Annals of Nuclear Medicine Vol. 16, No. 1, 55–59, 2002

New bone-seeking agent: Animal study of Tc-99m-incadronate

Makoto SHIGEMATSU,* Susumu SHIOMI,* Hiroshi IWAO** and Hironobu OCHI*

**Department of Nuclear Medicine and **Department of Pharmacology, Graduate School of Medicine, Osaka City University*

Objective: Disodium cycloheptylaminomethylenediphosphonate monohydrate (incadronate disodium) is a third-generation bisphosphonate compound which potently inhibits bone resorption, and a highly effective drug in the treatment of metastatic bone disease. We first labeled incadronate disodium with ^{99m}Tc, and examined its biodistribution and bone uptake after intravenous injection in rats to assess its potential for clinical use as a bone-seeking agent for judgment of the therapeutic effect of incadronate on bone metastases. Bone scan with ^{99m}Tc-labeled incadronate (^{99m}Tc-incadronate) may yield important information prior to the use of incadronate for treatment of bone metastases. ***Methods:*** Synthesis of ^{99m}Tc-incadronate was carried out by reduction of ^{99m}Tc-pertechnetate in the presence of $SnCl_2$ and N_2 gas. Normal rats were injected with 18.5 MBq (0.5 mCi) ^{99m}Tc-incadronate in a volume of 0.1 m*l* intravenously and then sacrificed at 15 min, 30 min, 1 h or 2 h (six rats at each time point) after injection. Samples of muscle, stomach, small intestine, kidney, liver and bone (femur) were taken and weighed. In addition, a 1-m*l* sample of blood was drawn from the heart, and urine was taken from the urinary bladder immediately after sacrifice. Samples were measured for radioactivity and expressed as percent uptake of injected dose per gram or per milliliter (% ID/g or m*l*). Bone-to-blood and bone-to-muscle uptake ratios were determined from the % ID/g or m*l* values for these organs. ***Results:*** The greatest accumulation of ^{99m}Tc-incadronate was found in bone. Radioactivity in bone was as high as 3.22 ± 0.68% ID/g at 2 hours after injection. Scintigraphic images of ^{99m}Tc-incadronate in normal rats revealed highly selective skeletal uptake. ***Conclusion:*** ^{99m}Tc-incadronate exhibited high uptake in bone, and relatively low uptake in soft tissue, suggesting that it may be useful as a bone-seeking agent for judgment of the therapeutic effect of incadronate on bone metastases, by determining the degree of its accumulation in metastatic bone lesions.

Key words: bone-seeking agent, bisphosphonate, ^{99m}Tc, incadronate, rats

INTRODUCTION

RADIONUCLIDE BONE SCANNING is an extremely sensitive method for the detection of various bone diseases, and is considered the most practical screening technique for assessing skeletal metastases in the whole body. Bisphosphonates have a P-C-P structure, and have been developed as stable analogues for inorganic pyrophosphoric acid. Bisphosphonates have long been known to inhibit bone resorption *in vitro* and *in vivo*.[1–4] Since the introduction of etidronate, a number of new bisphosphonates have been synthesized which inhibit bone resorption without causing mineralization defects. These compounds are referred to as new-generation bisphosphonates.[5–9]

Incadronate (disodium cycloheptylaminomethylenediphosphonate monohydrate) [Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan] (Fig. 1) is one of these new-generation bisphosphonates. This compound inhibits the increase in the concentration of free calcium in blood induced by carcinoma. It has been reported to have greater potency in this respect than pamidronate and alendronate, and to have a more prolonged effect than elcatonin.[10–12] Incadronate inhibits calcium release

Received September 5, 2001, revision accepted November 21, 2001.

For reprint contact: Susumu Shiomi, M.D., Department of Nuclear Medicine, Graduate School of Medicine, Osaka City University, 1–4–3 Asahimachi, Abeno-ku, Osaka 545–8585, JAPAN.

