

Pharmacokinetic and imaging evaluation of ^{99m}Tc -HBIDP as a potential bone imaging agent

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Abstract: Developing novel superior bone-seeking radiopharmaceutical for the detection of malignant bone lesions could further improve the diagnostic value of routine bone scanning and shorten the interval between injection and imaging. In order to further evaluate the bone imaging efficiency of ^{99m}Tc -HBIDP (1-hydroxy-2-(1-butyl-imidazol-2-yl)-ethane-1,1-diphosphonic acid), the pharmacokinetic in mice and single photon emission computed tomography (SPECT) bone scanning in rabbit for ^{99m}Tc -HBIDP was investigated. Kinetics of blood clearance showed that the distribution half-life ($T_{1/2\alpha}$) and elimination half-life ($T_{1/2\beta}$) of ^{99m}Tc -HBIDP are 2.73 and 24.87 min, respectively. Excellent bone images can be obtained at 1 h post injection with SPECT bone scanning, which is clearer and quicker than ^{99m}Tc -ZL (zoledronate) and ^{99m}Tc -MDP (methylenediphosphonate). All results indicate that ^{99m}Tc -HBIDP holds great potential as a novel improved bone imaging agent.

Keywords: ^{99m}Tc -HBIDP, Bone imaging agent, pharmacokinetic, SPECT.

INTRODUCTION

There is abundant hydroxyapatite (HA) in bone tissues, which is attractive to use HA as a target for bone selective drug delivery. Since diphosphonates (DPs) exhibit high affinities for calcified matrices such as HA (Jung *et al.*, 1973), DPs-containing drugs attract much attention. Zoledronic acid (ZL), which is the most potent member of the diphosphonates, is currently used in preclinical models of bone resorption. The research results indicate that ZL is at least 100 times more potent than either clodronate or pamidronate, what is more, at least 1000 times more potent than etidronate (Smith 2008).

In the field of nuclear medicine, a number of ^{99m}Tc -labeled DPs, such as ^{99m}Tc -MDP (Subramanian *et al.*, 1975), ^{99m}Tc -HMDP (Bevan *et al.*, 1980) and ^{99m}Tc -EHDP (Subramanian *et al.*, 1972), have been widely used for many years in bone scanning and provided an effective means of diagnosing primary bone cancer, metastatic bone disease, osteoporosis, bone trauma, etc (Paes and Serafini, 2010; Lewington, 2005; Pandit-Taskar *et al.*, 2004). In spite of the well-established clinical use as diagnostic agents, ^{99m}Tc -labeled DPs also present a set of clinical and chemical limitations, such as the recognized low specificity and relatively slow clearance from the blood and soft-tissues (Vasireddy *et al.*, 2003). To enable imaging at an earlier time after injection, a radiopharmaceutical with higher affinity for bone, lower tissue uptake and more rapid clearance from the blood is required (Ogawa *et al.*, 2006).

In the past few years, we have prepared a series of ^{99m}Tc -labeled DPs with well *in vivo* biological properties (Lin *et*

al., 2010; Wang *et al.*, 2011; Lin *et al.*, 2011; Qiu *et al.*, 2011; Qiu *et al.*, 2012; Qiu *et al.*, 2013). It is worthy to note that optimization of the alkyl substituent (R_1) in the imidazole ring (Scheme 1) can bring significant influences on the biological properties of ^{99m}Tc -labeled complexes, including the bone uptake, blood and soft-tissue clearance (Lin *et al.*, 2010; Wang *et al.*, 2011; Qiu *et al.*, 2012). Moreover, substituents at different positions in the imidazole ring of ZL may be another important factor for the biological properties of the compounds (Qiu *et al.*, 2012). Furthermore, as previous studies demonstrated, ^{99m}Tc -HBIDP displayed a highly selective uptake in the skeletal system and rapid clearance from the soft tissues (Wang *et al.*, 2012). Based on this, for a more in-depth study of the imaging properties of this radiotracer, the pharmacokinetics studies in mice and single photon emission computed tomography (SPECT) bone scanning in rabbit of ^{99m}Tc -HBIDP were continue investigated and reported herein.

