Nuclear Medicine and Biology 49 (2017) 1–7

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Preparation and evaluation of rhenium-188-pamidronate as a palliative treatment in bone metastasis

Mostafa Erfani [a,*], Nasim Rahmani [b], Alireza Doroudi [b], Mohammad Shafiei [a]

[a] Radiation Application Research School, Nuclear Science and Technology Research Institute (NSTRI), P.O.Box: 14395-836, Tehran, Iran
[b] School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, P.O.Box: 61357-33184, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 13 February 2017
Accepted 22 February 2017

Keywords:
^{188}Re-PAM
Pamidronic acid
Radiolabeling
Bone pain therapy

ABSTRACT

Objective: Rhenium-188-hydroxyethylidene diphosphonate (^{188}Re-HEDP) as a first generation bisphosphonate has been widely used for bone seeking radiopharmaceutical in cases of metastatic bone disease. No study has been yet reported on preparing a complex of ^{188}Re with pamidronate (3-aminohydroxypropylidene-1,1-bisphosphonic acid) (PMA) as a second generation bisphosphonate. Based on this fact, it was hypothesized that a bone-seeking ^{188}Re-PMA radiopharmaceutical could be developed as an agent for palliative radiotherapy of bone pain due to skeletal metastases.
Methods: Pamidronate was labeled with $^{188}ReO_4^-$ eluted from the alumina based ^{188}W/^{188}Re generator. Labeling was optimized, and radiochemical analysis was performed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Biodistribution of this radioconjugate was evaluated and verified further in mice.
Results: ^{188}Re-PMA was prepared successfully in a high labeling yield (˜95%) corresponding to a specific activity of 124 MBq/μmol and good in vitro stability, but it is likely to consist of multiple species. In biodistribution studies selective uptake and retention of activity in the skeletal system (0.81 ± 0.25% ID/g and 0.57 ± 0.16 at 4 and 48 h in bone post injection respectively) followed by clearance in the soft tissues were observed.
Conclusion: These results show that due to its biological capabilities it would be advantageous to use ^{188}Re-PMA for bone pain palliation therapy.

1. Introduction

In the advanced stages of breast and prostate cancers, bone is frequently affected by painful metastases. Metastatic disease poses significant problems for patients, their family and their quality of life. The major mechanism of pain from small metastases appears to be the stimulation of nerve endings in the endosteum by a variety of chemical mediators. Larger bone metastases produce stretching of the periosteum which leads to pain [1]. Autopsy studies showed that patients with advanced prostate cancer have a frequency of bone metastases of 65% to 85% [2].

Bisphosphonates have become the standard agents for treatment of malignant bone diseases. They are analogues of endogenous pyrophosphate and characterized by a P-C-P bond, which is resistant to enzymatic hydrolysis [3]. Most bisphosphonates contain a hydroxyl group at one position of the carbon giving high affinity binding to the calcium phosphate, and an essential determinate group with antiresorptive potency on the other position of the carbon. The earlier generations of these compounds like etidronate (HEDP) include short side chain. The second generation compounds like pamidronate have aliphatic chains of different lengths bearing terminal amino groups [4]. PMA is safe and more effective than HEDP in the treatment of cancer-related hypercalcemia [5]. In the treatment of osteolytic bone metastases in breast cancer and multiple myeloma, PMA helps reduce morbidity of bone metastases by inhibiting the accelerated bone resorption induced by the tumor [6].

Using suitable radionuclides linked to bone seeking ligands targeting radiotherapy is considered as a standard treatment in the management of bone metastasis, and found to be an effective treatment for the palliation of pain [7,8]. ^{99m}Tc complex of HEDP, a first generation biphosphonate, has been widely used as a radiopharmaceutical for bone scintigraphy in cases of metastatic bone diseases [9,10]. Similar chemical properties between technetium and rhenium made the development of a Rhenium-186 complex of HEDP as a bone seeking agent [11]. ^{186}Re-HEDP has been used in clinics to palliate the acute pain produced by cancer cells attacking the bone in cases of breast, prostate or other cancers [12–16]. Second generation bisphosphonates with an amine group side chain show higher affinity for bone minerals, and PMA, being one of this type, and using Rhenium radionuclide, could be chosen as a radioligand in developing bone pain palliation agents. $^{99m}Tc(CO)_3$-labeled pamidronate confirmed the overall adequate biological profile of the new radiotracers for bone imaging [17].

