Preparation, Characterization, and Preliminary Imaging Study of \[^{188}\text{Re}]\text{Re}-\text{Ibandronate as a Novel Theranostic Radiopharmaceutical for Bone Metastasis}

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Background. Bone is a common site of metastasis from a malignant tumor. Several radiopharmaceuticals are available to relieve bone pain in patients with cancer. However, every radiopharmaceutical has its own disadvantages, and there is still a need to investigate easily accessible and high bone affinity radiopharmaceuticals. Ibandronate (IBA) and \[^{188}\text{Re}]\text{Re}\) were used for radiolabeling to develop and evaluate a novel type of bone-seeking radiopharmaceutical. Methods. The preparation conditions of \[^{188}\text{Re}]\text{Re}\)-IBA were investigated, and thin-layer chromatography was used to analyze radiochemical purity. The stability, plasma protein binding rate, lipid-water distribution coefficient, safety and biodistribution in normal mice, and bone imaging of \[^{188}\text{Re}]\text{Re}\)-IBA in New Zealand rabbits were studied. In addition, the nude mice model of bone metastasis was established, and biodistribution and imaging characteristics of \[^{188}\text{Re}]\text{Re}\)-IBA in these nude mice were studied. Results. \[^{188}\text{Re}]\text{Re}\)-IBA was successfully prepared with radiochemical purity >95%. The optimum preparation conditions were as follows: IBA, 0.8–1.4 mg; ascorbic acid, 0.2–0.5 mg; stannous chloride, 0.14–0.18 mg; potassium perrhenate, 0.005 mg; and \[^{188}\text{Re}]\text{Re}\O\text{4}^{-}\ activity, 18.5–296 MBq, reacted for 30 min at pH 2. \[^{188}\text{Re}]\text{Re}\)-IBA demonstrated good stability, high plasma protein binding rate, good hydrophilicity, and low toxicity. The biodistribution and bone imaging in normal animals showed rapid blood clearance, high bone uptake, low uptake in the solid organs and soft tissue, and high contrast during imaging. The biodistribution and imaging of bone metastasis in nude mice showed that \[^{188}\text{Re}]\text{Re}\)-IBA has higher uptake in bone metastasis lesions than normal bone. Conclusions. Our study encompassed the successful preparation of \[^{188}\text{Re}]\text{Re}\)-IBA, a novel bone-seeking radiopharmaceutical, and confirmed it has potential in the treatment of bone metastasis and monitoring through imaging.
radiopharmaceuticals are available to relieve bone pain in patients with cancer, including \[^{90}Sr\]SrCl\(_2\), \[^{153}Sm\]Sm\-methyleneaminetetramethylene phosphonate (EDTMP), \[^{186}Re\]Re-bisphosphonate hydroxyethylenediphosphonate (HEDP), \[^{186}Re\]Re-HEDP, \[^{177}Lu\]Lu-EDTMP, and \[^{223}Ra\]RaCl\(_2\) [4, 5]. However, every radiopharmaceutical has its advantages and disadvantages. The choice is extremely dependent on the status of the patient, such as the renal function, bone marrow reserve, cancer extent, and physical properties of the radionuclides [6]. As the commercial availability of radiopharmaceuticals is limited, the availability of each radiopharmaceutical also needs to be considered. Moreover, most radionuclides used for treatment are produced through reactors; thus, they are extremely expensive. Therefore, it is important to choose an easily accessible and cost-effective radiopharmaceutical. Radioisotope \(^{188}\)Re has an advantage in this regard because of its commercial extraction from \(^{188}\)W/\(^{188}\)Re generators, which can be used on-demand and are cost-effective. \(^{188}\)Re has a physical half-life of 16.9 h and can produce \(\beta^-\) rays with a maximum energy of 2.1 MeV which can be used for treatment [7]. It also emits \(\gamma\) rays with an energy of 155 keV for imaging, which facilitates visualizing the distribution of radioactive tracers in the body during treatment [7]. \[^{188}\)Re-HEDP is one of the most widely studied bisphosphonate radiopharmaceuticals in nuclear medicine which can relieve bone pain caused by prostate cancer, breast cancer, or other tumors [8]. In addition, a variety of new \(^{188}\)Re labeled bisphosphonates have been synthesized, such as \[^{188}\)Re-diethylenetriamine-N,N,N',N"-pentakis acid [9], \[^{188}\)Re-nitriolotris [10], \[^{188}\)Re-ethylenediamine-N,N,N,N-tetras acid [11], \[^{188}\)Re-epamidronate (PMA) [6], \[^{188}\)Re-zoledronate (ZNA) [12], and \[^{188}\)Re-risedronate [13]. These drugs show potential for application in preclinical studies. It is still necessary to identify bisphosphonates with a stronger ability to target bones for \(^{188}\)Re labeling. Bisphosphonates are analogues of endogenous pyrophosphates, characterized by P-C-P bonds. There is a hydroxyl group attached to one side of the carbon atom, which has a high affinity for calcium phosphate, the primary mineral of the bone [6]. Moreover, there is a sidechain structure attached to the other side of the carbon atom that inhibits bone resorption [6]. The side-chain structure of the first-generation bisphosphonate does not contain nitrogen, and HEDP is one of its representative drugs. The second-and third-generation bisphosphonates contain nitrogen, and their ability to inhibit bone resorption is significantly stronger. Furthermore, third-generation bisphosphonates also contain a heterocyclic structure, and their bone affinity and ability to inhibit bone resorption are significantly stronger. However, nitrogen-containing bisphosphonates may have significant side effects, including renal failure, hypocalcemia, and osteonecrosis of the jaw [14]. The choice of drugs with lower toxicity is an important factor in determining the treatment for patients with renal insufficiency. Ibandronic acid and zoledronic acid are the most powerful and widely used third-generation bisphosphonates. The studies in [15, 16] on the effects of the aforementioned bisphosphonates on renal safety have reported on the possible occurrence of nephrotoxicity while using zoledronic acid. Nonetheless, the nephrotoxicity of ibandronic acid is extremely low and equivalent to that of the placebo. Another study [17] conducted on 44 patients treated with ibandronate (IBA) reported no impairment of renal function during an average follow-up of 18.5 months. In addition, Han et al. [18] noted that the pain relief rate and improvement in the quality of life in patients with bone tumors were higher in the ibandronic acid group than in the zoledronic acid group \((P < 0.05)\). Nonetheless, the rate of adverse reactions was lower in the ibandronic acid group than in the zoledronic acid group \((P < 0.05)\). There have been no reports on \(^{188}\)Re labeled with IBA. Therefore, IBA and \(^{188}\)Re were selected for radiolabeling to develop and evaluate a new radiopharmaceutical with potential bone-seeking properties and low toxicity, which may contribute to individualized treatment in the era of precision medicine. Light will be shed on the preparation, optimization of conditions, biological and safety evaluation, and imaging studies of \[^{188}\)Re-IBA.

