^{131}I . **Conclusion:** Both the β -particle- and α -particle-emitting compounds showed high in vivo stability and excellent affinity for osseous tissue. Further preclinical evaluation is therefore warranted.

Key Words: radiohalogenated bisphosphonates; bone therapy; bone pain

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Many cancer patients are affected by pain from skeletal metastases. Bony metastases develop in as many as 85% of patients with advanced lung, prostate and breast carcinoma (1,2). Established treatments for such patients include hormone therapy, chemotherapy and external radiotherapy, but, despite temporary responses, nearly all patients relapse (3).

There is, therefore, a strong demand for additional therapies to relieve pain and slow tumor progression. The treatment of metastases either with multifractional field external beam radiotherapy or β -emitting radionuclides is limited by myelotoxicity (4). Although these treatments frequently result in pain relief, they usually fail to make any substantial impact on the progression of the disease (5).

Bisphosphonates are biologically active molecules used to treat different clinical conditions, e.g., as inhibitors of osteoporosis (6) and as protectants against skeletal complications in cancer (7). They are distinguished by a nonhydrolyzable P-C-P bond and two side chains: one influencing the binding to bone, the other regulating the compounds' pharmacological properties. Radiolabeled derivatives have been investigated clinically for targeting radionuclides to bone (8,9). A first-generation ^{131}I -labeled bisphosphonate was reported to show elevated uptake in bone metastases with metastases-to-normal bone ranging from 2.5 to 7.4 and also to cause complete pain relief in 44% of patients with painful osseous sites (9). Since these studies were performed, a better understanding of the implications of labeling position in aromatic compounds has been presented (10). These studies have made it plausible to prepare bisphosphonates less susceptible to dehalogenation. β -irradiation from ^{131}I has shown therapeutic efficacy in treating thyroid carcinoma and non-Hodgkin's lymphoma (11,12). It may be interesting to compare more stable ^{131}I -labeled bisphosphonates with other β -emitters in the palliation of pain from osseous metastases. ^{131}I is well established in nuclear medicine; it is widely available and, compared with other β -emitters, is relatively inexpensive. Furthermore, its half-life ($t_{1/2}$) of 8.02 d compares favorably with the most widely used nuclide for bone pain palliation, ^{89}Sr ($t_{1/2}$ of 50 d).

Because of a high radiobiologic effectiveness and a range in tissue limited to approximately 2-5 cell diameters, α -particle emitters may be useful for delivering concentrated radiation doses to osseous metastases and reducing bone marrow toxicity (13). Because of its 7.2-h half-life, 100% α -emission and chemical resemblance to iodine, ^{211}At could be a valuable radionuclide for this application.

In this study, we describe four bisphosphonates synthesized by coupling radiolabeled acylating agents onto the

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For correspondence or reprints contact: Roy H. Larsen, PhD, Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo, Norway.

amino group of a commercially available and clinically well-known bisphosphonate, 3-amino-1-hydroxypropylidene bisphosphonate (APB). This bisphosphonate was selected as the basis for labeling with either ^{131}I or ^{211}At . We studied two labeling precursors for radiohalogenation, N-succinimidyl-3-(trimethylstannyl)benzoate (STMB) and the N-succinimidyl-5-(trimethylstannyl)-3-pyridinecarboxylate (STPC), to compare compounds of different lipophilicity.

MATERIALS AND METHODS

Radionuclides

^{211}At was produced by the $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$ reaction, using the internal target system at the Duke University Medical Center Cyclotron (Durham, NC), as previously described in detail (14). ^{131}I in the form of sodium [^{131}I]iodide in NaOH (pH 7–11) was obtained from DuPont-New England Nuclear (Billerica, MA).

Radiolabeling

The labeling precursors, STMB and STPC, were synthesized and purified as described (15,16) and labeled according to published procedures (16,17). The radiolabeled intermediates, N-succinimidyl 3- [^{131}I]iodobenzoate (SIB), N-succinimidyl 3- [^{211}At]astatobenzoate (SAB), N-succinimidyl 5- [^{131}I]iodo-3-pyridinecarboxylate (SIPC) and N-succinimidyl 5- [^{211}At]astato-3-pyridinecarboxylate (SAPC), were purified from these tin precursors using a silica gel Sep Pak (Waters, Milford, MA) cartridge (SIB, SAB) (17) or high-performance liquid chromatography (HPLC) (SAPC, SIPC) (16). Fractions containing the purified compounds were evaporated to dryness with argon, added to 100 μL of a solution of APB (30 mg/mL) in 0.1 mol/L borate (pH ~9) and agitated for 15 min. The final products (Fig. 1), 3- [^{131}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate (IBPB), 3- [^{211}At]astatobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate (ABPB), 5- [^{131}I]iodopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate (IPPB) and 5- [^{211}At]astatopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate (APPB), were purified by HPLC. The HPLC system was a C-18 Bondapak column (10- μm particles; Waters, MA) eluted with a mixture of 25 mmol/L tetrabutyl-ammoniumhydroxide in 0.1 mol/L phosphate-buffered saline (PBS) (60%) and ethanol (40%).