MATERIALS AND METHODS

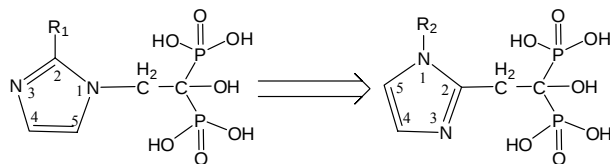
Materials

HBIDP and ^{99m}Tc -HBIDP were synthesized according to the previous work of our group (Wang *et al.*, 2012). A Packard-multi-prias γ Counter (Perkins Elmer, U.S.A) were used for pharmacokinetic study. Philips SKY Light emission computed tomography (ECT) (Philips, U.S.A) was used for bone imaging of rabbit. Institute of Cancer Research (ICR) mice (17-23g) and New Zealand rabbit (1.4-1.8kg) were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China). The animal experiment in this study was approved by the Animal Care and Ethics Committee of Jiangsu Institute of Nuclear Medicine.

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Pharmacokinetic study

About 7.4 MBq of ^{99m}Tc -HBIDP was injected through the tail vein into five mice for blood kinetic test. Blood samples were drawn from each mouse through the lateral tail vein by micro liter pipette (20 μL) at several time points between 5 min and 6 h after injection. The blood samples were measured for radioactivity by γ counter, and the radioactivity was expressed as a percentage of the injected dose per gram of blood ($\text{ID}\%/\text{g}$) (Zhou *et al.*, 2012). The data were analyzed using 3P97 pharmacokinetic software (Xue *et al.*, 1997) to fit the appropriate compartment model.



Scheme 1: ZL derivative with substituent at different imidazolyl positions (HBIDP, R_2 =butyl)

Bone imaging of rabbit

In order to better evaluate the whole body localization of ^{99m}Tc -HBIDP, SKY Light ECT was used to study the bone imaging in rabbit. Before imaging procedure, the rabbits were anesthetized with 8 mL 25% ethyl carbamate solution. When the rabbit can not move anymore, they were fixed on board. Then ^{99m}Tc -HBIDP (1.5 mL, 92.5 MBq) was injected intravenously into the rabbit through the marginal ear vein and bone scanning was carried out subsequently with the Philips SKY Light ECT. The whole body image was observed for 4 h. During the first hour, scanning images were collected every 5 min. Regions of interest (ROIs) were obtained on the SPECT composite image including bone, muscle, heart and liver. All subsequent images were kept constant shapes and sizes of ROIs, and the uptake ratios of bone to soft tissues (heart, liver and muscle) were calculated from it. Then, a series of static bone scanning images were collected at 1, 2, 3 and 4 h, respectively.

RESULTS**Pharmacokinetic of ^{99m}Tc -HBIDP**

The time-activity curve of radioactivity in blood of mice for ^{99m}Tc -HBIDP during 6 h post injection follows a double exponential curve (fig. 1), with $C = 8.655e^{-0.254t} + 5.106e^{-0.028t}$. A two-compartment model was used for the estimating of pharmacokinetic parameters. As shown in table 1, the distribution half-life ($T_{1/2\alpha}$) and elimination half-life ($T_{1/2\beta}$) of ^{99m}Tc -HBIDP were 2.73 and 24.87 min, respectively, with a total clearance rate of 1.70% $\text{ID}/\text{g}/\text{min}$.

Bone imaging of rabbit

Bone imaging efficiency and tissue clearance of ^{99m}Tc -HBIDP were evaluated by recording bone scanning

images of rabbit after the intravenous administration in the first hour. As shown in fig. 2, ^{99m}Tc -HBIDP mainly accumulated in the skeleton, kidney and urinary bladder. After injecting 30 min, a clear image of the rabbit skeleton can be obtained. Moreover, it can also learn from the dynamic images that ^{99m}Tc -HBIDP was metabolized through kidney. Noteworthy, the uptake of heart, liver and spleen are high at 5 min, but the clearance is very fast.

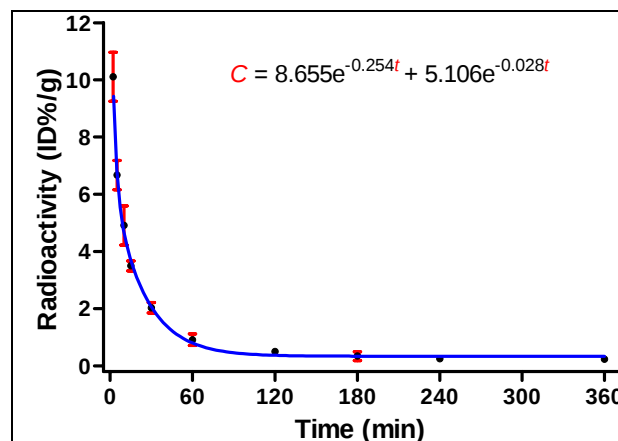


Fig. 1: Pharmacokinetic curve in mice for ^{99m}Tc -HBIDP (mean $\pm$ SD, $n = 5$, %ID/g)

