Nuclear Medicine and Biology 42 (2015) 465–469

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Drug composition matters: the influence of carrier concentration on the radiochemical purity, hydroxyapatite affinity and in-vivo bone accumulation of the therapeutic radiopharmaceutical ^{188}Rhenium-HEDP

R. Lange [a], J.M.H. de Klerk [b], H.J. Bloemendal [c], R.M. Ramakers [d], F.J. Beekman [d], M.M.L. van der Westerlaken [e], N.H. Hendrikse [f], R. ter Heine [g,*]

[a] Meander Medical Center, Department of Clinical Pharmacy, Amersfoort, The Netherlands
[b] Meander Medical Center, Department of Radiology & Nuclear Medicine, Amersfoort, The Netherlands
[c] Meander Medical Center, Department of Internal Medicine, Amersfoort, The Netherlands
[d] Section Radiation, Detection & Medical Imaging, TU Delft & MILabs B.V., Utrecht, The Netherlands
[e] Meander Medical Center, Department of Pharmacy, Amersfoort, The Netherlands
[f] Department of Clinical Pharmacology & Pharmacy, VU University Medical Center, Amsterdam, The Netherlands
[g] Meander Medical Center, Department of Clinical Pharmacy, Maatweg 3, 3813TZ, Amersfoort, The Netherlands

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 22 December 2014
Accepted 12 January 2015

Keywords:
Rhenium-188
Rhenium-HEDP
Rhenium-188-HEDP
Hydroxyapatite
Bone affinity

ABSTRACT

Introduction: ^{188}Rhenium-HEDP is an effective bone-targeting therapeutic radiopharmaceutical, for treatment of osteoblastic bone metastases. It is known that the presence of carrier (non-radioactive rhenium as ammonium perrhenate) in the reaction mixture during labeling is a prerequisite for adequate bone affinity, but little is known about the optimal carrier concentration.
Methods: We investigated the influence of carrier concentration in the formulation on the radiochemical purity, in-vitro hydroxyapatite affinity and the in-vivo bone accumulation of ^{188}Rhenium-HEDP in mice.
Results: The carrier concentration influenced hydroxyapatite binding in-vitro as well as bone accumulation in-vivo. Variation in hydroxyapatite binding with various carrier concentrations seemed to be mainly driven by variation in radiochemical purity. The in-vivo bone accumulation appeared to be more complex: satisfactory radiochemical purity and hydroxyapatite affinity did not necessarily predict acceptable bio-distribution of ^{188}Rhenium-HEDP.
Conclusions: For development of new bisphosphonate-based radiopharmaceuticals for clinical use, human administration should not be performed without previous animal bio-distribution experiments. Furthermore, our clinical formulation of ^{188}Rhenium-HEDP, containing 10 μmol carrier, showed excellent bone accumulation that was comparable to other bisphosphonate-based radiopharmaceuticals, with no apparent uptake in other organs.
Advances in knowledge: Radiochemical purity and in-vitro hydroxyapatite binding are not necessarily predictive of bone accumulation of ^{188}Rhenium-HEDP in-vivo.
Implications for patient care: The formulation for ^{188}Rhenium-HEDP as developed by us for clinical use exhibits excellent bone uptake and variation in carrier concentration during preparation of this radiopharmaceutical should be avoided.

1. Introduction

^{188}Rhenium-HEDP is a therapeutic radiopharmaceutical that can be used for treatment of painful bone metastases. It is a complex of the radionuclide ^{188}Rhenium, which decays with a half-life of 17 hours by emission of β^- (2.2 MeV) and γ (155 keV) radiation, with the bisphosphonate HEDP (disodiumetidronate) and stannous chloride in the presence of gentisic acid [1–3]. The radiochemical properties and bio-distribution of ^{188}Rhenium-HEDP are very similar to those of other radionuclide-bisphosphonate complexes that are routinely used, like the diagnostic agents ^{99m}Technetium-oxidronate and ^{99m}Technetium-medronate [4,5]. The pharmacodynamic action of ^{188}Rhenium-HEDP is explained by its high affinity for hydroxyapatite, that is abundantly present in osteoblastic bone metastases, resulting in accumulation and local delivery of a high radiation dose and subsequent destruction of malignant surrounding tissue [6]. Carrier-free ^{188}Rhenium (as sodium perrhenate solution) can be easily obtained from a ^{188}Tungsten/^{188}Rhenium-generator, which operates similar to the widely used ^{99}Molybdenum/^{99m}Technetium-generator. Therefore, ^{188}Rhenium-HEDP can be produced directly on-site upon presentation of a patient with painful bone metastases. Hence, ^{188}Rhenium-HEDP has advantages over commercially available therapeutic bone-seeking radiopharmaceuticals, like ^{89}Strontium chloride, ^{153}Samarium-lexidronam and ^{223}Radium

* Corresponding author. Tel.: +31 338502335.
E-mail address: rob@terheine.nl (R. ter Heine).

http://dx.doi.org/10.1016/j.nucmedbio.2015.01.007
0969-8051/

chloride, which have a delivery period of 1 to 2 weeks. Furthermore, it has a favorable toxicity profile with a relatively short half-life of 17 hours, but with a high energetic β^- radiation of 2.2 MeV [3,6].

