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Development of superior bone scintigraphic agent from a series of ^{99m}Tc-labeled zoledronic acid derivatives

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ABSTRACT

Two novel zoledronic acid (ZL) derivatives, 1-hydroxy-4-(1H-imidazol-1-yl)butane-1,1-diyldiphosphonic acid (IBDP) and 1-hydroxy-5-(1H-imidazol-1-yl)pentane-1,1-diyldiphosphonic acid (IPeDP), were prepared and labeled with the radionuclide technetium-99m in a high labeling yield. In vitro stabilities of these radiolabeled complexes were measured by the radio-HPLC analysis as a function of time, which showed excellent stability with the radiochemical purity of over 95% at 6 h post preparation. Their in vivo biological performances were evaluated and compared with those of ^{99m}Tc-ZL and ^{99m}Tc-MDP (methylenediphosphonic acid). The biodistribution in mice and scintigraphic images of the rabbit showed that the tracer agent ^{99m}Tc-IPeDP had highly selective uptake in the skeletal system and rapid clearance from the blood and soft tissues and an excellent scintigraphic image can be obtained in a shorter time post injection with clear visualization of the skeleton and low soft tissue activity. These preclinical studies suggest that ^{99m}Tc-IPeDP would be a novel superior bone scintigraphic agent.

1. Introduction

Technetium-99 m, with its excellent physical characteristics (E_γ=142 keV, $t_{1/2}$=6.02 h) and easy availability from a generator, has become the most important nuclide for organ imaging in nuclear medicine (Dilworth and Parrott, 1998; Jurisson and Lydon, 1999; Pauwels and Stokkel, 2001; Méndez-Rojas et al., 2006; Bartholom et al., 2010). In the past few years, several ^{99m}Tc-labeled diphosphonate compounds have been developed for skeletal imaging in attempts to diagnose various pathological conditions, which usually result in widespread metastases to the skeletal system, such as neoplastic diseases, metabolic disorders, infections and cancers (El-Mabhouh et al., 2006; Mari et al, 1999; Palma et al., 2007; Shigematsu et al., 2002). Some agents have proven useful in bone imaging and provide an effective means for diagnosing primary bone cancer, bone metastases, bone trauma, Paget's disease, etc. For example, ^{99m}Tc-labeled methylenediphosphonate (MDP) and hydroxymethylenediphosphonate (HMDP) have been widely used for many years as radiopharmaceuticals for bone scintigraphy (Subramanian et al., 1975; Domstad et al., 1980).

In order to achieve images of high definition, however, with these ^{99m}Tc-labeled diphosphonates (DPs) an interval of 2–6 h is needed normally between injecting the agent into the patient and performing the bone scanning (Love et al., 2003). Therefore, reducing this interval is highly desirable which could lead to worthwhile increases in the convenience to patients and physicians and in the efficiency of running the nuclear medicine units. To enable imaging at an earlier time post injection, a radiopharmaceutical with higher affinity for bone, larger uptake ratios of bone-to-soft tissues and more rapid clearance from the blood and soft tissues (such as muscle, liver and kidney) are required accordingly (Ogawa et al., 2006).

The nature of the ligand (diphosphonic acid) becomes a key factor in determining the characteristics and advantages of the radiopharmaceuticals. zoledronic acid and its derivatives have a variety of important therapeutic uses in patients with two main types of disorders, such as ectopic calcification and ossification (Rogers et al., 2000; Green, 2004; Marma et al., 2007); they also apply as ligands for radiometals in bone-seeking diagnostic and therapeutic agents (Asikoglu and Durak, 2009; Majkowska et al., 2009). Recently, several novel complexes of the radionuclide-labeled third-generation DPs have been prepared and reported (Asikoglu and Durak, 2009; Majkowska et al., 2009; Shigematsu et al., 2002).

In a continuing effort to search better bone-imaging agents, we have started to optimize the linker chain between the imidazolyl and geminal diphosphonate group in the ZL (Lin et al., 2011). It was found that when the number of the methylene chains were extended from one to two (i.e., from ^{99m}Tc-ZL to ^{99m}Tc-IPrDP), there was a significant influence on biological properties. Encouraged by these primary

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studies, we decided to further investigate about further extending the number of the methylene chains between the imidazolyl and geminal diphosphonate groups (see Table 1), in an attempt to develop a superior ^{99m}Tc-labeled diphosphonate bone scintigraphic agent with possible imaging as early as 1 h post injection. Thus, in the present work, two novel ZL derivatives (IBDP and IPeDP) with different lengths of the linker chain were synthesized and labeled successfully with the radionuclide ^{99m}Tc. In vitro and in vivo biological performances of them were investigated systematically and compared with those of ^{99m}Tc-MDP, ^{99m}Tc-ZL as well as ^{99m}Tc-IPrDP.

2. Experimental

2.1. Reagents, instruments and animals

All analytical chemical reagents employed were purchased from commercial sources and used without further purification. $Na^{99m}TcO_4$ was supplied by Jiangsu Institute of Nuclear Medicine. Ketamine hydrochloride injection and diazepam injection were purchased from Jiangsu Hengrui Medicine Co. Ltd. and Jiangsu Jumpcan Pharmaceutical Co. Ltd., respectively. Melting point (m.p.) was determined on Yanaco MP-500 melting point apparatus (Japan). Elemental analysis was carried out using an Elementar Vario EL III analyzer. Electron spray ion mass spectra (ESI-MS) were measured using a Waters Platform ZMD4000 LC/MS. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DRX-500 spectrometer, and the chemical shifts were given relative to the internal tetramethylsilane (TMS). The Packard-multi-prias γ Counter (USA) and Philips SKYLight emission computed tomography (ECT) (USA) were used in the radioactivity counting and bone scanning, respectively.