For better comparison, time-uptake ratios of bone to heart, liver and muscle within the dynamic imaging procedure are shown in fig. 3 for ^{99m}Tc -HBIDP, ^{99m}Tc -ZL and ^{99m}Tc -MDP, respectively (Lin *et al.*, 2011). It is clear that the bone-to-muscle uptake ratios of ^{99m}Tc -HBIDP at 15, 35, and 55 min were 6.87, 9.87, and 10.91, respectively, whereas those of ^{99m}Tc -ZL and ^{99m}Tc -MDP are 3.33, 4.45, 4.89, and 5.17, 8.86, and 9.62, respectively.

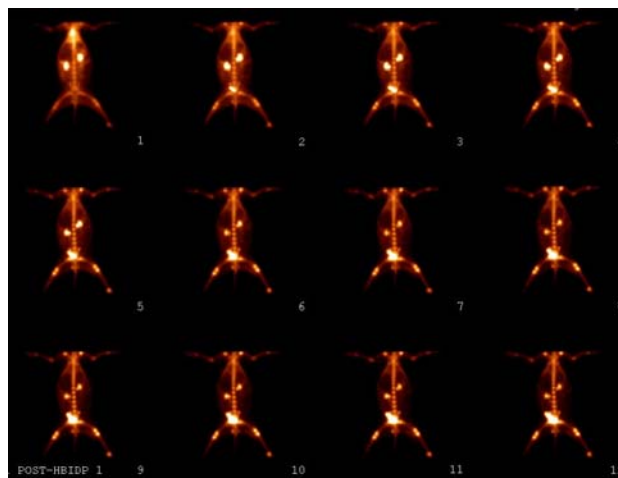


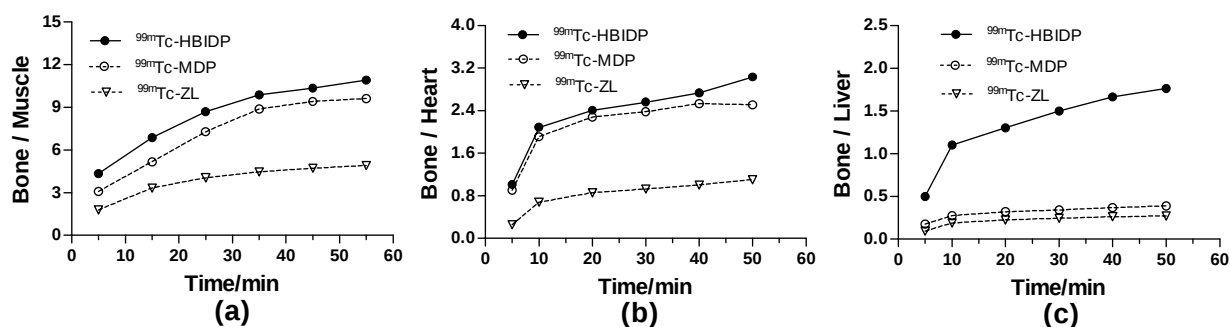
Fig. 2: Dynamic images of rabbit bone scanning in 1 h post injection of ^{99m}Tc -HBIDP.

Fig. 4 shows the static images of the rabbit bone scanning after intravenous administration of ^{99m}Tc -HBIDP, ^{99m}Tc -ZL and ^{99m}Tc -MDP (Lin *et al.*, 2011) from 1 to 4 h. From these images, we can see that all radiotracers are excreted

Table 1: Pharmacokinetics parameters of ^{99m}Tc -HBIDP calculated by bi-exponential equation

Parameter	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	K_e (min^{-1})	K_{12} (min^{-1})	K_{21} (min^{-1})	CL (%ID/g/min)
Values	2.729	24.871	0.063	0.107	0.112	1.70

$T_{1/2\alpha}$, distribution half-life; $T_{1/2\beta}$, elimination half-life; K_e , elimination rate constant; K_{12} , rate constant of central compartment to peripheral compartment; K_{21} , rate constant of peripheral compartment to central compartment; CL, total clearance rate.

**Fig. 3:** Time-ratio curves of ^{99m}Tc -HBIDP, ^{99m}Tc -MDP and ^{99m}Tc -ZL in the bone/muscle, heart and liver within the dynamic imaging procedure.