2. Materials and Methods

2.1. Materials. \[^{188}\)Re\]NaReO\(_4\) was eluted from the alumina based \(^{188}\)W/\(^{188}\)Re generator (OncoBeta, Germany) with saline solution (0.9% NaCl). IBA was purchased from Twbio Technology Co., Ltd., Beijing, China. Ascorbic acid, potassium perrhenate (KReO\(_4\)), and stannous chloride (SnCl\(_2\)) were purchased from Macklin Biochemical Co., Ltd., Shanghai, China. Xinhua No. 1 chromatography paper (Xinhua Paper Industry Co., Ltd., Hangzhou, China) was used for paper chromatography (PC). Other equipment, chemicals, and animals used in the experiment were provided by the Nuclear Medicine and Molecular Imaging Key Laboratory of Sichuan Province. All studies were approved by the Ethics Committee of Southwest Medical University.

2.2. Methods

2.2.1. Radiolabeling and Quality Control. Various reaction parameters were studied by using the control variables method (changing one parameter at a time) to determine the effects of IBA (Figure 1), the antioxidant (ascorbic acid), carrier (KReO\(_4\)), reducing agent (SnCl\(_2\)), \[^{188}\)Re\]ReO\(_4\) activity, pH, temperature, and reaction time on the radiochemical purity (RCP) of \[^{188}\)Re-IBA. First, 0.1–1.8 mg, 0–0.5 mg, 0.02–0.4 mg, and 0–0.019 mg of IBA, ascorbic acid, SnCl\(_2\), and KReO\(_4\) were mixed sequentially. Then, the fresh eluted \[^{188}\)Re\]ReO\(_4\) solution 18.5–444 MBq was added. Subsequently, the pH value was adjusted to 0.5–4 with 1 N sodium acetate solution and 1 N hydrochloric acid. The reaction occurred at room temperature (25 ± 2°C), 60°C, or 95°C for 10–60 min, respectively. After the reaction was completed, the solution was cooled to room temperature (25 ± 2°C). The pH value of each tube was adjusted to 6–7. An aseptic filter membrane of 0.22 \(\mu\)m was used for sterilization and filtration. In this study, the specific fixed values of all parameters were as follows: IBA, 1.0 mg; ascorbic acid,
0.3 mg; SnCl₂, 0.14 mg; KReO₄, 0.005 mg; and [¹⁸⁸Re]ReO₄⁻ activity, 37 MBq, reacted for 30 min at 95°C and pH = 2. The RCP of [¹⁸⁸Re]Re-IBA was determined by PC. Acetone and 0.9% NaCl were used as the eluents. A TLC scanner was used to measure the distribution of radioactivity on the TLC strips. The RCP of [¹⁸⁸Re]Re-IBA was calculated from the peak area measurements as follows:

\[ \text{RCP} = 100\% - \left( \frac{\text{[¹⁸⁸Re]ReO₄}^- + \% \times 100\%}{\% \times 100\%} \right) \]

2.2.2. In Vitro Stability. Freshly prepared [¹⁸⁸Re]Re-IBA was incubated in 0.9% NaCl and fresh human serum at 37°C. The RCP of the tubes was determined by PC at 1 h, 3 h, 6 h, 12 h, and 24 h. The experiment was repeated thrice. The results are expressed as mean ± standard deviation (X ± S).

2.2.3. Plasma Protein Binding Rate. 0.1 mL fresh human plasma and freshly prepared 1.85 MBq [¹⁸⁸Re]Re-IBA were added in a tube labeled A and incubated at 37°C for 2 h. Then, 25% trichloroacetic acid solution (1 mL) was added to tube A and centrifuged under a centrifugal force of 587 g for 5 min. The supernatant was collected into another tube labeled B. Centrifugation was repeated, and the supernatant was collected thrice. A γ counter was used to measure the radioactivity counts of tubes A and B. After repeating the experiment thrice, the plasma protein binding (PPB) rate was calculated as follows: PPB = [(A − background)/((A + B − background) × 2)] × 100%. The results are expressed as mean ± standard deviation (X ± S).