E-mail: shiomis@med.osaka-cu.ac.jp

induced by bone resorption stimulants more potently than do other bisphosphonates. Incadronate is therefore a highly effective drug in the treatment of metabolic bone diseases such as hypercalcemia of malignancy, bone pain due to skeletal metastases, and osteoporosis.[10,11] It has been reported that bisphosphonates are effective in the treatment of bone metastases.[13] ^{99m}Tc-labeled incadronate (^{99m}Tc-incadronate) is therefore considered potentially useful as a radiotracer for judgment of the therapeutic effect of incadronate on bone metastases, by determining the degree of accumulation in metastatic bone lesions.

For the above mentioned reasons, we labeled incadronate disodium with ^{99m}Tc-pertechnetate sodium. This study was conducted to evaluate the biodistribution of ^{99m}Tc-incadronate in normal rats, in order to determine whether ^{99m}Tc-incadronate is useful as a bone-seeking agent for the determination of therapeutic policy prior to the use of incadronate for treatment of bone metastases.

MATERIALS AND METHODS

Labeled compounds

The synthesis of ^{99m}Tc-complex with incadronate was carried out by reduction of ^{99m}Tc-pertechnetate in the presence of $SnCl_2$ and N_2 gas. One milliliter of $SnCl_2$ (0.19 mg/m*l*) was first added to 1 m*l* of a solution of incadronate (0.38 mg/m*l*) [Lot. No. GEPYA001] and mixed. The ^{99m}Tc-complex of incadronate was prepared by adding 1 m*l* of ^{99m}Tc (Nihon Medi-Physics Co., Ltd., Nishinomiya, Japan) as pertechnetate (370 MBq/m*l*) to 1 m*l* of the incadronate solution prepared previously, and mixed well. The labeling efficiency of ^{99m}Tc-incadronate was determined by thin-layer chromatography (TLC Silica gel 60, methylethylketone).

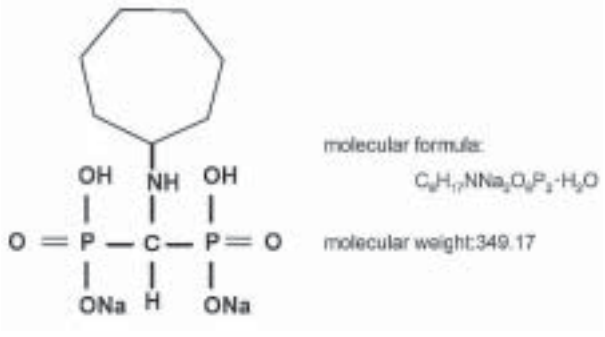

Fig. 1 Structural formula of incadronate disodium.

Biodistribution method

Twenty-four female rats (Wistar, six weeks), each weighing 130–160 g, were used to determine the organ distribution of ^{99m}Tc-incadronate. The rats were sacrificed at 15 min, 30 min, 1 h or 2 h (six rats at each time point) after injection of 18.5 MBq (0.5 mCi) ^{99m}Tc-incadronate in a volume of 0.1 m*l* via the tail vein. Samples of muscle, stomach, small intestine, kidney, liver and bone (femur) were taken and weighed. In addition, a 1-m*l* sample of blood was drawn from the heart immediately after sacrifice. Samples of different organs were counted in a well-type gamma scintillation counter (AUTO WELL GAMMA SYSTEM ARC-2000, Aloka Co., Ltd., Tokyo, Japan) to calculate resident activity in different organs. Tissue concentrations were calculated and expressed as percent uptake of injected dose per gram or per milliliter (% ID/g or m*l*). Bone-to-blood and bone-to-muscle uptake ratios were determined from the % ID/g or % ID/m*l* values for the organs.

An imaging study was performed in a normal rat (Wistar, six weeks) weighing 160 g at 1 h after intravenous injection administration of 18.5 MBq (0.5 mCi) of ^{99m}Tc-incadronate. Bone scan was performed with a gamma camera (BODY SCAN, Siemens-Asahi Medical Technologies Co., Ltd., Tokyo, Japan) equipped with a 141 keV (20%), LEHR (low-energy, high-resolution) collimator.