High performance liquid chromatography (HPLC) was equipped with a Waters 1525 Binary HPLC pump, a Waters 2487 dual λ absorbance detector and a Perkin Elmer Radiomatic 610TR radioactivity detector, which were operated by Breeze and proFSA software. HPLC analyses of $Na^{99m}TcO_4$ and the complexes were performed on a SinoChrom ODS-BP C18 reversed-phase column (10 μm, 250 × 4.6 mm^2). The flow rate was 0.9 mL/min, and the mobile phase was isocratic with 70% solvent A (1% acetic acid in water) and 30% solvent B (acetonitrile).

Normal institute of cancer research (ICR) mice (weighing 18–20 g) and New Zealand rabbits (weighing 1.7–1.8 kg) were supplied by Shanghai SLAC Laboratory Animal Co. Ltd. The animal experiments in this work were approved by the Animal Care and Ethics Committee of Jiangsu Institute of Nuclear Medicine.

2.2. Synthesis of diphosphonic acid

Diphosphonic acids (**4a–4d**) were synthesized according to the procedure outlined in Scheme 1, where **4a** and **4b** had been reported previously (Lin et al., 2011; Widler et al., 2002).

1-Hydroxy-4-(1H-imidazol-1-yl)butane-1,1-diyldiphosphonic acid (*IBDP*, **4c**). Yield: 62%; m.p.: 194–196 °C; ^{1}H NMR (500 MHz, D_2O): δ7.46 (s, 1H, CH-ring), 6.96 (d, 1H, CH-ring), 6.74 (d, 1H, CH-ring), 3.74 (t, 2H, N–C$\underline{H}_2$), 1.82 (t, 2H, C$\underline{H}_2$COH) and 1.60 (q, 2H, CH_2CH_2COOH) and ESI-MS, m/z (%): 300 = *M*, 299 = *M* − *H*. Anal. calculated for $C_7H_{14}N_2O_7P_2$ (%): C, 28.01; H, 4.70; N, 9.33; found (%): C, 29.12; H, 4.82; N, 9.51.

1-Hydroxy-5-(1H-imidazol-1-yl)pentane-1,1-diyldiphosphonic acid (*IPeDP*, **4d**). Yield: 72%; m.p.: 182–185 °C; ^{1}H NMR (500 MHz, D_2O): δ7.46 (s, 1H, CH-ring), 7.44 (d, 1H, CH-ring), 7.37 (d, 1H, CH-ring), 4.19 (t, 2H, N–CH_2), 1.91 (t, 2H, CH_2COH), 1.85 (m, 2H, ring-CH_2CH_2), 1.55 (m, 2H, ring-$CH_2CH_2CH_2$) and ESI-MS, m/z (%): 314 = *M*, 313 = *M* − *H*. Anal. calculated for $C_8H_{16}N_2O_7P_2$ (%): C, 30.58; H, 5.13 and N, 8.92; found (%): C, 31.62; H, 5.32 and N, 9.05.

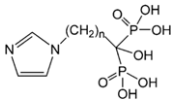
2.3. Radiolabeling of ^{99m}Tc-DPs

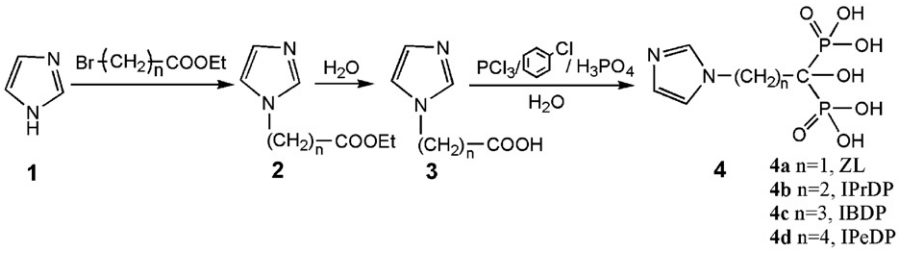
In the presence of the reducing agent stannous chloride, the solution of DPs (**4**) and the freshly eluted pertechnetate were mixed to prepare the complex ^{99m}Tc-DPs (**5**).

2.4. In vitro stability

To evaluate the in vitro stability of ^{99m}Tc-DPs, 3.7 MBq freshly prepared ^{99m}Tc-labeled compounds in 100 μL water were diluted with 0.1 M phosphate buffered saline (PBS, pH = 7.0), and the solutions were incubated at 37 °C. At different time intervals post incubation (1–6 h), the RCP was measured and analyzed by the radio-HPLC to determine the stability of these complexes.

Table 1
Structures of zoledronic acid derivatives.

Structure	*n*	Generic name	Abbreviation
	1	1-hydroxy-2-(1H-imidazol-1-yl)ethane-1,1-diyldiphosphonic acid	ZL
	2	1-hydroxy-3-(1H-imidazol-1-yl)propane-1,1-diyldiphosphonic acid	IPrDP
	3	1-hydroxy-4-(1H-imidazol-1-yl)butane-1,1-diyldiphosphonic acid	IBDP
	4	1-hydroxy-5-(1H-imidazol-1-yl)pentane-1,1-diyldiphosphonic acid	IPeDP



Scheme 1. Synthesis of zoledronic acid derivatives.