2.2.4. Lipids and Water Distribution Coefficient. Freshly prepared 1.85 MBq [¹⁸⁸Re]Re-IBA was added to a tube labeled A and it was shaken for 20 min with a vortex mixer, followed by centrifugation at 587 g for 5 min. The 0.1 mL upper liquid (organic phase) was collected into a tube labeled B. The 0.1 mL lower liquid (water phase) was collected into a tube labeled C. The radioactivity counts of the organic phase and water phase were measured by a γ counter. After repeating the experiment thrice, the lipid-water partition coefficient (logP) was calculated by using the following formula: \[ \log P = \log \left( \frac{\text{B-background}}{\text{C-background}} \right) \]

The results are expressed as mean ± standard deviation (X ± S).

2.2.5. Toxicity Test of Mice. Sixteen Kunming mice were randomly divided into four groups (equal number of males and females): normal control group and low-, middle-, and high-dose [¹⁸⁸Re]Re-IBA groups. The control group was injected with 0.9% NaCl, and the experimental groups were injected with [¹⁸⁸Re]Re-IBA solution of 3.7 MBq, 18.5 MBq, and 37 MBq, respectively. The body weight and general condition of the mice in each group were measured. After 4 weeks, the blood of the mice in each group was drawn for routine blood examination and liver and kidney function. A pathological examination of important tissue and organs was performed.

2.2.6. The Biodistribution of [¹⁸⁸Re]Re-IBA and [¹⁸⁸Re]ReO₄⁻ in Mice. Twenty Kunming mice (originated from Swiss albino mice and introduced into Kunming, China.) were randomly divided into five groups (equal number of males and females). Each group was injected with 3.7 MBq [¹⁸⁸Re]Re-IBA. Then, the mice were sacrificed by CO₂ asphyxiation at 1 h, 3 h, 6 h, 24 h, and 48 h. The blood and important tissue and organ samples were collected to measure the radioactivity count by γ counter. Following the time attenuation correction, the percentage injection dose rate per gram of tissue (%ID/g) was calculated at each time point. The result is expressed as mean ± standard deviation (X ± S). The in vivo distribution of [¹⁸⁸Re]ReO₄⁻ was studied using the aforementioned method.

2.2.7. Imaging of New Zealand Rabbits with [¹⁸⁸Re]Re-IBA. The New Zealand rabbit was anesthetized by intraperitoneal injection of 2% pentobarbital sodium (40 mg/kg). Then, 100 MBq [¹⁸⁸Re]Re-IBA was injected. Bone imaging was performed at different time points (acquisition equipment: American GE Infinia T4 double probe single-photon emission computed tomography, high energy collimator; scanning parameters: energy peak: 155 keV, posture: supine position, matrix: 128 × 128, and window width: ±10%). Following image acquisition, the images were processed by using the software of the postprocessing workstation.

2.2.8. Biodistribution and Imaging of Bone Metastasis Nude Mice. The nude mice of the bone metastasis model were established by tibial bone marrow injection. The specific methods were as follows: 25 μL of prostate cancer PC-3 and breast cancer MDA-MB-231 cell culture medium were injected into the left tibial bone marrow cavity of healthy male and female nude mice, respectively. At approximately 3-4 weeks after inoculation, they were scanned by...
microcomputed tomography (CT, SIEMENS InveonTM, Munich, Germany) to determine the condition of the model. If CT showed bone destruction in the left tibia (osteolytic, osteogenic, or mixed types); it suggested that the model was successful. Bone metastases were initially evaluated by CT and confirmed by pathology after the completion of related studies.

Ten PC-3 and ten MDA-MB-231 nude mice with bone metastasis were randomly divided into five groups. The mice were sacrificed by CO2 asphyxiation at 1 h, 3 h, 6 h, 24 h, and 48 h. The important tissue and organ samples were collected for the biodistribution study.

The nude mice were injected with $[^{188}\text{Re}]\text{Re-IBA}$, $[^{99m}\text{Tc}]\text{Tc-methylene diphosphonate (MDP)}$, and $[^{201}\text{Na}]\text{NaF}$, $[^{99m}\text{Tc}]\text{Tc-methylene diphosphonate (MDP)}$, and $[^{201}\text{Na}]\text{NaF}$, and confirmed by pathology after the completion of related studies.

The bone uptake of $[^{188}\text{Re}]\text{Re-IBA}$ is relatively high, and the %ID/g reaches the maximum at 6 h ($7.724 \pm 2.292\%\text{ID/g}$), and %ID/g remains $5.239 \pm 0.029$ at 48 h. In addition, the radioactivity ratio of the bone to heart, liver, and muscles is rather high at 48 h. In addition to the bone, the highest uptake of $[^{188}\text{Re}]\text{Re-IBA}$ occurred in the kidneys, which is related to the kidney as the primary excretory organ. Table 3 outlines the distribution of $[^{188}\text{Re}]\text{ReO}_4^-$ in mice. The highest uptake of $[^{188}\text{Re}]\text{ReO}_4^-$ occurs in the stomach and thyroid gland. However, the uptake in the bone is negligibly low. Furthermore, the radioactivity ratio of bone to heart, liver, and muscle tissue is low, and the highest ratio is as low as 2.372 (bone/muscle, 3 h).