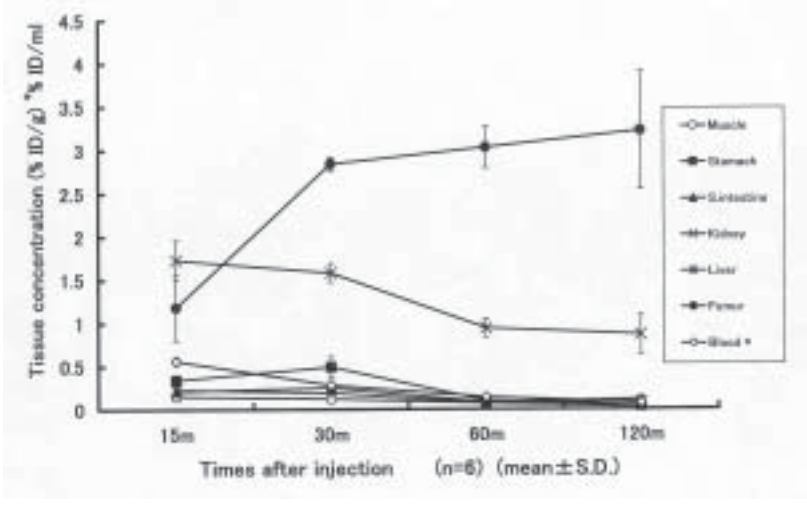

Fig. 2 Biodistribution of ^{99m}Tc-incadronate in normal rats.

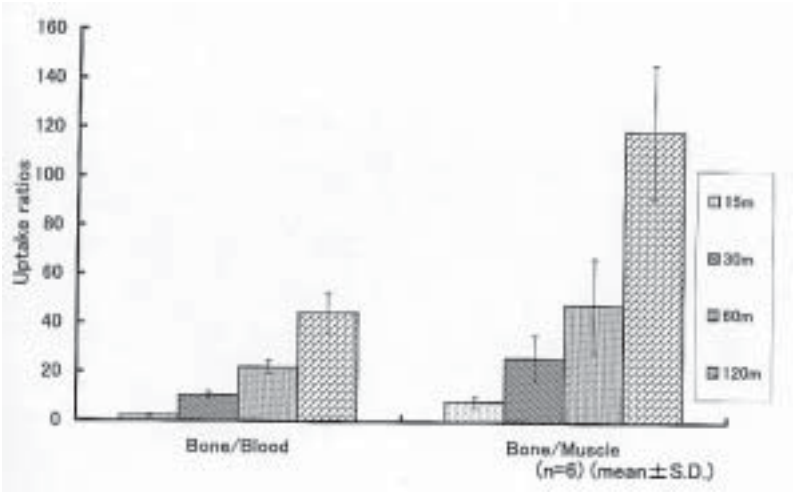

Fig. 3 Bone-to-blood and bone-to-muscle uptake ratios in normal rats at different times after injection of ^{99m}Tc-incadronate.

Excretion in urine

Six rats were used to calculate the percentage of the injected dose excreted in urine. All of the animals were placed in individual cages equipped for urine collection. By 2 hours after injection of 18.5 MBq (0.5 mCi) of ^{99m}Tc-incadronate into the tail vein, radioactivity in urine was almost all collected. All of the urine was counted in a well-type gamma scintillation counter to calculate radioactivity.

RESULTS

The labeling efficiency of ^{99m}Tc-incadronate, as determined by TLC, was 93% on average, and the final pH ranged from 4.3 to 4.5. The biodistribution in various organs is shown in Figure 2 (n = 6). Radioactivity in bone tissue was as high as 2.84 ± 0.08% ID/g (mean ± S.D., n = 6) at 30 minutes after injection, increasing to 3.03 ± 0.24% ID/g at 1 hour and 3.22 ± 0.68% ID/g at 2 hours. Activity in kidney was highest at 15 minutes but declined rapidly throughout the experiment. The radioactivities in muscle, stomach, small intestine, liver and blood were all lower than 0.6% ID/g at 15 minutes and also declined rapidly. The bone-to-blood and bone-to-muscle uptake ratios are shown in Figure 3. The bone-to-blood ratio and the bone-to-muscle ratio increased to 44.34 ± 8.37 and 118.73 ± 27.38 (mean ± S.D., n = 6), respectively, at 2 hours. Most of the radiotracer was excreted by the urinary system. The percentage of the injected dose of ^{99m}Tc-incadronate excreted in the urine of rats at 2 hours after injection was 45.47 ± 6.41% (mean ± S.D., n = 6). Scintigraphic images of ^{99m}Tc-incadronate in normal rats revealed highly selective skeletal uptake (Fig. 4).