The following HPLC retentions for the various products were observed: IPPB, 7.4 min; APPB, 8.1 min; IBPB, 9.2 min; ABPB, 10.0 min. After HPLC purification, the ethanol was evaporated with a stream of argon. Before injection into animals, a final buffer exchange was performed using a Sephadex (Pharmacia, Sweden) G-25 PD10 column eluted with 0.1 mol/L PBS (pH 7.4). Details of this chemistry, including nuclear magnetic resonance data for the iodinated products, have been presented (18).

Radiation Precautions

The chemistry procedures should be performed in a charcoal-filtered hood. An advantage of ^{211}At is that, because of low energy

or low levels of x-ray and gamma emissions, only a small amount of lead shielding is needed. Although not mandatory, an air mask was used as an extra safety precaution to protect against any radioisotope released into the air. Also, a detector sensitive to α and β radiation was used to survey the air inside and outside the hood. A disposable body suit and three layers of gloves were used to protect from contamination. Conventional finger and body dosimeters were used to monitor penetrating radiation components. The outer layer of gloves was changed frequently during the procedure. After completing the labeling procedure, personnel conducted a body scan with an α - and β -sensitive probe. In case of accidental body contamination, a safety procedure including body decontamination and protective thyroid gland blocking with supersaturated potassium chloride can be initiated. Most of these procedures and precautions are similar to those used when working with therapeutic levels of ^{131}I .

Biodistribution

White male Balb/C mice with a body weight in the range of 19–22 g were used in the biodistribution experiments. The four compounds, IBPB, ABPB, IPPB and APPB, were administered by tail vein injections of 100 μL for each animal. The compounds were administered in paired-label arrangement (IBPB versus ABPB and IPPB versus APPB), using approximately 125 kBq ^{211}At and 50 kBq ^{131}I for each animal. Animals were killed, and tissue distributions were determined at 0.5, 2, 6 and 24 h after injection, using groups of 5 mice at each time point. After each tissue sample was weighed, the ^{211}At and ^{131}I activity levels were measured using an automated gamma counter (LKB Wallac 1282; Wallac, Turku, Finland) with a dual-channel setting, with windows circumscribing the 77- to 92-keV Po x-rays from ^{211}At and the 364-keV gamma ray of ^{131}I . Data were automatically corrected for the 11% crossover of ^{131}I in the ^{211}At channel, and physical decay of radionuclides was accounted for by normalizing to standard samples of radionuclides. Samples with a single nuclide and mixtures of the nuclides were used as references.

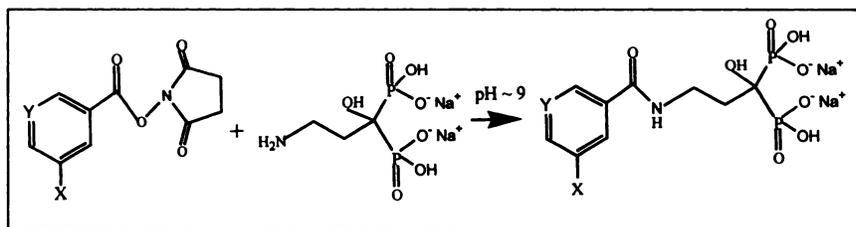
Dose Estimates

Absorbed dose estimates were performed using standard MIRD methodology applied to the mouse model according to Hui et al. (19). However, Monte Carlo techniques were used to calculate the bone-surface dose for both ^{131}I and ^{211}At over a 10- μm thickness according to Nelson et al. (20). Because of the anticipated uptake on the bisphosphonate compounds on the bone surfaces, bone dosimetry calculations were performed assuming uptake and retention on bone surfaces.

Statistical Evaluation

Biodistribution data were compared using the Student *t* test; $P = 0.05$ was defined as the significance limit.

FIGURE 1. Schematic presentation of preparation sequence of radiohalogen-labeled bisphosphonates. X = ^{211}At or ^{131}I ; Y = C or N. ABPB (X = ^{211}At , Y = C); IBPB (X = ^{131}I , Y = C); APPB (X = ^{211}At , Y = N); IPPB (X = ^{131}I , Y = N).



