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Preliminary Evaluation of a New Radiolabelled Bisphosphonate.

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Summary

A new bisphosphonate was synthesised using N-succinimidyl-3-[^{125}I]iodobenzoate and 3-amino-1-hydroxypropylidene as the reagents. After purification by reverse phase chromatography the product, 3-[^{125}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate, was injected intravenously into nude mice and the radioactivity content in various normal tissues was measured. Both at 3 h and at 17 h after injection a highly selective bone uptake was observed. Average ratios femur/blood and skull/blood were 35.9 and 36.6 at 3 h and 111.6 and 128.0 at 17 h, respectively. While clearing was observed for all other tissues, the bone radioactivity content did not decrease significantly from 3 h to 17 h after injection, indicating a high stability of this bisphosphonate in bone tissue. This preliminary study implies that pharmacokinetics, biological activity and clinical applications of amidebisphosphonates in general, and the described compound in particular, should be further evaluated.

Key words: amidebisphosphonate, bone seeking agent, radiohalogen

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Introduction

Primary bone cancers and skeletal metastases typified by a high turnover of bone matrix can be targeted with radioactive bisphosphonates, usually with several times higher uptake in osseous tumours vs normal bone (1). Bisphosphonates are a class of compound which have a high affinity for metabolically active bone. They have been studied extensively for their ability to interact with bone metabolism. Clinically, bisphosphonates have been approved for the treatment of hypercalcemia and for the palliation of pain from skeletal metastases (2,3). Several analogues have been investigated and the chemical structures of the side groups have been shown to be important determinants for the biological effects as well as the pharmacokinetics of the compounds (4). Radioactive bisphosphonates have been developed for bone scintigraphy (5) and as palliative agents for the targeting of radiation to painful sites caused by cancers affecting the skeleton (6,7).

There is considerable debate as to which type of radiation would be the optimal for a bone-seeking antineoplastic agent. In radiotherapy high energy β-particles may ensure a more homogeneous dose distribution in solid tumours (8). Because of reduced crossfire of radiation to the bone marrow from a radiation source localized in the bone matrix, short range (< 100 μm) α-particle emitters have been proposed to be more suitable (9). In scintigraphy, nuclides emitting moderately energetic γ-radiation are usually preferred. Nevertheless, because of reduced background contribution from distant sources, nuclides emitting low energetic X-rays have shown promise in some special applications (10) and may therefore be useful in short range detection of osseous metastases in soft tissues (11).

In the current study we report on the preparation sequence and an initial evaluation of a radiohalogenated bisphosphonate, *i.e.*, an aromatic amidebisphosphonate labelled with ^{125}I, which has interesting properties as a bone-seeking agent. Analogues based on other halogen isotopes, e.g., ^{131}I or ^{211}At, may also be prepared by the described methods allowing the provision of bone-seeking agents with a broad range of radiation properties.

Materials and methods

Reagents for the synthesis of amidebisphosphonate

Iodine-125 in NaOH was obtained commercially (Amersham, UK), and N-succinimidyl-3-[^{125}I]iodobenzoate (NS[^{125}I]IB) was prepared by destannylation of the precursor N-succinimidyl-3-(trimethylstannyl)benzoate and purified as previously described (12). The disodium form of 3-amino-1-hydroxypropylidene bisphosphonate (APB), which was commercially available as a 3 mg/ml solution for infusion (Pamidronate, Ciba-Geigy, Switzerland), was concentrated on a rotary evaporator, recrystallized from ethanol and dissolved in 0.1 M borate buffer prior to its use.

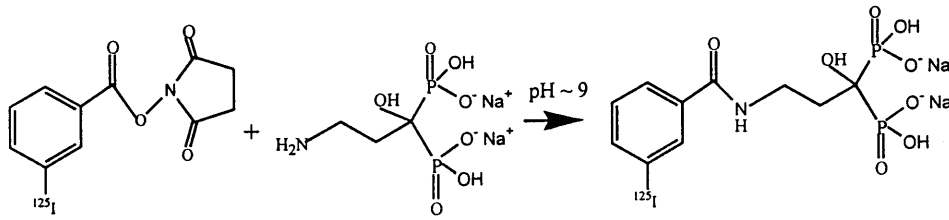

Figure 1 Synthesis of 3-[^{125}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate.

Synthesis of the new bisphosphonate

Two concentrations of APB were used, *i.e.*, 5 mg/ml and 30 mg/l. APB in 75 µl of borate was transferred to a 1 ml glass vial containing dry NS[^{125}I]IB. This mixture was incubated on a Whirlmixer (Fisons, UK) for 30 min before injection onto the HPLC. Fractions of 0.25 ml were collected from the HPLC using a Helirack fraction collector (LKB, Sweden).

HPLC system for the detection and purification of the product.