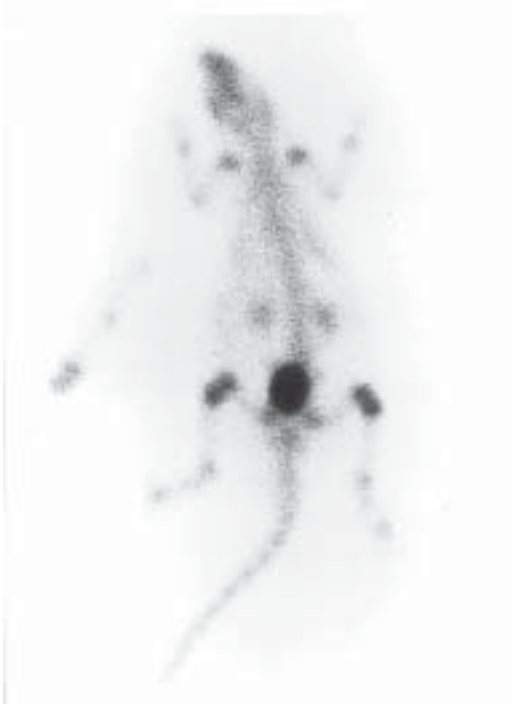

Fig. 4 Whole-body image of rat obtained 1 h after injection of 18.5 MBq (0.5 mCi) of ^{99m}Tc-incadronate. This image shows selective uptake of radiotracer in normal bone tissue.

DISCUSSION

Since technetium has many chemical properties, it is possible to obtain high chelates with diphosphonate compounds such as 1-hydroxy-methylene-1,1-diphosphonate (HMDP) and methylene-diphosphonate (MDP).[14,15] Good stability both *in vitro* and *in vivo*, good availability, and low cost are all very important for clinical application of a radiopharmaceutical. ^{99m}Tc-incadronate is a very attractive radiopharmaceutical for bone scan since it has several

advantages including good stability, gamma ray emissions, and, in particular, the ready availability of a generator which permits on-site "milking" of the radioisotope.[16] ^{99m}Tc-incadronate was prepared by reduction of ^{99m}Tc as pertechnetate in the presence of $SnCl_2$ and N_2 gas. ^{99m}Tc-incadronate was labeled immediately after mixing well. We have achieved 93% labeling efficiency of ^{99m}Tc-incadronate on average, with pH ranging from 4.3 to 4.5. The method of labeling used in this study was considered to be as accurate and valid as any other technique used for labeling pertechnetate,[17] but since the labeling efficiency of ^{99m}Tc-incadronate was not satisfactory for clinical use, it is necessary to improve the labeling conditions for it.

Incadronate is a third-generation bisphosphonate compound which potently inhibits bone resorption, and is expected to be useful in the clinical treatment of hypercalcemia of malignancy, bone pain due to skeletal metastases, and osteoporosis.[10,11] At present, diphosphonates such as HMDP and MDP labeled with ^{99m}Tc are widely used in clinical practice for bone scanning.[18–20] Incadronate has been successfully used in the clinical setting for hypercalcemia of malignancy and osteoporosis.[21–23] It is therefore possible that ^{99m}Tc-incadronate concentrates in bone metastases of cancer as much as ^{99m}Tc-HMDP, since incadronate potently inhibits bone resorption. It has been reported that the femur tissue distributions of ^{99m}Tc-HMDP and ^{99m}Tc-MDP in normal rats are 2.579% ID/g and 1.484% ID/g, respectively at 2 hours after intravenous injection.[24] In our study, the femur tissue distribution of ^{99m}Tc-incadronate was 3.22% ID/g, and higher than those of ^{99m}Tc-HMDP and ^{99m}Tc-MDP.