Figure 9 depicts the bone imaging of the New Zealand rabbits in different time points. There was an obvious accumulation in bones and mild tracer uptake in soft tissue at 20 min after the injection. Over time, the soft tissue accumulation gradually faded and disappeared, and the bone imaging was clear with a high contrast between the bone and the background. In addition, the elimination of most of the $[^{188}\text{Re}]\text{Re-IBA}$ activity was through the kidneys, which was consistent with the biodistribution in mice.

3.6. Biodistribution and Imaging of Bone Metastasis Nude Mice. Table 4 summarizes the biodistribution of $[^{188}\text{Re}]\text{Re-IBA}$ in mice. A rapid blood clearance and low uptake of $[^{188}\text{Re}]\text{Re-IBA}$ in the soft tissue, brain, liver, lung, and spleen were observed. The bone uptake of $[^{188}\text{Re}]\text{Re-IBA}$ is relatively high, and the %ID/g reaches the maximum at 6 h ($7.724 \pm 2.292\%\text{ID/g}$), and %ID/g remains $5.239 \pm 0.029$ at 48 h. In addition, the radioactivity ratio of the bone to heart, liver, and muscles is rather high at 48 h. In addition to the bone, the highest uptake of $[^{188}\text{Re}]\text{Re-IBA}$ occurred in the kidneys, which is related to the kidney as the primary excretory organ. Table 3 outlines the distribution of $[^{188}\text{Re}]\text{ReO}_4^-$ in mice. The highest uptake of $[^{188}\text{Re}]\text{ReO}_4^-$ occurs in the stomach and thyroid gland. However, the uptake in the bone is negligibly low. Furthermore, the radioactivity ratio of bone to heart, liver, and muscle tissue is low, and the highest ratio is as low as 2.372 (bone/muscle, 3 h).

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Figure 2: The proposed structure of $^{188}$Re-IBA.

Figure 3: Effects of IBA (a), ascorbic acid (b), SnCl$_2$ (c), $^{188}$ReO$_4^-$ activity (d), KReO$_4$ (e), temperature and reaction time (f), and pH (g) on the radiochemical yield of $^{188}$Re-IBA.
IBA in bone metastasis nude mice. A rapid blood clearance and low uptake in soft tissue, brain, liver, lung, and spleen of \([^{188}\text{Re}]\text{Re-IBA}\) were observed, which is consistent with the biodistribution in mice. The comparison between the model bone and the contralateral bone shows that the uptake of the model bone is lower than that of the contralateral bone at 1–3 h but higher than that of the contralateral bone from 6 to 48 h.

\([^{188}\text{Re}]\text{Re-IBA}\) bone imaging of nude mice with bone metastasis showed that, at 1–3 h after injection, the uptake in the model side was higher than that of the control side, but the concentration was mainly distributed in the surrounding soft tissue; at 6 h, 16 h, and 32 h, the bone uptake of the model bone was higher than that of the contralateral bone. Table 5 shows the ROI ratio of model bone/contralateral bone in nude mice of bone metastasis model at 6 h, 16 h, and 32 h.

### Table 1: The in vitro stability of \([^{188}\text{Re}]\text{Re-IBA}\) under different methods.

| Method   | 1 h (%)       | 3 h (%)       | 6 h (%)       | 12 h (%)      | 24 h (%)      |
|----------|--------------|--------------|--------------|--------------|--------------|
| 0.9% NaCl | 98.14 ± 0.2  | 97.82 ± 0.6  | 96.99 ± 1.3  | 95.33 ± 1.1  | 91.44 ± 0.8  |
| Serum    | 99.01 ± 0.5  | 98.67 ± 0.9  | 98.15 ± 0.8  | 96.90 ± 1.4  | 93.06 ± 1.6  |

Figure 4: Typical PC distribution of \([^{188}\text{Re}]\text{Re-IBA}\) in acetone (a) and saline (b).

![Typical PC distribution](image)

**Figure 5:** Weight change of mice injected with normal saline or different doses of \([^{188}\text{Re}]\text{Re-IBA}\) within 4 weeks.

### 4. Discussion

The amount of ligand IBA is an important evaluation parameter in the labeling process. A sufficient number of ligands must be provided in the formula to achieve high RCP complex formation. However, using excessive quantities of bisphosphonate ligand should be avoided to achieve high specific activity labeling and avoid the formation of...
Figure 6: Routine blood (WBC, RBC, HGB, and PLT), liver function (ALT and AST), and renal function (blood urea nitrogen and serum creatinine) of normal mice at 4 weeks after injection of normal saline or different doses of [188Re]Re-IBA. The results are expressed as mean ± standard deviation (x ± s). (LLN: lower limit of normal; ULN: upper limit of normal).

Figure 7: Pathology of mice at 4 weeks after injection of 0.9% NaCl (normal control group): (a) heart; (b) liver; (c) spleen; (d) lung; (e) stomach; (f) kidney; (g) brain; (h) small intestine; (i) muscle; (j) bone marrow. (a)–(i) HE × 200; (j) HE × 400.
The percentage injection dose rate per gram of tissue (%ID/g) is expressed as mean ± standard deviation (±).
undesired side product, which increases the impurity and inversely affects the labeling yield [6, 12]. Our study found that the highest RCP complex formation can be obtained when the number of ligands in the formula is 0.8–1.4 mg. With further increase of the dosage, the RCP gradually decreased, which may be due to the nonlabeled ligand which interfered with the labeling process or the presence of too many ligands making the reaction solution supersaturated.

