The influence of carrier addition on the biodistribution of bone-seeking agent «\(^{188}\text{Re-oxa-bis(ethylenenitrilo)-tetramethylenephosphonic acid}\)»

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Abstract. Bone-seeking radiopharmaceuticals are known to be the most promising agents for the palliative treatment of the pain of bone metastases. A generator-produced radionuclide rhenium-188 \(^{188}\text{Re}\) is an attractive candidate for therapeutic use due to its nuclear characteristics. The goal of the current work was the investigation of the biodistribution of \(^{188}\text{Re-oxa-bis(ethylenenitrilo)tetramethylenephosphonic acid}\) \(^{188}\text{Re-OENTMP}\) with gallium carrier and without carrier and to compare them. A comparative accumulation study of both \(^{188}\text{Re-OENTMP}\) formulations was performed in normal rats after intravenous administration up to 48 hours. It was revealed that for carrier-added \(^{188}\text{Re-OENTMP}\) the bone samples from femur, skull, ribs, spine and knee joint all had higher uptake in comparison to that of \(^{188}\text{Re-OENTMP}\) without carrier. The values of carrier-added \(^{188}\text{Re-OENTMP}\) in skeleton were ranged from 7.82 % to 57.37 % of injected dose (ID), when the amount of \(^{188}\text{Re-OENTMP}\) without carrier varied from 5.76 % to 22.75 % of ID. Among the soft tissue organs, only thyroid gland and kidneys had a relatively high uptake. Most of the activity excreted via the urinary tract. In conclusion, it should be pointed out that carrier addition strongly affects bone uptake of \(^{188}\text{Re-OENTMP}\), so carrier-added \(^{188}\text{Re-OENTMP}\) could be useful to deliver radiation to skeletal metastases from soft tissue cancer.

1. Introduction

Systemic radionuclide therapy is the effective method of disseminated bone metastases treatment. Phosphonates are widely used for local delivery of a high radiation dose to skeleton [1]. The mechanism of their action is explained by high affinity for hydroxyapatite and suppression of osteolysis and bone resorption.

Rhenium-188 \(^{188}\text{Re}\) is a generator-produced radionuclide with appropriate properties for radionuclide therapy. \(^{188}\text{Re}\) decays with a half-life of 16.7 h. \(^{188}\text{Re}\) decays to stable \(^{188}\text{Os}\), yielding \(\beta^-\) radiation \((E_{\beta\text{max}} = 2.1\ \text{MeV})\) and \(\gamma\)-radiation \((E_{\gamma} = 155\ \text{keV}, 15\%)\), which can be used for imaging. Post-treatment imaging is important in order to evaluate the distribution of the radiopharmaceuticals. The \(^{188}\text{W}/^{188}\text{Re}\) generator is an ideal source for the long term (4-6 months) continuous availability of \(^{188}\text{Re}\) by saline elution.
The adequate quality and stability of bone-targeting radiopharmaceuticals depend on large number of factors such as the amount of agents in reaction mixture, the presence or absence of carrier, pH, ionic strength of solution, heating time and temperature [2]. It is known that carrier addition is essential for stability and biodistribution of many bone-seeking radiopharmaceuticals [3-11].

Toegel S et al. [11] showed that carrier addition significantly increased the binding affinity of $^{68}$Ga-EDTMP to hydroxyapatite and human cortical matrix pre vivo. Also carrier-added $^{90}$Y-EDTMP augmented the binding affinity to inorganic part of bone tissue [10]. It was observed that bone uptake of carrier-added radiolabelled phosphonates was higher than non-carrier-added formulation in vivo [3, 5, 8, 12].

The aim of this study was the comparative investigation of biodistribution of $^{188}$Re-oxa-bis(ethylenenitrilo)tetramethylene phosphonic acid ($^{188}$Re-OENTMP) prepared with gallium carrier and without carrier.

2. Methods and discussion

Biodistribution studies of $^{188}$Re-OENTMP were carried out in healthy wild-type rats each weighing 160 ± 40 g. The animals were divided into 2 groups (20 rats in each group). Pharmacokinetic properties of carrier-added and non-carrier-added $^{188}$Re-OENTMP were studied in the animals of the first and the second groups, respectively. A volume of 0.1 ml containing 0.37 MBq of radioactivity was injected through the tail vein.

The animals were sacrificed by decapitation at selected times after injection (5 min, 1, 3, 24 and 48 h). Four rats were used for each time point. The samples of organs and tissues were removed and placed in preweighed tubes for gamma-counter. The radioactivity was measured by automated gamma-counter «Wizard». The distribution of activities was calculated as the percentage of injected dose per gram (% ID/g). Activity in the femur was considered for obtaining the total skeletal uptake assuming the skeleton to be 10% of the total body weight.

Descriptive statistical analyses were performed using mean values and standard errors (M ± m). Hypothesis tests among two data sets were made by comparison of two means by t-test. A value of p < 0.05 was considered significant.