The liquid chromatographic equipment used for identification and purification included a LC-6A pump (Shimadzu, Japan), a Rheodyne Model 7125 injector (Rheodyne, USA) a SPD-6A UV-detector (Shimadzu, Japan) and a Radiomatic A-100 radioactivity detector (Canberra, USA). A reverse phase system with a Hibar pre-packed column RT-250-4 (Merck, Germany), and a mobile

phase of methanol combined with 0.2 M phosphate with 25 mM tetrabutylammonium hydroxide. Methanol was added in a 4:6 ratio to the aqueous buffer and the pH adjusted to 6.0. During HPLC runs the flow rate was kept at 1 ml/min.

Preparation of standards used for the identification of HPLC peaks.

The following radioactive products were expected to be observed in the crude reaction mixture of NS[^{125}I]IB and APB, unreactive NS[^{125}I]IB, 3-[^{125}I]iodobenzoic acid due to hydrolysis of the ester and possibly the desired product, 3-[^{125}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate. 3-[^{125}I]iodobenzoic acid was prepared by incubation of N-succinimidyl-3-[^{125}I]iodobenzoate in a 0.1 M borate solution, pH 9.2, overnight, and afterwards diluted 10 times with HPLC mobile phase. Dry N-succinimidyl-3-[^{125}I]iodobenzoate was dissolved in HPLC buffer immediately before injection into the HPLC system.

Animal experiments

Prior to the animal experiments the HPLC fractions containing 3-[^{125}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate were concentrated with a stream of nitrogen gas to remove most of the methanol and subsequently diluted 10 times with 0.1 M phosphate buffered saline (pH 7.4). Adult male nude mice (BALB/c) 4 months old with an average weight of 30.1 g were used to study uptake and retention of the bisphosphonate in various tissues. An injection volume of 100 µl and an activity of 80 kBq was injected into the tail vein. Tissue distribution was studied after 3 and 17 hours respectively, using two animals at each time point. The count rates in the samples were measured using a multi well Multigamma 1640 counter (LKB, Sweden).

Results

Retention time of the compounds on the HPLC column.

After injections of the standards the 3-[^{125}I]iodobenzoic acid and NS[^{125}I]IB peaks were observed at 7.1 min and 10.5 min respectively. At the low concentration of APB in the reaction

mixture these two compounds were observed but also a new compound with a retention time of 5.7 min appeared. At the higher concentration of APB only the 5.7 and 7.1 min peaks were observed indicating a complete consumption of the radioactive labelling reagent. The yield of the desired product improved with an increase in APB concentration used. At the highest concentration tested, 30 mg/ml, 82% yield was observed.

Biodistribution

In Table 1 bone to tissue ratios are presented. Bone samples from the femur and skull were measured. Bone to soft tissue ratios increased significantly from 3 h to 17 h after injection. Among the soft tissues kidneys and liver had the highest radioactivity level but the clearance rate was similar for all the soft tissue organs with a reduction at 17 h to approximately half the value at 3 h. The absolute radioactivity in bone was similar at both time points indicating a rapid uptake and a high retention. The absolute uptakes in bone were on average 5.6% and 6.0% of injected dose per gram at 3 h and 5.2% and 6.0% of injected dose at 17 h for femur and skull respectively.

Table 1 Bone to tissue ratios for 3-[^{125}I]iodobenzamide-N-3-hydroxypropylidene-3,3-bisphosphonate.

	3 h		17 h	
	mean	range	mean	range
femur/blood	35.9	26.3-46.6	111.6	101.1-122.0
skull/blood	36.6	34.4-38.8	128.0	107.5-148.5
femur/liver	3.5	2.1-4.9	6.2	5.8-6.5
scull/liver	3.5	2.8-4.1	7.3	5.1-9.5
femur/kidneys	1.8	1.7-1.9	5.5	5.1-5.9
skull/kidneys	1.9	1.5-2.2	6.4	5.2-7.5
femur/spleen	19.2	13.8-24.5	23.3	16.3-30.2
skull/spleen	19.3	18.1-20.4	29.4	14.4-44.3
femur/lungs	10.4	9.4-11.3	18.1	15.8-20.4
skull/lungs	10.9	9.4-12.4	22.1	14.0-30.1
femur/heart	26.8	24.0-29.6	52.6	50.1-55.0
skull/heart	28.1	24.7-31.5	60.1	48.4-73.5
femur/muscle	34.4	11.3-57.5	39.9	28.7-50.0
skull/muscle	42.5	9.4-75.3	49.8	26.2-73.3

Discussion

In this initial screening study of a novel bisphosphonate with an aromatic side group we are using the activated ester method to add a radiolabelled intermediate onto an amino group on a small molecule. This method has previously been used for the labelling of larger compounds, *i.e.*, proteins and microspheres (12,13). The biodistribution data indicate that the amide bond is sufficiently stable *in vivo* and that favorable radioactivity ratios between bone and soft tissues can be achieved. It should be noted that the presented study was conducted using adult animals and that bone in younger animals are likely to show significantly higher relative uptake of bone-seeking agents (14). Based on experience with uptake of radiolabelled bisphosphonate and tetraphosphonate in humans (5,15) it can be anticipated that this new bisphosphonate may be useful for targeting osseous sites clinically.