RESULTS

A schematic presentation of the method of synthesis for the bisphosphonate conjugates is shown in Figure 1. Yields for the labeling of ABPB, IBPB, APPB and IPPB from their corresponding labeled N-succinimidyl esters, determined by HPLC, were greater than 80%.

The distribution of ^{211}At and ^{131}I activity, expressed as percentage injected dose per gram tissue (%ID/g), for ABPB and IBPB are presented in Table 1; Table 2 shows the distribution values for IPPB and APPB. In both experiments, no significant difference in uptake for the two radionuclides was observed in bone. On the other hand, when results for the same radionuclide were compared, differences in the distribution of the benzoyl and pyridyl conjugates were observed. At each time point and with both labels, significantly higher bone uptake was seen with the benzoyl compounds. Peak uptakes in femur of 34.2 ± 7.4 %ID/g and 34.9 ± 8.2 %ID/g were observed with IBPB and ABPB, respectively, whereas IPPB and APPB had peak uptakes of 15.7 ± 3.3 %ID/g and 16.6 ± 4.2 %ID/g, respectively. The average radioactivity values decreased slightly between 2 and 6 h, but because of relatively large SDs, this decrease was not statistically significant ($P > 0.05$). No significant difference in %ID/g bone values was observed between 6 and 24 h after injection, suggesting that once incorporated into bone, the tracers were retained in stable fashion. Similar degrees of tracer accumulation were observed in the skull and in rib samples (data not presented), suggesting a general accumulation in osseous tissue.

All compounds cleared rapidly from the blood pool; less than 0.4 %ID/g was found by 2 h after injection. The iodine analogs cleared from blood significantly faster than the

corresponding astatine analogs, and the pyridine analogs cleared more rapidly than benzoate compounds labeled with the same radionuclide. Although metabolic cages were not used, urine samples collected from some of the animals confirmed that the four compounds were rapidly eliminated. Among nontarget tissues, the spleen and liver had the highest retention of radiolabeled bisphosphonates at later time points. At 0.5 and 2.0 h, IBPB and ABPB had significantly lower uptake ($P < 0.05$) in spleen than IPPB and APPB; however, no significant differences among the four compounds were observed at 24 h.

The effect of exchanging astatine for iodine was investigated through paired-label administration of astatate- and iodo-bisphosphonate conjugates in the same groups of animals. The tissue distribution of the two radionuclides was generally similar; however, significant differences were observed by paired *t* test in some tissues. When ABPB and IBPB were compared, ^{211}At levels were significantly higher at some time points in lungs, stomach, intestines and blood and were significantly lower in liver and kidneys. In the APPB and IPPB study, ^{211}At levels were significantly higher at some time points in lungs, stomach, intestines, blood, brain and muscle and were significantly lower in liver. In general, differences between radionuclides were small except at 24 h in stomach, where ^{211}At levels were more than 10 times higher than ^{131}I .

Dehalogenation of radioiodinated and radioastatinated compounds in vivo was monitored by measuring accumulation of radioactivity in the thyroid gland and stomach. Although animals were not given blocking agents, the thyroid and stomach levels of ^{211}At and ^{131}I were low for both the benzoyl and pyridyl conjugates. The thyroid itself was not