Table 4: The biodistribution of $^{188}$Re-IBA in nude mice of bone metastasis model ($n = 4$).

| Tissue          | 1 h  | 3 h  | 6 h  | 24 h | 48 h  |
|-----------------|------|------|------|------|-------|
| Heart           | 0.595 ± 0.099 | 0.326 ± 0.175 | 0.183 ± 0.097 | 0.052 ± 0.027 | 0.035 ± 0.013 |
| Liver           | 1.236 ± 0.219 | 0.431 ± 0.312 | 0.368 ± 0.054 | 0.089 ± 0.027 | 0.044 ± 0.013 |
| Spleen          | 0.530 ± 0.124 | 0.227 ± 0.141 | 0.176 ± 0.029 | 0.066 ± 0.024 | 0.022 ± 0.013 |
| Lung            | 1.135 ± 0.209 | 0.298 ± 0.250 | 0.257 ± 0.030 | 0.101 ± 0.038 | 0.047 ± 0.005 |
| Kidney          | 6.004 ± 0.523 | 2.591 ± 1.120 | 2.587 ± 0.126 | 0.640 ± 0.175 | 0.121 ± 0.034 |
| Stomach         | 3.056 ± 0.321 | 2.064 ± 0.207 | 1.176 ± 0.190 | 0.192 ± 0.092 | 0.027 ± 0.019 |
| Thyroid gland   | 0.625 ± 0.309 | 0.282 ± 0.149 | 0.354 ± 0.217 | 0.082 ± 0.030 | 0.020 ± 0.010 |
| Small intestine | 0.718 ± 0.073 | 0.404 ± 0.047 | 0.242 ± 0.118 | 0.049 ± 0.003 | 0.030 ± 0.018 |
| Blood           | 1.704 ± 0.348 | 0.531 ± 0.092 | 0.302 ± 0.092 | 0.045 ± 0.020 | 0.021 ± 0.008 |
| Brain           | 0.098 ± 0.046 | 0.082 ± 0.017 | 0.042 ± 0.042 | 0.037 ± 0.020 | 0.021 ± 0.009 |
| CB              | 9.331 ± 0.541 | 7.717 ± 4.349 | 7.662 ± 2.934 | 4.413 ± 2.225 | 4.737 ± 0.863 |
| MB              | 8.329 ± 0.329 | 5.922 ± 2.126 | 8.417 ± 1.820 | 6.403 ± 0.247 | 6.503 ± 0.010 |
| CS              | 0.578 ± 0.043 | 0.524 ± 0.172 | 0.153 ± 0.083 | 0.081 ± 0.050 | 0.041 ± 0.022 |
| Gonad           | 0.439 ± 0.162 | 0.231 ± 0.068 | 0.106 ± 0.022 | 0.069 ± 0.018 | 0.028 ± 0.001 |
| CB/heart        | 15.682     | 23.637 | 41.876 | 85.157 | 133.599 |
| CB/liver        | 7.549     | 17.896 | 20.818 | 49.848 | 106.617 |
| CB/CS           | 16.144     | 14.731 | 50.044 | 54.620 | 115.161 |
| MB/heart        | 14.003     | 18.139 | 45.999 | 123.563 | 183.399 |
| MB/liver        | 6.738     | 13.734 | 22.867 | 72.330 | 146.359 |
| MB/CS           | 14.411     | 11.305 | 54.970 | 79.255 | 158.088 |
| MB/CB           | 0.893     | 0.767 | 1.099 | 1.451 | 1.373 |

The percentage injection dose rate per gram of tissue (\%ID/g) is expressed as mean ± standard deviation ($\bar{x} \pm s$). CB, contralateral bone; MB, model bone; CS, contralateral muscle.

Table 5: ROI ratio of model bone/contralateral bone in nude mice of bone metastasis model ($n = 4$).

| ROI ratio | 6h  | 16h  | 32h  |
|-----------|-----|------|------|
| Mean      | 1.245 | 1.633 | 1.529 |
| SD        | 0.295 | 0.472 | 0.187 |

Figure 9: Bone imaging of New Zealand rabbits injected with $^{188}$Re-IBA at 20 min–48 h.
and thus affecting the labeling. In this study, ascorbic acid was used as the antioxidant and stannous ion stabilizer. The results showed that when the quantity of ascorbic acid in the formula was 0.15–0.5 mg, [188Re]Re-IBA could be prepared in >95% RCP. An inorganic compound of tin, SnCl2, has been widely used in nuclear medicine as a reducing agent for pharmaceutical products radiolabeled with 99mTc or 188Re [19]. In our study, SnCl2 was chosen as the reducing agent to reduce [188Re]ReO4 to a lower oxidation state and facilitate its reaction with IBA. Despite its widespread use, there are several side effects associated with this ion and its derivatives described in the literature, for example, irritation of oral mucosa in rats [20], deterioration in semen quality of male rabbits [21], free radical induction and damage to liver and kidney function in rabbits [22], and marked hazardous alterations in some enzymatic activities and biochemical parameters in rabbits [23]. Furthermore, genotoxicity, mainly related to SnCl2, has been reported [24], which could mediate single-strand breaks in plasmid DNA through reactive oxygen species (ROS) formation [19]. Our research showed that the RCP is high enough when the amount of SnCl2 in the formula reaches 0.14–0.18 mg. An increase in the amount of SnCl2 beyond this dosage range is not beneficial to the labeling yield and might lead to the formation of colloidal impurities in the product. However, an insufficient amount of SnCl2 will not reduce all [188Re]ReO4. Therefore, it is necessary to ensure an optimum ratio of SnCl2 with IBA and [188Re]ReO4, and the excess SnCl2 should be removed as much as possible. In addition, avoiding the potential toxicity of SnCl2 in the formula is also a problem worth discussing. Several studies showed that ascorbic acid, a well-known antioxidant and water-soluble ROS scavenger, is capable of detoxifying the hazardous effects of SnCl2, including alleviation of reproductive toxicity [21] and the toxicity to some enzyme activities and oxidative damage [22], as well as increase in the activities of antioxidant enzymes [23]. Therefore, the ascorbic acid used in our formula may also act as a protectant against the toxicity of SnCl2. The 188Re obtained in the generator was carrier-free. In previous studies [9, 25–32], [188Re]Re-bisphosphonate, without cold rhenium, had a poor stability and little bone uptake. Hence, nonradioactive KReO4 was added as a cold perhenate carrier to ensure the stability and bone uptake.