3. Results and discussion

The results of carrier-added and carrier-free $^{188}$Re-OENTMP are represented in table 1. They showed higher bone uptake of carrier-added $^{188}$Re-OENTMP than carrier-free preparation after their intravenous administration. The peak levels of radioactivity in femur were 2.71 % ID/g at 1 h and 1.20 % ID/g at 1 h for carrier-added and non-carrier-added $^{188}$Re-OENTMP, respectively. In other bones (skull, spine, ribs) and knee joints specific activity of $^{188}$Re-OENTMP with carrier was also higher than carrier-free agent. In joints the amount of $^{188}$Re-OENTMP with carrier was about 2.5 – 6 times higher than carrier-free preparation within 3 h post-injection (p < 0.001-0.02).

The same influence of carrier addition on biodistribution of phosphonates labeled with $^{188}$Re was reported in many papers [3-9]. For example, Pervez et al. [12] found that the bone uptake of carrier-added $^{188}$Re-EDTMP was almost twice as high (2.0 % ID/g after 24 h) as the carrier-free product (1.1 %ID/g after 24 h). This influence of carrier addition as well as the general influence of the radionuclide on elevated bone binding affinity could be explained by the rearrangement of complex structures or formation of polymeric structures initiated by the carriers and the radionuclides themselves [13].

A comparison of total skeletal uptake of both agents in rats at different time intervals post-injection is given in figure 1. The total amount of carrier-added $^{188}$Re-OENTMP in skeleton was 34.83 % ID at 5 min and reached 57.37 % ID at 1 h post-injection. Then the levels of radioactivity dropped to 47.22 % ID, 10.14 % ID and 7.82 % ID at 3 h, 24 h and 48 h, respectively. At the same time a bone uptake of non-carrier-added $^{188}$Re-OENTMP didn’t exceed 22.75 % ID.
In blood the specific activity of carrier-added $^{188}$Re-OENTMP was little more as compared with carrier-free preparation, but didn’t exceed 1.95 % ID/g. Furthermore, both formulations were rapidly washed out from the circulation and taken up in bones within 3 h after administration.

Table 1. Biodistribution of $^{188}$Re-OENTMP with or without gallium carrier addition in rats after intravenous injection (in % ID/g).

| Organ/tissue | 5 min | 1 h  | 3 h  | 24 h | 48 h |
|--------------|-------|------|------|------|------|
| 1 Blood      | 1.95±0.42* | 0.56±0.14 | 0.41±0.07 | 0.027±0.006 | 0.042±0.004 |
|              | 0.76±0.14** | 0.24±0.05 | 0.10±0.01 | 0.042±0.005 | 0.041±0.007 |
|              | p<0.05 | p>0.05 | p<0.01 | p>0.1 | p>0.5 |
| 2 Thyroid    | 8.51±0.98 | 7.88±2.06 | 2.25±0.19 | 0.27±0.02 | 0.17±0.03 |
|              | 13.97±2.39 | 1.86±0.28 | 1.01±0.20 | 0.34±0.06 | 0.97±0.46 |
|              | p>0.05 | p>0.05 | p>0.01 | p>0.25 | p>0.1 |
| 3 Lungs      | 1.44±0.28 | 0.34±0.07 | 0.19±0.03 | 0.019±0.003 | 0.013±0.004 |
|              | 0.53±0.09 | 0.15±0.02 | 0.07±0.01 | 0.028±0.005 | 0.020±0.001 |
|              | p<0.05 | p<0.05 | p<0.01 | p>0.1 | p>0.1 |
| 4 Liver      | 0.59±0.15 | 0.75±0.37 | 0.14±0.02 | 0.015±0.002 | 0.013±0.003 |
|              | 0.26±0.03 | 0.09±0.01 | 0.05±0.01 | 0.026±0.002 | 0.023±0.004 |
|              | p>0.05 | p>0.05 | p>0.01 | p>0.05 | p>0.05 |
| 5 Kidneys    | 5.37±1.02 | 2.97±0.43 | 3.10±0.56 | 0.56±0.08 | 0.45±0.01 |
|              | 1.20±0.24 | 1.78±0.08 | 1.48±0.07 | 0.84±0.20 | 0.67±0.05 |
|              | p<0.01 | p>0.05 | p>0.05 | p>0.1 | p<0.01 |
| 6 Spleen     | 0.37±0.08 | 0.12±0.02 | 0.08±0.01 | 0.015±0.003 | 0.012±0.003 |
|              | 0.17±0.01 | 0.06±0.01 | 0.04±0.01 | 0.019±0.005 | 0.014±0.001 |
|              | p<0.05 | p<0.05 | p<0.05 | p>0.5 | p>0.5 |
| 7 Muscle     | 0.44±0.06 | 0.130±0.034 | 0.034±0.006 | 0.004±0.001 | 0.008±0.002 |
|              | 0.10±0.02 | 0.023±0.003 | 0.014±0.003 | 0.007±0.002 | 0.006±0.001 |
|              | p>0.002 | p<0.05 | p<0.05 | p>0.1 | p>0.25 |
| 8 Knee joint | 3.48±0.62 | 5.32±0.86 | 5.05±0.41 | 0.91±0.08 | 0.68±0.05 |
|              | 0.55±0.03 | 2.55±0.20 | 2.11±0.25 | 1.01±0.15 | 0.94±0.14 |
|              | p<0.01 | p>0.02 | p>0.001 | p>0.5 | p>0.1 |
| 9 Femur      | 1.86±0.37 | 2.71±0.43 | 2.27±0.07 | 0.47±0.03 | 0.39±0.03 |
|              | 0.34±0.03 | 1.20±0.09 | 0.96±0.14 | 0.71±0.09 | 0.56±0.11 |
|              | p<0.01 | p<0.02 | p<0.001 | p<0.05 | p>0.1 |
| 10 Skull     | 1.68±0.24 | 2.15±0.52 | 1.87±0.14 | 0.37±0.02 | 0.33±0.04 |
|              | 0.31±0.04 | 0.91±0.07 | 0.72±0.08 | 0.63±0.07 | 0.42±0.08 |
|              | p>0.002 | p>0.05 | p<0.001 | p<0.02 | p>0.25 |
| 11 Ribs      | 1.84±0.12 | 2.08±0.42 | 1.88±0.17 | 0.39±0.02 | 0.29±0.04 |
|              | 0.29±0.03 | 0.89±0.10 | 0.68±0.08 | 0.51±0.07 | 0.34±0.01 |
|              | p>0.001 | p>0.05 | p<0.001 | p>0.1 | p>0.25 |
| 12 Spine     | 1.71±0.33 | 2.43±0.50 | 2.03±0.16 | 0.41±0.03 | 0.29±0.02 |
|              | 0.32±0.02 | 1.01±0.06 | 0.81±0.10 | 0.58±0.06 | 0.43±0.06 |
|              | p>0.01 | p>0.05 | p<0.001 | p<0.05 | p>0.05 |