The concentration of ^{99m}Tc-incadronate determines the increase or decrease in the % ID/g tissue in bone and other organs. In our study with normal rats, ^{99m}Tc-incadronate accumulated significantly in bone tissue. Radioactivity in muscle and blood, on the other hand, was low and declined quickly. The bone-to-muscle uptake ratio was 8.26 at 15 minutes and continuously increased to a peak of 118.73 at 2 hours. In addition, ^{99m}Tc-incadronate accumulated in normal bone, as shown in Figure 4. Like all diphosphonates used for bone scintigraphy, ^{99m}Tc-incadronate was mainly excreted by the urinary system. High uptake of ^{99m}Tc-incadronate was thus observed in bone, whereas uptake in soft tissue was relatively low.

Incadronate potently inhibits bone resorption, and is highly effective in the treatment of bone metastases. The *in vivo* behavior of ^{99m}Tc-incadronate suggests that it could be used clinically for the determination of therapeutic policy prior to the use of incadronate for treatment of bone metastases, and as an effective means to judge treatment of bone metastases. Bone scan with ^{99m}Tc-incadronate may yield important information prior to the use of incadronate for treatment of bone metastases. Furthermore, detailed studies will be necessary to more fully assess the clinical usefulness of ^{99m}Tc-incadronate.

In conclusion, ^{99m}Tc-incadronate appears to be a very good potential candidate for clinical use in bone-seeking agents for judgment of the therapeutic effect of incadronate on bone metastases, since it displays highly selective uptake in the skeletal system and has low non-target uptake and rapid clearance in nonosseous tissue. Scintigraphic images of ^{99m}Tc-incadronate in normal rats revealed highly selective skeletal uptake.

REFERENCES

1. Fleisch H, Russell RGG, Francis MD. Diphosphonates inhibit hydroxyapatite dissolution *in vitro* and bone resorption in tissue culture and *in vivo. Science* 1969; 165: 1262–1264.
2. Reynolds JJ, Minkin C, Morgan DB, Spycher D, Fleisch H. The effect of two diphosphonates on the resorption of mouse calvaria *in vitro. Calcif Tissue Res* 1972; 10: 302–313.
3. Fleisch H. Bisphosphonates—History and experimental basis. *Bone* 1987; 8 (Suppl 1): S23–S28.
4. Flanagan AM, Chambers TJ. Dichloromethylenebisphosphonate (Cl_2MBP) inhibits bone resorption through injury to osteoclasts that resorb Cl_2MBP-coated bone. *Bone Mineral* 1989; 6: 33–43.
5. Russell RGG, Fleisch H. Pyrophosphate and diphosphonates in skeletal metabolism. *Clin Orthop* 1975; 108: 241–263.
6. Shinoda H, Adamek G, Felix R, Fleisch H, Schenk R, Hagan P. Structure-activity relationships of various bisphosphonates. *Calcif Tissue Int* 1983; 35: 87–99.
7. Schenk R, Eggli P, Fleisch H, Rosini S. Quantitative morphometric evaluation of the inhibitory activity of new aminobisphosphonates on bone resorption in the rat. *Calcif Tissue Int* 1986; 38: 342–349.
8. Pluijm GVD, Binderup L, Bramm E, Wee-Pals LVD, Groot HD, Binderup E, et al. Disodium 1-hydroxy-3-(1-pyrrolidinyl)-propylidene-1,1-bisphosphonate (EB-1053) is a potent inhibitor of bone resorption *in vitro* and *in vivo. J Bone Min Res* 1992; 7: 981–986.
9. Sato M, Grasser W. Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. *J Bone Min Res* 1990; 5: 31–40.
10. Kawamuki K, Kudo M, Ouchi N, Abe T. Inhibitory effect of YM175 on bone resorption *in vitro. Basic Clinical Rep* 1994; 28: 2895–2904. (in Japanese)
11. Takahashi K, Fukushima S, Kokubo S, Teramura K, Tanaka T. Effect of YM175 on hypercalcemia induced by human squamous cell carcinoma of oral cavity. *Basic Clinical Rep* 1994; 28: 2905–2919. (in Japanese)
12. Takeuchi M, Sakamoto S, Yoshida M, Abe T, Isomura Y. Studies on novel bone resorption inhibitors. I. Synthesis and pharmacological activities of aminomethylenebisphosphonate derivatives. *Chem Pharm Bull* 1993; 41: 688–693.
13. Hamdy NAT, Papapoulos SE. The palliative management of skeletal metastases in prostate cancer: use of bone-seeking radionuclides and bisphosphonates. *Semin Nucl Med* 2001; 31: 62–68.
14. Bevan JA, Tofe AJ, Benedict JJ, Francis MD, Barnett BL. Tc-99m HMDP (hydroxymethylene diphosphonate): a radiopharmaceutical for skeletal and acute myocardial infarct imaging. I. Synthesis and distribution in animals.