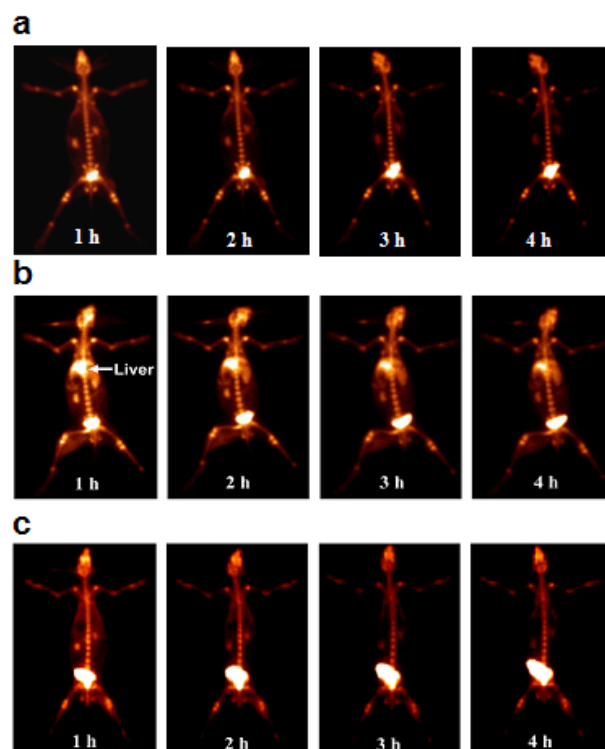
from the kidney, which is similar to the dynamic images studying result. In addition, the highest selective skeletal uptake is seen at different time intervals post injection of ^{99m}Tc -HBIDP.

DISCUSSION

^{99m}Tc -HBIDP was obtained according to our reported method with high labeling efficiency and the radiochemical purity was larger than 95% in 6 h, which indicated that it was stable enough to allow further pharmacokinetic and imaging studies. As can be seen from the pharmacokinetic results, the total blood clearance rate (1.70 %ID/g/min) was higher than those of ^{99m}Tc -labeled zoledronic acid derivatives (Lin *et al.*, 2012). It is obvious that ^{99m}Tc -HBIDP has a rapid blood clearance rate embodying in quickly absorbed and eliminated in blood (Liu *et al.*, 2009). In general, it will contribute to shorten the interval between injection and bone imaging for radiopharmaceutical with quicker clearance from soft tissues. Consequently, the designed tracer ^{99m}Tc -HBIDP was expected to be more suitable for clinical use as a novel bone imaging agent for its enabling imaging at an earlier time after injection.

As mentioned above, ^{99m}Tc -HBIDP mainly accumulated in the skeleton, kidney and urinary bladder and metabolized through kidney with quick imaging of the rabbit skeleton after 30 min post injecting. This reveals that ^{99m}Tc -HBIDP has highly selective bone uptake and rapid clearance from soft tissues in the rabbit and more satisfy for imaging, which agrees well with the biodistribution studies in mice of our previous study (Wang *et al.*, 2012). It displays some advantages not only in the bone-to-muscle uptake ratio but also the uptake ratios of bone to other soft tissues, such as heart and liver,

compared with ^{99m}Tc -ZL and ^{99m}Tc -MDP. As well-known, the high uptake ratios of bone to soft tissues are very important for obtaining a good quality of the skeletal imaging. However, utilizing a bone pathology model to compare the bone lesion with the normal bone uptake will be necessary to more fully assess the further clinical utility of ^{99m}Tc -HBIDP.

**Fig. 4:** Static images of rabbit bone scanning after injection of (a) ^{99m}Tc -HBIDP, (b) ^{99m}Tc -ZL and (c) ^{99m}Tc -MDP from 1 to 4 h.

CONCLUSIONS

Pharmacokinetic and bone imaging studies of ^{99m}Tc -HBIDP show that the radiotracer ^{99m}Tc -HBIDP washed out quickly from the blood and excellent scintigraphic images of the rabbit skeleton can also be quickly obtained, which was faster than ^{99m}Tc -ZL and the clinical widely used bone imaging agent ^{99m}Tc -MDP. This may shorten the interval and lessen the burden on patients in terms of the total time of examination and the dose of radiation absorbed. In the present case, ^{99m}Tc -HBIDP did reveal attractive biological features as a superior bone scanning agent, justifying further investigations in animals and humans with bone pathology.

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