* _$^{188}$Re-OENTMP with carrier addition_  
** _$^{188}$Re-OENTMP without carrier addition_
The influence of carrier addition on the stability of phosphonates labeled with $^{188}$Re is still controversial. Some authors found out that carrier addition enhanced the stability of radiolabelled compounds [6, 14, 15], and others didn’t reveal this regularity [3, 4, 12]. In this study the stability of $^{188}$Re-OENTMP in vivo was evaluated by thyroid uptake as free $^{188}$Re has high affinity to thyroid tissue mediated by the sodium-iodide symporter [16]. The highest uptake of carrier-free $^{188}$Re-OENTMP was 13.97 % ID/g at 5 min post-injection. The amount of carrier-added preparation of $^{188}$Re-OENTMP didn’t exceed 8.51 % ID/g (table 1). However, carrier-free formulation removed faster than carrier-added $^{188}$Re-OENTMP from thyroid. All these findings didn’t permit to make a single-valued conclusion about the influence of gallium carrier on stability of $^{188}$Re-OENTMP in vivo.

The excretion of radiolabelled phosphonates is occurred via urinary tract [3, 7, 8]. In our study kidneys also showed high uptake of $^{188}$Re-OENTMP.

The amounts of radioactivity in soft organs were low. Only in lungs up to 1.44 % ID/g of carrier-added $^{188}$Re-OENTMP was accumulated. The levels of carrier-added $^{188}$Re-OENTMP in liver, muscle and spleen didn’t exceed 0.75 %, 0.44 % and 0.37 % ID/g, respectively. The activity levels of carrier-free $^{188}$Re-OENTMP in soft organs were all lower than 0.53 % ID/g (table 1). Moreover, the uptake in soft organs and tissues were found to reduce with time.

4. Summary
Biodistribution studies of carrier-added and carrier-free $^{188}$Re-OENTMP in wild-type rats demonstrated rapid and selective skeletal uptake, fast clearance from blood and low uptake in any of the major organs and tissues. However, carrier-added $^{188}$Re-OENTMP showed higher skeletal accumulation (up to 57.37 % ID) compared to that of carrier-free formulation (up to 22.75 % ID) throughout the time point studied. The highest uptakes of $^{188}$Re-OENTMP in all bones were observed at 1 h. In soft organs and tissues the amounts of carrier-added formulation were also slightly higher than carrier-free complex, especially within 3 h post-injection. The excretion of $^{188}$Re-OENTMP was occurred via urinary tract. In conclusion, the addition of gallium carrier had a great influence on biodistribution of $^{188}$Re-OENTMP, particularly in skeleton.

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