A potential application of ^{125}I-labelled bisphosphonate would be as an aid in the surgical removal of metastates from osseous sarcomas in soft tissues, *i.e.*, as an agent for radioguided surgery. A detection probe for radioguided surgery has been developed for clinical procedures (Neoprobe model 1000, Neoprobe Corp., USA), and is specially designed to be used for the detection of low energy X-rays from iodine-125 decay (10).

Preliminary experiments indicate that the coupling to APB works similarly with N-succinimidyl[^{211}At]astatobenzoate as with the NS[^{125}I]IB. Thus an α-particle emitting bone seeking agent can be produced which can deliver relatively short range radiation to bone with limited radiation of bone marrow. Studies with the ^{211}At-labelled version of this bisphosphonate are therefore in progress. Some possible uses of analogues based on other nuclides also seem appealing. The ^{131}I- labelled product may be useful for palliation of pain from osseous cancer and nonradioactive analogues may be studied as potential biologically active compounds. The use of the amide bonding allows the preparation of a diversity of structures varying in lipophilicity, both radioactive and nonradioactive. Modified products of this type may be made from other protein labelling agents, *e.g.*, Bolton-Hunters reagent and radiolabelled pyridine- and furane carboxylates (16-18).

In the current study very dilute samples were injected and no toxicity from the solvent was observed. A further refinement of the purification procedure is warranted since the use of the toxic compounds methanol and tetrabutylammonium hydroxide may be a potential problem if less dilute samples are used for injections.

In conclusion, we have presented a new radiolabelled bisphosphonate that shows excellent affinity for bone. We therefore suggest further optimization of the chemistry and extended biological evaluation of this compound labelled with various radiohalogens.

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References

1. Fleish, H. – Bisphosphonate in bone disease. From the laboratory to the patient. 2nd edition, The Parthenon Publishing Group, New York, 1995.
2. Kanis, J.A. – Bone 17: 101S (1995)
3. Vinholes, J., Guo, C.Y., Purohit, O.P., Eastell, R. and Coleman, R.E. – Br. J. Cancer 73: 1089 (1996).
4. Keen, R.W. and Spector, T.D. – Br. J. Clin. Pract. 50: 211 (1996)
5. Fogelman, I., Maisey, M.N. and Clarke, S.E.M. – An atlas of clinical nuclear medicine. Mosby, St. Louis, pp. 1-110, 1994.
6. Eisenhut, M., Berberich, R., Kimmig, B. and Oberhausen, E. – J. Nucl. Med. 27: 1255 (1986)
7. Silberstein, E.B. – J. Nucl. Med. 37: 249 (1996)

8. Wheldon, T.E. and O'Donnoghue, J.A. – Int. J. Radiat. Biol., 58: 1, (1990)

9. Hassfjell, S.P., Hoff, P., Bruland, Ø.S. and Alstad, J. – J. Label. Compds. Radiopharm. 34: 717 (1994)

10. Di Carlo, V., Stella, M., De Nardi, P. and Fazio, F. – Tumori 81: (Suppl.) 98 (1995)

11. Hassfjell, S.P., Skretting, A., Bruland, Ø.S. and Hoff, P. – Nucl. Med Comm. (In press)

12. Larsen, R.H., Hoff, P., Alstad, J. and Bruland, Ø.S. – J. Label. Compds. Radiopharm. 34: 773 (1994)

13. Larsen, R.H., Hassfjell, S.P., Hoff, P., Alstad, J., Olsen, E., Vergote, I.B., De Vos, L.N., Bjørgum, J. and Nustad, K. – J. Label. Compds. Radiopharm. 33: 977 (1993)

14. Hassfjell, S.P., Bruland, Ø. and Hoff, P. – Nucl. Med. Biol. 24: 231 (1997)

15. Bruland, Ø.S., Skretting, A. Solheim, Ø.P. and Aas, M. – Acta Oncol. 35: 381 (1996)

16. Bolton, A.E. and Hunter, W.M. – Biochem. J. 133: 529 (1973)

17. Garg, S., Garg, K., Zhao, X.G., Friedman, H., Bigner, D.D. and Zalutsky, M.R. – Bioconjugate Chem. 2: 50 (1991)

18. Garg, S., Garg, P.K., Bigner, D.D. and Zalutsky, M.R. – J. Label. Compds. Radiopharm. 30: 207 (abstract) (1991)