TABLE 1
Tissue Distribution of IBPB and ABPB in Balb/c Mice

Tissue	30 min		2 h		6 h		24 h	
	IBPB	ABPB	IBPB	ABPB	IBPB	ABPB	IBPB	ABPB
Liver	3.24 ± 1.11	2.48 ± 0.83	3.06 ± 0.35	$1.12 \pm 0.24^*$	2.37 ± 0.30	$0.78 \pm 0.24^*$	2.77 ± 0.91	1.55 ± 0.64
Spleen	2.74 ± 1.72	3.16 ± 1.48	3.13 ± 0.53	3.41 ± 0.53	4.14 ± 1.06	4.35 ± 1.17	6.42 ± 3.47	6.52 ± 3.35
Lungs	2.51 ± 0.83	4.31 ± 1.33	0.82 ± 0.16	$1.49 \pm 0.33^*$	0.52 ± 0.09	$0.94 \pm 0.18^*$	0.61 ± 0.08	$1.30 \pm 0.20^*$
Heart	2.11 ± 1.13	3.02 ± 1.17	0.98 ± 1.12	1.23 ± 1.12	0.24 ± 0.20	0.44 ± 0.19	0.61 ± 0.08	1.24 ± 1.43
Kidneys	12.05 ± 3.32	8.55 ± 2.51	6.39 ± 0.98	$2.89 \pm 0.81^*$	2.35 ± 0.25	$1.46 \pm 0.24^*$	1.00 ± 1.38	0.85 ± 0.34
Bladder	3.52 ± 3.80	3.94 ± 3.45	8.93 ± 12.44	9.05 ± 11.69	1.77 ± 1.97	2.22 ± 1.88	0.68 ± 0.13	4.28 ± 5.74
Stomach	0.85 ± 0.49	1.48 ± 0.64	0.42 ± 0.17	$2.65 \pm 0.38^*$	0.17 ± 0.08	$1.88 \pm 0.92^*$	0.93 ± 0.95	$2.54 \pm 0.68^*$
Small intestine	0.63 ± 0.18	1.09 ± 0.32	0.33 ± 0.046	$0.50 \pm 0.07^*$	0.16 ± 0.04	0.31 ± 0.10	0.22 ± 0.04	0.34 ± 0.10
Large intestine	0.80 ± 0.62	1.52 ± 1.23	0.23 ± 0.14	0.35 ± 0.14	0.26 ± 0.17	0.38 ± 0.22	0.14 ± 0.03	$0.53 \pm 0.19^*$
Muscle	0.59 ± 0.28	0.90 ± 0.37	0.42 ± 0.15	0.52 ± 0.13	0.16 ± 0.04	0.23 ± 0.13	0.37 ± 0.15	0.20 ± 0.18
Blood	2.28 ± 0.64	4.32 ± 1.18	0.11 ± 0.03	$0.37 \pm 0.08^*$	0.06 ± 0.01	$0.18 \pm 0.04^*$	0.03 ± 0.02	$0.13 \pm 0.03^*$
Brain	0.18 ± 0.15	0.25 ± 0.14	0.14 ± 0.15	0.16 ± 0.16	0.24 ± 0.40	0.17 ± 0.23	0.03 ± 0.01	0.12 ± 0.08
Skull	18.10 ± 2.76	16.65 ± 2.69	24.28 ± 4.16	24.25 ± 3.92	19.2 ± 1.77	21.07 ± 1.71	19.55 ± 3.70	20.96 ± 3.33
Femur	18.22 ± 3.75	16.63 ± 3.56	34.23 ± 7.35	34.94 ± 8.21	22.59 ± 7.73	25.33 ± 9.07	20.30 ± 4.97	23.88 ± 6.14

*Significant difference.

IBPB = 3-[^{131}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate; ABPB = 3-[^{211}At]astato-benzamide-N-3-hydroxypropylidene-3,3-bisphosphonate.

Values expressed as % injected dose per gram tissue and mean \pm SD of five samples.

TABLE 2
Tissue Distribution of IPPB and APPB in Balb/c Mice

Tissue	30 min		2 h		6 h		24 h	
	IPPB	APPB	IPPB	APPB	IPPB	APPB	IPPB	APPB
Liver	3.19 ± 1.15	2.60 ± 1.09	2.85 ± 0.73	2.13 ± 0.70	3.18 ± 1.65	2.63 ± 1.81	1.91 ± 0.16	1.47 ± 0.16
Spleen	7.83 ± 3.10	9.39 ± 3.25	6.61 ± 1.63	7.47 ± 1.65	5.11 ± 1.31	5.74 ± 1.17	5.67 ± 3.41	6.83 ± 4.53
Lungs	1.46 ± 0.38	2.51 ± 0.60*	0.79 ± 0.64	1.25 ± 0.82	0.58 ± 0.23	0.90 ± 0.26	0.52 ± 0.23	0.78 ± 0.22
Heart	1.23 ± 0.44	1.51 ± 0.45	0.84 ± 0.59	1.04 ± 0.61	0.32 ± 0.46	0.43 ± 0.47	0.21 ± 0.12	0.30 ± 0.16
Kidneys	5.43 ± 0.94	4.23 ± 0.78	3.43 ± 0.52	2.23 ± 0.48	2.59 ± 0.23	1.63 ± 0.29*	0.58 ± 0.09	0.34 ± 0.12*
Bladder	2.03 ± 1.77	2.24 ± 1.65	5.63 ± 10.46	5.81 ± 10.20	0.67 ± 0.79	0.86 ± 0.76	0.20 ± 0.16	0.61 ± 0.38
Stomach	3.29 ± 0.703	2.12 ± 0.77	2.38 ± 1.09	3.50 ± 1.43	1.61 ± 1.40	2.80 ± 1.90	0.08 ± 0.05	1.09 ± 0.30*
Small intestine	0.76 ± 0.57	0.87 ± 0.52	1.63 ± 3.13	1.95 ± 3.58	0.10 ± 0.03	0.17 ± 0.05	0.06 ± 0.02	0.14 ± 0.04*
Large intestine	0.26 ± 0.06	0.36 ± 0.08	0.45 ± 0.59	0.51 ± 0.62	0.22 ± 0.06	0.25 ± 0.04	0.08 ± 0.04	0.13 ± 0.05
Muscle	0.37 ± 0.14	0.42 ± 0.14	0.10 ± 0.08	0.15 ± 0.10	0.18 ± 0.22	0.21 ± 0.24	0.11 ± 0.13	0.49 ± 0.83*
Blood	0.91 ± 0.22	0.89 ± 0.25	0.07 ± 0.03	0.12 ± 0.05	0.03 ± 0.01	0.07 ± 0.01*	0.01 ± 0.01	0.06 ± 0.02*
Brain	0.16 ± 0.13	0.89 ± 0.24*	0.35 ± 0.41	0.48 ± 0.54	0.23 ± 0.25	0.31 ± 0.33	0.59 ± 0.58	0.10 ± 0.09
Skull	9.51 ± 2.33	12.35 ± 3.09	8.96 ± 1.76	11.69 ± 2.25	8.54 ± 1.48	11.14 ± 2.11	12.39 ± 2.57	12.75 ± 2.75
Femur	12.94 ± 4.78	16.91 ± 6.11	10.74 ± 3.40	13.95 ± 4.29	8.28 ± 3.11	10.92 ± 4.21	15.74 ± 3.30	16.58 ± 4.20