J Nucl Med 1980; 21: 961–966.
15. Subramanian G, McAfee JG, Blair RJ, Kallfelz FA, Thomas FD. Technetium-99m-methylene diphosphonate—a superior agent for skeletal imaging: Comparison with other technetium complexes. *J Nucl Med* 1975; 16: 744–755.
16. Lin WY, Lin CP, Yeh SJ, Hsieh BT, Tsai ZT, Ting G, et al. Rhenium-188 hydroxyethylidene diphosphonate: A new generator-produced radiotherapeutic drug of potential value for the treatment of bone metastases. *Eur J Nucl Med* 1997; 24: 590–595.
17. Deutsch E, Libson K, Vanderheyden JV, Ketring AR, Maxon HR. The chemistry of rhenium and technetium as related to the use of isotopes of these elements in therapeutic and diagnostic nuclear medicine. *Nucl Med Biol* 1986; 13: 465–477.
18. Rudd TG, Allen DR, Hartnett DE. Tc-99m methylene diphosphonate versus Tc-99m pyrophosphate: biologic and clinical comparison. *J Nucl Med* 1977; 18: 872–876.
19. Lin WY, Wang SJ. The influence of two bone agents (Tc-99m pyrophosphate and Tc-99m methylenediphosphonate) on quantitative sacroiliac joint scintigraphy. *Nucl Med Commun* 1996; 17: 1035–1038.
20. Imanishi Y, Mitogawa Y, Takizawa M, Konno S, Samura H, Ohsawa A, et al. Relapsing polychondritis diagnosed by Tc-99m MDP bone scintigraphy. *Clin Nucl Med* 1999; 24: 511–513.
21. Fukumoto S, Matsumoto T, Takebe K, Onaya T, Eto S, Nawata H, et al. Treatment of malignancy-associated hypercalcemia with YM175, a new bisphosphonate: Elevated threshold for parathyroid hormone secretion in hypercalcemic patients. *J Clin Endocrinol Metab* 1994; 79: 165–170.
22. Tsunematsu R, Saito T, Iguchi H, Fukuda T, Tsukamoto N. Hypercalcemia due to parathyroid hormone-related protein produced by primary ovarian clear cell adenocarcinoma: Case report. *Gynecol Oncol* 2000; 76: 218–222.
23. Koizumi M, Kobayashi M, Furukawa M, Yamashita T, Ogawa E. The bisphosphonate incadronate for bone metastases of breast cancer. *Int J Clin Oncol* 2000; 5: 241–246.
24. Wang TST, Fawwaz RA, Johnson LJ, Mojdehi GE, Johnson PM. Bone-seeking properties of Tc-99m carbonyl diphosphonic acid and monohydroxy-methylene phosphonic acid: concise communication. *J Nucl Med* 1980; 21: 767–770.