The properties of ^{188}Re that make it attractive for therapy including T1/2 = 16.9 h, Eβ(max) = 2.12 MeV, Eγ = 155 keV (15%, its availability from an ^{188}W/^{188}Re generator system with a long useful shelf life, and its chemical properties, similar to those of ^{99m}Tc, prompted study of ^{188}W/^{188}Re generator labeled compounds. Additionally, its

* Corresponding author.
E-mail address: mgandomkar@aeoi.org.ir (M. Erfani).

http://dx.doi.org/10.1016/j.nucmedbio.2017.02.001
0969-8051/

155-KeV γ-emission (15% abundance) makes imaging of its distribution possible to facilitate dose calculation. Also the short physical half-life of rhenium-188 allows for higher activity doses compared to longer-lived radionuclides [18]. The twofold higher beta particles energy of ^{188}Re compared to ^{186}Re provides a threefold longer maximum penetration range in tissues, 11 mm versus 3.6 mm respectively, made it more useful for treating larger tumors (>1 cm in diameter) or tumors in which there is incomplete targeting of radiolabeled compound to cancer cells [19,20]. A recent study about ^{188}Re tricarbonyl bisphosphonate complexes in a metastatic breast cancer cell line showed that the ^{188}Re complex could be considered an attractive candidate for further preclinical evaluation and systemic radionuclide therapy of bone metastases [21]. There are other reports that indicate therapeutic efficacy of ^{188}Re-HEDP in skeletal metastases [22–24]. In addition compared with ^{186}Re which emits γ-radiation 137 keV, 9%, ^{188}Re also emits γ-rays of 155 keV, 15% which produces better image qualities than ^{186}Re.

The limiting factor in radionuclide therapy for bone pain palliation is bone marrow toxicity, leading to decreasing of blood cells, specially platelet and leukocyte counts [25]. This toxicity is mainly related to high beta energy emitters that have been used for bone pain therapy. Due to this, the uses of radionuclides with low maximum beta energy are recommended by many researchers [26,27]. However in a comparative study performed by Liepe et al. no differences in the grade of bone marrow toxicity were found between the different investigated radiopharmaceuticals (^{188}Re-HEDP, ^{186}Re-HEDP,^{153}Sm-EDTMP and ^{89}Sr) [28].

Due to the specific advantages of using ^{188}Re in palliative radiotherapy of bone pain, the aim of this study was to develop ^{188}Re-PMA as a new bone seeking radiopharmaceutical. The present work describes the preparation of ^{188}Re-PMA complex, biological evaluation and imaging studies to estimate its efficacy as a novel bone pain palliation radiopharmaceutical.

2. Materials and methods

Pamidronate disodium and all other chemicals reagents were purchased from Sigma/Aldrich and used without further purification. $^{188}ReO_4^-$ was eluted from the alumina based $^{188}W/^{188}Re$ generator (Polatom, Poland) with saline solution (0.9% NaCl), and its activity was determined in a dose calibrator (Isomed, Germany). ITLC-SG chromatography paper (Agilent Technologies US) was used for radiochromatography. Labeling was monitored using an analytical reverse-phase high performance liquid chromatography (RP-HPLC) on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwavelength detector and a flow-through Raytest-Gabi γ-detector. CC 250/4.6 Nucleosil 120–5 C-18 column from Teknokroma was used for HPLC. Quantitative gamma counting was performed using an EG&G/ORTEC Model 4001 M Mini Bin & Power Supply NaI(Tl) counter.