*Significant difference.

IPPB = 5-[¹³¹I]iodopyridine-3-amide-N-3-hydroxypropylidene-3,3-biphosphonate; APPB = 5-[²¹¹At]astatopyradine-3-amide-N-3-hydroxypropylidene-3,3-biphosphonate.

Values expressed as % injected dose per gram tissue and mean ± SD of five samples.

dissected, but samples from the neck containing thyroid tissue were excised from the animals. At 24 h, 0.69 ± 0.41 %ID and 0.45 ± 0.36 %ID were found in the neck with ABPB and IPPB, respectively, whereas a maximum of 0.37 ± 0.13 %ID and 0.66 ± 0.14 %ID were measured for APPB and IPPB, respectively. In the stomach, the astatine compounds showed only slightly elevated uptake compared with the iodine compounds. With ABPB, a maximum of 2.5 ± 0.7 %ID/g at 24 h was observed in stomach, whereas with APPB, a maximum of 3.5 ± 1.4 %ID/g (at 2 h) was observed. For comparison, stomach uptake of [²¹¹At]astatide in this mouse strain is approximately 50 %ID/g at 1 and 4 h (21). The low values in thyroid and stomach found in this study are consistent with a low degree of dehalogenation for these bisphosphonate conjugates.

To compare the selectivity of bone localization for the four compounds, femur-to-normal-tissue uptake ratios were calculated for the data obtained at the 30 min, 2 and 24 h time points. As summarized in Figure 2, bone-to-normal tissue ratios greater than unity were observed for all tissues at all time points, reflecting the strong bone affinity of this class of radiopharmaceuticals. Femur-to-blood and femur-to-muscle ratios were particularly high, and, consistent with the urinary excretion of these compounds, relatively low ratios were observed in the kidneys at 30 min. With IPPB and APPB, relatively low femur-to-spleen ratios were observed.

Cumulated activities were calculated on the basis of the data presented in Tables 1 and 2 and for an injected activity of 1 kBq/g of body weight (~20 kBq/animal). It was assumed that the tissue activities were determined by physical decay only after the 24 h time point. Table 3 presents a summary of the estimated absorbed doses to organs and different bone segments. The bone surfaces by

far received the highest radiation doses from the four compounds. IBPB and ABPB delivered a similar dose to the bone marrow; IPPB and APPB also delivered a similar dose to the bone marrow. It is noteworthy, though, that the bone-surface doses were about three times higher with the astatine analogs than with their respective iodine analogs, i.e., bone surface-to-bone marrow dose ratios of approximately 15 were estimated for ABPB and APPB, whereas they were only approximately 5–6 for IBPB and IPPB.