In addition, [188Re]Re-IBA, like [188Re]Re-HEDP [33], [188Re]Re-ZNA [12], and [188Re]Re-ABP [34], may be an anionic six-coordinated complex with one metal atom bound to two IBA ligand molecules. Figure 2 shows the proposed structure of [188Re]Re-IBA. However, the structure we provide is only a basic coordination structure. In fact, [188Re]Re-IBA is likely to exist in a form with a complex spatial structure. Elder et al. [35] carefully studied the structure of Re-HEDP and found that medically effective species of Re-HEDP with Re-Re bonds may exist in two forms of complex mixture: as a linear tetramer of rhenium atoms bridged by HEDP ligands, HEDP ligands which also bind an equivalent number of tin atoms with additional HEDP ligands, or as a triangular cluster of rhenium atoms bicapped by two HEDP ligands and bridged to three tin atoms by HEDP to form a complex. As a similar compound of Re-HEDP, we speculate that [188Re]Re-IBA may also exist in a similar structural form. Therefore, its structure may contain Re-Re bonds and tin atoms, bridged by HEDP ligands, forming a complex spatial structure. However, at present, little is known about the specific structure of this radiopharmaceutical. To determine the exact structure of [188Re]Re-IBA, further research should be pursued.

The stability of radiopharmaceuticals is extremely important for therapy. Our study showed that the [188Re]Re-IBA has good stability, which is beneficial for further research. The differences in stability between [188Re]Re-IBA and other drugs were also compared, and the results showed that there was no significant difference in stability between [188Re]Re-IBA and [188Re]Re-HEDP (91.44 ± 0.8% versus 91.2% at 24h) in 0.9% NaCl [32], but its stability improved compared to [188Re]Re-risedronate (80.3% at 24h) and [188Re]Re-PMA (81.9% at 24h) [6, 13]. In addition, the stability of [188Re]Re-IBA (93.06 ± 1.6% at 24h) in human serum was improved compared to those of [188Re]Re-ZNA (91.53 ± 1.39% at 24h) and [188Re]Re-risedronate (73 ± 1.21% at 24h) [12, 13].

Radiopharmaceuticals should achieve ideal therapeutic effects and minimize the related toxicity to gain efficacy as therapeutic drugs [4]. Toxicity test of mice showed that [188Re]Re-IBA is safe and has low toxicity; thus it can be used for later imaging and therapeutic research. The biodistribution and imaging of normal animals indicated that [188Re]Re-IBA highly targets the bone and can remain in the bone for a long time. As expected, the in vivo distribution of [188Re]ReO4 revealed that the free [188Re]ReO4 was poorly targeted to the bone, thus confirming the success and bone-targeting capability of the labeled [188Re]Re-IBA. In addition, the imaging results of the New Zealand rabbits revealed fast soft tissue clearance and low nontarget tissue uptake, and the overall image quality was good. Hence, [188Re]Re-IBA has potential for the treatment of bone pain and can also be used to monitor therapeutic imaging.

The bone uptake of [188Re]Re-IBA in normal mice was also compared with those of other 188Re labeled bisphosphonates [6, 12, 36–38]. Although the mouse strain used for evaluation of [188Re]Re-IBA (Kunming mice) is not completely the same as those used for evaluation of other 188Re labeled bisphosphonates (Swiss mice or Kunming mice), we believe that these data can be compared considering that Kunming mice also originate from the Swiss mice strain. We noted that, among the 188Re labeled third-generation bisphosphonates (i.e., IBA and ZNA), [188Re]Re-IBA had the highest bone uptake. The highest femur bone uptake value of [188Re]Re-IBA in Kunming mice was 7.724 ± 2.292%ID/g (6h), and the minimum value was 5.239 ± 0.029%ID/g (48h). The bone uptake of [188Re]Re-ZNA in Swiss mice reached the maximum at 1h (1.08 ± 0.14%ID/g); however, with the extension of time, the bone uptake decreased gradually and reached the lowest value of 0.36 ± 0.06%ID/g at 24h [12]. In addition, the highest and lowest bone uptakes of 188Re labeled second-generation bisphosphonate PMA in Swiss mice were 0.81 ± 0.25%ID/g (4h) and 0.40 ± 0.05%ID/g (1h).
uptake of \([^{188}\text{Re}]\text{Re-IBA}\) in the joints of the New Zealand rabbits confirmed the following: rapid blood clearance, high affinity to the bone, long retention time in the bone, low uptake in the solid organs and soft tissue, and high contrast of imaging. The biodistribution and imaging of bone metastasis nude mice confirmed that \([^{188}\text{Re}]\text{Re-IBA}\) has a higher bone affinity to bone metastasis lesion than normal bone. Therefore, \([^{188}\text{Re}]\text{Re-IBA}\) is a suitable candidate for the treatment of bone metastasis and monitoring the therapeutic effects. However, the method of modelling should be further improved to obtain an osteogenic metastasis model that is more suitable for research. In addition, the efficacy of \([^{188}\text{Re}]\text{Re-IBA}\) in the treatment of animal models of bone metastasis and comparisons with other radiopharmaceuticals should be explored to completely clarify its value. This is the goal of our future research.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