2.1. ^{188}Re radiolabeling of PMA

A stock solution was prepared by dissolving PMA (concentration 30 mg/mL) in water. Samples of different amounts of PMA (2–20 mg), $SnCl_2.2H_2O$ (0.1–2.0) and ascorbic acid (0.5–5 mg) were combined in vials. Each vial was added 0.01–0.1 mg potassium perrhenate as a carrier and then was labeled by 370–3700 MBq of $^{188}ReO_4^-$ in 1 mL saline. Final pH was adjusted (pH = 1–9) by addition of 2 N HCl. The mixture was shaken vigorously for 30 s. The incubation is carried out in sealed container for 30 min at 95 °C.

2.2. Radiochemical analysis

The radiochemical purity of the ^{188}Re-PMA was determined by ITLC-SG strips developed in acetone and saline as mobile phases. The radioactivity was quantified by cutting the strips (1.5 × 10 cm^2) into 1 cm pieces and counting in a well type gamma counter. With the acetone as an eluting solvent, $^{188}ReO_4^-$ migrates with the solvent front, while the colloidal impurity $^{188}ReO_2$ and ^{188}Re-PMA both remain at the origin of the TLC strip. With the water as an eluting solvent, the colloidal impurity $^{188}ReO_2$ remains at the origin, while $^{188}ReO_4^-$ and ^{188}Re-PMA moves with the solvent front.

To verify the complex formation and likely multiple species, reaction mixture was analyzed by monitoring the elution profile in the gradient HPLC system after incubation at room temperature for 24 h. Triethylammonium phosphate (TEAP) 0.05 M and pH = 2.25 (solvent A) and methanol (solvent B) were used as a mobile phase in the following gradient: 0–5 min 100% A, 5–6 min 0–25% B, 6–9 min 25–34% B, 9–20 min 34–100 B, 20–30 min 100% B, 30–31 min 100% B to 100% A, 31–35 min 100% A, flow rate = 0.5 mL/min.

2.3. Serum stability

Stability of ^{188}Re-PMA was evaluated in saline and human serum. Aliquots were taken out at 1, 4, 6, 12 and 24 h post reconstitution at room temperature and analyzed by ITLC strip. 100 μl of labeled formulation was added to 1 ml of freshly prepared human serum, and the mixture was incubated in a 37 °C environment. 100 μl aliquots were removed at the different time points (1, 4, 6, 12, 24 h after reaction) and treated with 100 μl of ethanol. Samples were centrifuged for 5 min at 3000 rpm to precipitate serum proteins. Supernatants were removed for both activity counting in a γ-well counter, and chromatography performing to determine serum stability for radiocomplex. The sediments were counted after washing twice with 1 mL of EtOH, and the activity in the supernatant was compared with the activity in the sediment in order to get the percentage of radiocomplex bound to proteins.

2.4. Lipophilicity

For lipophilicity determination, 0.1 mL of the ^{188}Re-PMA was mixed with 1 mL of octanol and 1 mL of water in a 3 mL micro tube at pH value of 7.0. The tube was vigorously vortexed over a period of 5 min and centrifuged for 5 min at 500×*g*. Then, three aliquots of 50 μL of each phase were taken for radioactivity counting. The partition coefficient (log Po/w) was calculated by dividing the net radioactivity counting of the organic phase by that of the aqueous phase. The results presented are the means of three determinations (with SD of <0.01).

2.5. Biodistribution studies

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. A group of three male white Swiss albino mice (30–35 g, 8 week) received 3.7 MBq of ^{188}Re-PMA in volume of 100 μl intravenously via a tail vein. The mice were sacrificed at different post injection times (1, 2, 4 and 24 h) and the tissues and organs of interest were collected, immediately weighed and counted in a NaI well-type γ-counter. Blood was taken from the heart in a pre-weighted 1 ml syringe, activity was measured and the syringe was reweighed. Subsequently, percentage uptake of the ^{188}Re-PMA in one gram of the bone, blood, heart, lung, stomach, intestine, thyroid, liver, spleen, kidney of mice were calculated as the percentage of the injected dose per gram tissue (%ID/g tissue). The total counts injected per animal were determined by extrapolation from counts of an aliquot taken from the injected solution as a standard.

3. Results and discussion

3.1. Radiolabeling

^{188}Re-PMA was prepared via the reduction of $^{188}ReO_4^-$ by addition of an aqueous solution of stannous chloride and then its subsequent