DISCUSSION

²¹¹At has considerable potential in the treatment of metastatic cancers, because the maximum range of its α-particles in tissue (~70 μm) is well suited to the treatment of small tumor foci. As a consequence of this short range, crossfire into bone marrow from radiation sources localized on the bone surfaces should be greatly reduced compared with the β-emitting bone-seekers, ³²P, ⁸⁹Sr, ¹⁵³Sm and ¹⁸⁶Re (maximum ranges 2.7, 2.4, 0.55 and 1.06 mm, respectively) and the conversion electron emitter ^{117m}Sn (maximum range 0.29 mm) used currently in patients (22). Also, the relatively short half-life of ²¹¹At may have some benefit in clinical settings, because it could reduce the hospitalization needed by patients with sustained radioactivity after treatment. Furthermore, ²¹¹At and its decay products are a relatively pure α-source: More than 99% of the radiation energy emitted during the decay is related to α-particles. Because of the short range of the α-particles of ²¹¹At, special shielding of patients would not be required to protect hospital staff from radiation. With the ¹³¹I-labeled compounds, precautions similar to that for other pharmaceuticals based on this nuclide should be followed.

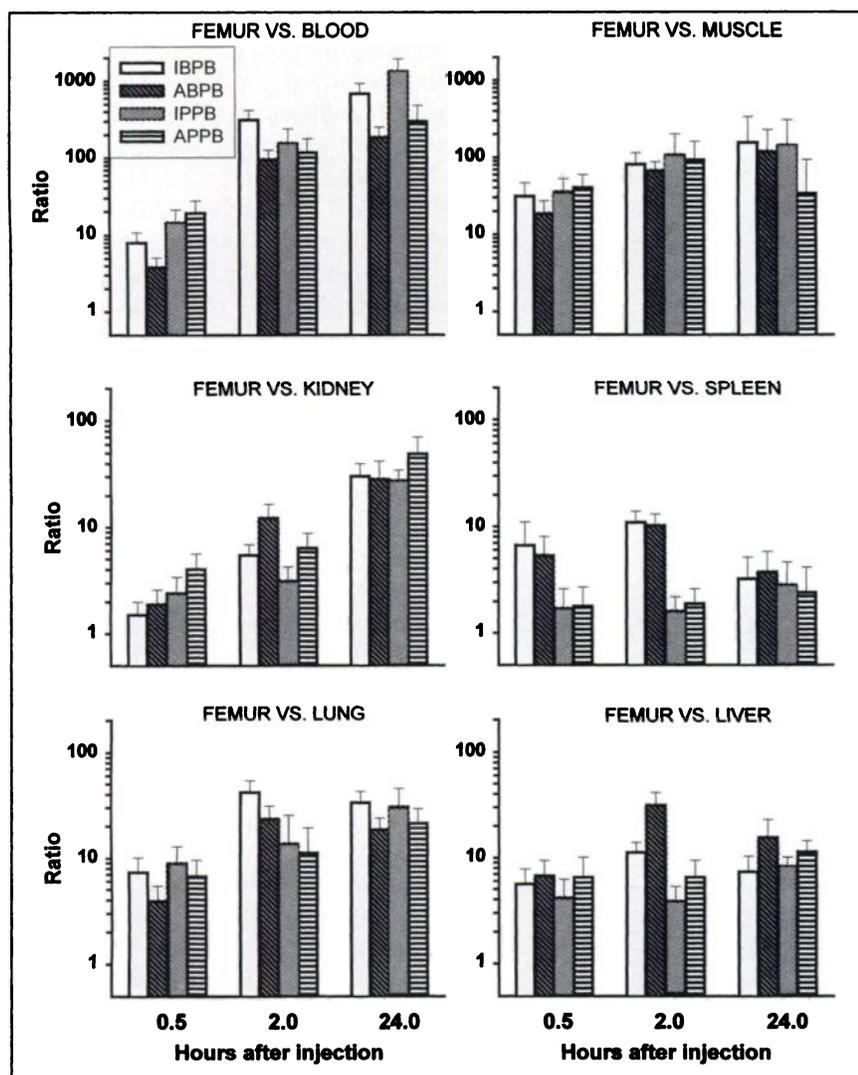


FIGURE 2. Ratios between percentage injected dose per gram for femur and some tissues. Error bars represent \pm SD.

Currently, ^{211}At must be produced in the vicinity of the end user. Initial clinical trials would have to be performed in centers with a cyclotron. According to recent estimates, it should be possible to produce ^{211}At at a cost of approximately \$0.57/MBq in intermediate-size cyclotrons (23). ^{211}At could be marketed on a large scale from a few central production units. Because ^{211}At itself has no pronounced bone affinity, its usefulness for bone cancer applications is dependent on approaches for attaching ^{211}At covalently to bone-seeking molecules.