TX, YW, and ZC contributed to the study design and TX wrote the manuscript. HL, SY, GL, YZ, WF, LL, KX, and DP are responsible for the integrity of the data and the accuracy of the data analysis. YC was responsible for revising of important intellectual content. All authors read and approved the final manuscript. TX, YW, and ZC contributed equally to this work.

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References

[1] M. Meckel, V. Kubicek, P. Hermann, M. Miederer, and F. Rossch, “A DOTA based bisphosphonate with an albumin binding moiety for delayed body clearance for bone targeting,” Nuclear Medicine and Biology, vol. 43, no. 11, pp. 670–678, 2016.
[2] N. Ayati, K. Aryana, A. Jalilian et al., “Treatment efficacy of (153)Sm-EDTMP for painful bone metastasis,” Asia Oceania journal of nuclear medicine & biology, vol. 1, pp. 27–31, 2013.
[3] L. Florimonte, L. Dellavedova, and L. S. Maffioli, “Radium-223 dichloride in clinical practice: a review,” European Journal of Nuclear Medicine and Molecular Imaging, vol. 43, no. 10, pp. 1896–1909, 2016.
[4] Q. Xu, S. Zhang, Y. Zhao et al., “Radiolabeling, quality control, biodistribution, and imaging studies of 177 Luibandronate,”
M. El-Kolaly, M. Motaleb, and M. Nassar, “Study of labeling 188Re and study of its stability,” *Journal of Nuclear Medicine and Molecular Imaging*, vol. 27, no. 2, pp. 123–130, 2000.

R. Sharma, C. Kumar, H. Bender et al., “Cellular inactivation induced by a radiopharmaceutical kit: role of stannous chloride,” *Toxicology Letters*, vol. 99, no. 3, pp. 199–205, 1998.

V. K. Shiryeva, V. M. Petrov, A. A. Bryukhanova, O. A. Smoryzanova, V. G. Skvortsov, and O. E. Shrever, “Evaluation of the influence of preparation conditions on pharmacokinetics of bone-seeking radiopharmaceutical 188Re-labeled hydroxyethylidenediphosphonic acid monophosphate in rats,” *Pharmacological Journal*, vol. 46, no. 7, pp. 443–448, 2012.

K. Hashimoto, “Synthesis of a 188Re-HEDP complex using carrier-free 188Re, and a study of its stability,” *Applied Radiation and Isotopes*, vol. 49, no. 4, pp. 351–356, 1998.

S. J. Oh, K. S. Won, D. H. Moon et al., “Preparation and biological evaluation of 188Re-ethylidenediamine-N,N,N,N-tetrakis (methylene phosphonic) acid complex,” *Arab J Nucl Sci Appl*, vol. 36, no. 2, pp. 491–500, 2018.

M. El-Kolaly, M. Nassar, M. Tabataabei, A. Doroudi, and M. Shafiei, “Radiolabeling of zoledronic acid with 188Re as a new palliative agent radiotracer in treatment of bone tumors,” *Journal of Radioanalytical and Nuclear Chemistry*, vol. 316, no. 2, pp. 491–500, 2018.

M. Erfani, A. Doroudi, M. A. Dinari, and S. P. Shirmard, “Preparation of a rhenium-188 labeled bisphosphonate for bone pain palliation therapy,” *Journal of Radioanalytical and Nuclear Chemistry*, vol. 303, no. 3, pp. 2027–2032, 2015.

Y. Kucuksezgin, Y. Kucukzeybek, G. Gorumlu, E. Cengiz, C. Erten, and R. Uslu, “Bisphosphonate (Zoledronic acid) associated adverse effects: single center experience,” *UHOD*, vol. 20, pp. 135–140, 2010.

I. J. Diehl, R. Weide, H. Köppler et al., “Risk of renal impairment after treatment with ibandronate versus zoledronic acid: a retrospective medical records review,” *Supportive Care in Cancer*, vol. 17, no. 6, pp. 719–725, 2009.

M. Duh, R. Weide, H. Köppler et al., “Renal toxicity in patients with multiple myeloma receiving zoledronic acid vs. ibandronate: a retrospective medical records review,” *Journal of Cancer Research and Therapeutics*, vol. 6, no. 1, pp. 31–35, 2010.

I. Meattini, A. Bruni, V. Scotti et al., “Oral ibandronate in metastatic bone breast cancer: the Florence University experience and a review of the literature,” *Journal of Chemotherapy*, vol. 22, no. 1, pp. 58–62, 2010.

J. Han, L. Han, L. Zhang et al., “Comparison of clinical effect in treatment of bone tumor between zoledronic acid needle and ibandronate needle,” *Pakistan Journal of Pharmaceutical Sciences*, vol. 31, pp. 1683–1686, 2018.

F. J. S. Dantas, M. O. Moraes, J. C. P. de Mattos et al., “Stannous chloride mediates single strand breaks in plasmid DNA through reactive oxygen species formation,” *Toxicology Letters*, vol. 110, no. 3, pp. 129–136, 1999.

A. Larsson, B. Kinnyb, R. Könsberg, M. J. Peszkowski, and G. Warevinge, “Irritant and sensitizing potential of copper, mercury and tin salts in experimental contact stomatitis of rat oral mucosa,” *Contact Dermatitis*, vol. 23, no. 3, pp. 146–153, 1990.

M. I. Yousef, “Protective role of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride,” *Toxicology*, vol. 207, no. 1, pp. 81–89, 2005.

F. M. El-Demerdash, M. I. Yousef, and M. A. Zoheir, “Stannous chloride induces alterations in enzyme activities, lipid peroxidation and histopathology in male rabbit: antioxidant role of vitamin C,” *Food and Chemical Toxicology*, vol. 43, no. 12, pp. 1743–1752, 2005.

M. I. Yousef, T. I. Awad, F. A. Elhag, and F. A. Khaled, “Study of the protective effect of ascorbic acid against the toxicity of stannous chloride on oxidative damage, antioxidant enzymes and biochemical parameters in rabbits,” *Toxicology*, vol. 235, no. 3, pp. 194–202, 2007.

M. L. B. Assis, M. R. Caceres, J. C. P. De Mattos, A. Caldeira-de-Araújo, and M. Bernardo-Filho, “Cellular inactivation induced by a radiopharmaceutical kit: role of stannous chloride,” *Toxicology Letters*, vol. 99, no. 3, pp. 199–205, 1998.

K. Hashimoto, “Synthesis of a 188Re-HEDP complex using carrier-free 188Re, and a study of its stability,” *Applied Radiation and Isotopes*, vol. 49, no. 4, pp. 351–356, 1998.

S. J. Oh, K. S. Won, D. H. Moon et al., “Preparation and biological evaluation of 188Re-ethylidenediamine-N,N,N,N-tetrakis (methylene phosphonic acid) as a potential agent for bone pain palliation,” *Nuclear Medicine Communications*, vol. 23, no. 1, pp. 75–81, 2002.

Q. N. Li, X. D. Zhang, R. Sheng, and W. X. Li, “Preparation of (188Re) Re-AEDP and its biodistribution studies,” *Applied Radiation and Isotopes*, vol. 53, no. 6, pp. 993–997, 2000.

E. S. Verdera, J. Gaudiano, A. Léon et al., “Rhenium-188-HEDP kit formulation and quality control,” *Ract*, vol. 79, no. 2, pp. 113–118, 1997.

M. Y. Nassar, M. T. El-Kolaly, and M. R. H. Mahran, “Synthesis of a 188Re-HEDP complex using carrier-free 188Re and a study of its stability and biological distribution,” *Journal of Radioanalytical and Nuclear Chemistry*, vol. 287, no. 3, pp. 779–785, 2011.

F. M. El-Demerdash, M. I. Yousef, and M. A. Zoheir, “Stannous chloride induces alterations in enzyme activities, lipid peroxidation and histopathology in male rabbit: antioxidant role of vitamin C,” *Food and Chemical Toxicology*, vol. 43, no. 12, pp. 1743–1752, 2005.

M. I. Yousef, T. I. Awad, F. A. Elhag, and F. A. Khaled, “Study of the protective effect of ascorbic acid against the toxicity of stannous chloride on oxidative damage, antioxidant enzymes and biochemical parameters in rabbits,” *Toxicology*, vol. 235, no. 3, pp. 194–202, 2007.
[35] R. C. Elder, J. Yuan, B. Helmer, D. Pipes, K. Deutsch, and E. Deutsch, "Studies of the structure and composition of rhenium−1,1-hydroxyethylenediphosphonate (HEDP) analogues of the radiotherapeutic agent 186ReHEDP," *Inorganic Chemistry*, vol. 36, no. 14, pp. 3055–3063, 1997.

[36] B. L. Faintuch, S. Faintuch, and E. Muramoto, "Complexation of 188Re-phosphonates: in vitro and in vivo studies," *Radiocimica Acta*, vol. 91, no. 10, pp. 607–612, 2003.

[37] S. Jiang, S. Luo, H. Deng, W. Bin, W. Wang, and H. Wei, "Preparation and biodistribution of 188Re-TCTMP (in Chinese)," *Journal of Nuclear and Radiochemistry*, vol. 25, no. 1, pp. 26–30, 2003.

[38] D. Yin, Z. Guo, J. Zhang, X. Min, J. Yu, and B. Li, "Experimental study of 188Re-HEDP (in Chinese)," *Chinese Journal of Nuclear Medicine*, vol. 18, no. 3, p. 189, 1998.

[39] A. El-Mabhouh and J. R. Mercer, "188Re-labeled bisphosphonates as potential bifunctional agents for therapy in patients with bone metastases," *Applied Radiation and Isotopes*, vol. 62, no. 4, pp. 541–549, 2005.

[40] T. Xu, Y. Wang, Z. Chen et al., *A study on the preparation, evaluation of biological characteristics, and preliminary imaging of [188Re]Re-ibandronate*, Research Square, Durham, North Carolina, USA, 2021.