The strategy investigated in this study was to couple well-characterized, radiohalogenated, acylation agents to bisphosphonates, a class of compounds used in stable form to treat bone cancer (6,24). The chemical procedure to prepare the final product is reasonably rapid and simple to perform, and a kit based on the STMB and APB could be used with a disposable column for product purification in routine production of ABPB.

As displayed in this study, the rapid bone accumulation and normal tissue elimination of these bisphosphonate conjugates are well suited to the relatively short half-life of

^{211}At . An additional feature observed with ABPB and APPB was their low degree of deastatination in vivo. The uptake in the thyroid gland, and to some extent in stomach, is a strong indicator of in vivo dehalogenation for compounds labeled with astatine and iodine, because of these elements' extreme affinity for thyroid, when test animals are not pretreated with blocking agents. We therefore chose to perform the experiments without any thyroid blocking and to use the distribution profiles in thyroid and stomach as indicators of the stability of the compounds toward dehalogenation. Stability toward other possible catabolic processes, such as cleavage of the labeled aromatic ring from the bisphosphonate, will be investigated in future studies. The low degree of dehalogenation observed with ABPB, IBPB, APPB and IPPB markedly contrasted with the results reported with most other low-molecular weight ^{211}At -labeled compounds, which show considerably higher dehalogenation in vivo than their corresponding radioiodinated analogs (25–28), an effect consistent with the lower bond strength of the C-At versus C-I bond. The stability of the present compounds, compared with other small astatinated compounds, has been signifi-

TABLE 3
Absorbed Dose Estimates

Organ	IBPB	ABPB	IPPB	APPB
Liver	23.8	6.8	16.6	11.0
Spleen	54.4	26.6	48.6	36.0
Lungs	5.2	7.6	4.4	5.4
Heart	5.2	5.8	1.8	3.0
Kidneys	9.2	10.4	5.4	7.4
Bladder	6.4	23.6	2.0	9.4
Stomach	7.8	12.0	1.0	11.6
Small intestine	1.8	2.2	0.6	2.8
Large intestine	1.2	2.8	0.8	1.4
Muscle	3.2	1.6	1.0	1.8
Brain	0.2	0.8	5.0	1.6
Bone marrow*	264.2	269.8	150.4	151.2
Bone surface*	1309.0	4174.8	876.2	2323.6
Bone*	171.0	125.2	114.6	69.6

*Assuming that the bone radioactivity is deposited on the bone surfaces.

IBPB = 3-[¹³¹I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate; ABPB = 3-[²¹¹At]astato-benzamide-N-3-hydroxypropylidene-3,3-bisphosphonate; IPPB = 5-[¹³¹I]iodopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate; APPB = 5-[²¹¹At]astatopyridine-3-amide-N-3-hydroxypropylidene-3,3-bisphosphonate.

Values expressed as milligray per kilobecquerel per gram of body weight.

cantly improved. For example, in previous studies (26), 4-[²¹¹At]astato-N-piperidinoethyl benzamide had a stomach uptake of 27.68 %ID/g and a thyroid uptake of 1.89 %ID 2 h after injection in mice. Likewise, 5-[²¹¹At]astato-2'-deoxyuridine (27) had an uptake of approximately 2% in the thyroid gland and approximately 15% in the stomach at 1 h, and biotinyl-3-[²¹¹At]astatoanilide (24) had an uptake of 0.66% in the thyroid and 6.02% in the stomach at 1 h. In comparison, ABPB had 2.65 %ID/g (1.04 %ID) in the stomach and 0.36 %ID in the thyroid, while APPB uptake was 3.50 %ID/g (1.59 %ID) and 0.31 %ID, respectively, 2 h after injection. Further studies are needed to determine the factors responsible for the low deastatination rates for these bisphosphonate conjugates. One can only speculate that the relatively low level of metabolic activity in bone may play a role.

We have explored the possibility of using ²¹²Pb ($t_{1/2}$ of 10.6 h) coupled to a bone-seeking carrier molecule to generate the α -source ²¹²Bi in vivo. ²¹²Pb was bound to the tetraphosphonate ethylene-diamine-tetra(methylene-phosphonic acid). The maximum uptake of ²¹²Bi in the femurs of mice was 13 %ID/g 13 h after injection (13). However, femur-to-kidney ratios of only 1.5, as well as peak femur-to-blood ratios of only 20, were seen, suggesting some instability of the complex in vivo. Considerably higher ratios were seen with the ²¹¹At-labeled bisphosphonate conjugates: Femur-to-kidney ratios of 17.3 and 6.7 and femur-to-blood ratios of 140.7 and 156.0 were reached with ABPB and APPB, respectively, by 6 h after injection.

Because the use of cold bisphosphonates in the treatment

of cancer-related bone complications has become a well-established procedure, radiolabeled bisphosphonate is likely to be applied to patients also treated with cold bisphosphonates. The effects of such pretreatment may influence the biodistribution of radioactive bisphosphonate. Biodistribution of ¹⁴C-labeled APB in mice and rats has been reported to be affected by the amount of unlabeled APB that was co-injected. The uptake in bone and liver, which together accounted for a large proportion of the dose, appeared to increase proportionally with the dose of bisphosphonate, i.e., the percentage injected dose per gram of tissue did not change significantly, whereas in kidneys a strong increase in percentage injected dose per gram of tissue was observed above a threshold dose (29). The possible influence of cold bisphosphonate with a different chemical structure on the use of radiolabeled bisphosphonate should be addressed in future studies.

The high bone accumulation of the radiolabeled bisphosphonates in this study hopefully can be exploited for the management of bone cancers showing elevated turnover of bone matrix. Furthermore, their rapid clearance from blood should minimize dose to hematopoietic cells from circulating radioactivity. The ¹³¹I-labeled compounds may be useful as palliation agents against pain mediated from bone metastases. Several other β -emitters are currently used clinically for this purpose, and some are commercially available (4). ¹³¹I has some distinct advantages compared with other radionuclides, i.e., it is widely available, has a relatively low cost and is used frequently in hospitals throughout the world.

Another ¹³¹I-labeled bisphosphonate, the amino-(4-hydroxybenzylidene)-diphosphonate (BDP3), has been studied clinically (9), and significant palliative effects have been documented in some patients. However, preclinical data suggest that the in vivo stability of this compound may be somewhat lower than that of IBPB or IPPB. Thyroid-to-femur ratios in rat for ¹³¹I-BDP3 have been reported to be approximately 7.7:1 (30) compared with 4.0:1 for IBPB and 6.8:1 for IPPB (assuming a mouse thyroid gland weight of 5 mg). The femur uptake of ¹³¹I-BDP3 in the rat was 245 %g dose/g (%ID/g tissue \times animal body weight) at 24 h (31). Expressed in this fashion, femur uptakes were 406 %g dose/g (IBPB) and 315 %g dose/g (IPPB) in mice in this study. Although comparison of results obtained in different species must be done with caution, the new bisphosphonate conjugates described in this article may offer advantages in comparison with the first-generation ¹³¹I-labeled bisphosphonate.

Clinical studies (5) indicate that β -emitting nuclides incorporated in bone can induce significant pain relief in patients with cancer metastases to the skeleton. The major drawback with bone-seeking β -emitting radiopharmaceuticals is that the range of these radiations is sufficient to produce crossfire irradiation of the bone marrow, limiting the level of activity that can be administered (32). The use of α -particle emitters against these types of lesions may therefore be appealing, because the dose delivered is then

more strongly focused to the area where the decay occurs, i.e., within 70 μm of the point of decay for ^{211}At . Provided the uptake in red bone marrow is low, it may be possible to irradiate the site of the lesion selectively with high linear energy transfer radiation while sparing the majority of the bone marrow cells. As indicated by the dosimetric estimates in this study, a significant improvement in bone surface-to-bone marrow dose ratio may be achieved with the α -emitter ^{211}At compared with β -emitters such as ^{131}I and ^{89}Sr if radionuclide localization on bone surfaces is achieved. The dosimetry in this study, however, should be considered only as preliminary estimates, because microdosimetric studies will be required to fully understand the dose distribution of the α -emitter in the various bone segments. ^{211}At may have significant potential in the treatment of micrometastases to the bone but would probably not be effective in bulky metastatic lesions unless a high homogeneous uptake could be achieved. Studies of radionuclide microdistribution, toxicity and therapeutic efficacy in relevant models are therefore warranted to further elucidate the usefulness of bisphosphonate conjugated to α -emitters as treatment against osseous neoplasms.

CONCLUSION

These results demonstrate that these ^{211}At -labeled bisphosphonate conjugates have excellent bone accumulation and exhibit rapid clearance from normal tissues in a time frame compatible with the half-life of ^{211}At . The ^{131}I -labeled bisphosphonate analogs also were taken up selectively in bone. The compounds may therefore be used to investigate whether a short-lived α -emitter offers any advantages compared with β -emitters in the treatment of osseous cancers. These compounds will be investigated further in relevant tumor models.

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