In contrast to commercially available bone-targeting radiopharmaceuticals, generators and labeling kits for the production of ^{188}Rhenium-HEDP are not available as products with a marketing authorization. Also, there is no standardized labeling method available. It is known, however, that presence of carrier (non-radioactive Rhenium as perrhenate) in the reaction mixture during labeling is a prerequisite for adequate bone accumulation *in vivo* [2,7]. Although these previous studies showed that addition of carrier (perrhenate) to the reaction mixture was essential for adequate complex quality and bone affinity, the results are likely not comparable with ours, because the composition of the HEDP-cold kit (containing HEDP, stannous chloride and gentisic acid or ascorbic acid) as well as the reaction conditions (like elution volume, heating time and temperature) were different. Furthermore, previously no thorough in-vitro hydroxyapatite binding experiments were performed to investigate the optimal carrier concentration.

At our institute, we have recently developed a standardized production method for GMP grade ^{188}Rhenium-HEDP for clinical use [3]. During pharmaceutical development, we chose to add 10 μmol of ammonium perrhenate to the reaction mixture, together with 200 μmol of HEDP, 78 μmol of stannous chloride and 97 μmol of gentisic acid, to obtain a molar carrier-to-bisphosphonate ratio in the range of 1:50 to 1:20 as described previously [8,9]. This resulted in excellent bone uptake *in vivo* [3]. Other groups have, however, used a wide range of different formulations, with carrier-to-bisphophonate ratios as low as even 1:200 or 1:600 [2,10]. To date, the optimal carrier concentration for adequate bone affinity of ^{188}Rhenium-HEDP is unknown. This knowledge, however, is crucial to assess the comparability between the different ^{188}Rhenium-HEDP formulations that are used in research and clinical practice. Furthermore, in order to guarantee a pharmaceutical product with reproducible bone affinity, it is vital to know the influence of variation in carrier concentration during labeling of ^{188}Rhenium-HEDP on bone affinity. Lastly, the gold standard for quality control of bisphosphonate-based radiopharmaceuticals is the determination of the radiochemical purity. It is unknown, however, whether radiochemical purity alone is predictive of hydroxyapatite affinity or bone accumulation of ^{188}Rhenium-HEDP. Therefore, we investigated the influence of variation in carrier concentration on the radiochemical purity and on the in-vitro hydroxyapatite binding of ^{188}Rhenium-HEDP and also compared the hydroxyapatite binding of ^{188}Rhenium-HEDP to that of commercially available bisphosphonate-based radiopharmaceuticals. Furthermore, the influence of varying carrier concentrations on the *in vivo* bone accumulation of ^{188}Rhenium-HEDP was studied in mice.

2. Methods

2.1. Preparation and quality control of ^{188}Rhenium-HEDP

Preparation of ^{188}Rhenium-HEDP was performed as described previously (3), but with varying amounts of carrier (as ammonium perrhenate), without changing other reaction conditions. Directly after preparation, the radiochemical purity of the complex was determined using thin layer chromatography as described previously (3). The resulting radioligands were not purified before use in the experiments.

2.2. Preparation of other bone-targeting radiopharmaceuticals

The skeletal imaging agents ^{99m}Technetium-oxidronate and ^{99m}Technetium-medronate were obtained ready-made from the GE Healthcare Radiopharmacy (Eindhoven, The Netherlands) with a radiochemical purity for both compounds of ≥ 95%, as specified in the product leaflet [11,12]. The therapeutic bone-targeting radiopharmaceutical ^{153}Samarium-lexidronam was obtained ready-made from IBA Molecular (Louvain-la-Neuve, Belgium), with a radiochemical purity of ≥ 99% as specified by the manufacturer [13].

2.3. Hydroxyapatite binding experiments

The hydroxyapatite binding assay was carried out as described previously [3,14,15]. In short, Bio-Gel HTP hydroxyapatite crystals were obtained from Bio-Rad (Veenendaal, The Netherlands), and phosphate buffered saline (PBS) was obtained from VWR (Amsterdam, The Netherlands). Then, 0.10 mL of the studied radiopharmaceutical was added to 3.0 mL of phosphate buffered saline with a predetermined amount of the hydroxyapatite crystals in a 5 mL polypropylene test tube. The tube was then closed, inverted a few times, vortex-mixed for 3 seconds and subsequently incubated at 37 °C for 120 minutes. Thereafter, the radioactivity was measured in each test tube, and the contents of the test tube were filtered through a 0.2 μm syringe filter (Sartorius-Stedim, Nieuwegein, The Netherlands). Then, the activity of 1.0 mL of the filtrate was measured, and the hydroxyapatite binding was calculated as described in Eq. (1).

$$\text{Hydroxyapatite binding}(\%) = 100 - \frac{100 \times \text{activity of 1.0 ml filtrate}}{[\text{activity of tube after incubation}/3.1]} \quad (1)$$

To assure that variability in hydroxyapatite binding was a result of variation in hydroxyapatite affinity of the radiopharmaceutical and not variability in the total binding capacity of the hydroxyapatite, initially experiments were performed with increasing amounts of hydroxyapatite crystals (0–500 mg) to establish the quantity in which an excess of hydroxyapatite was present. Then, using the established amount of hydroxyapatite, the hydroxyapatite binding was determined in triplicate for ^{99m}Technetium-oxidronate, ^{99m}Technetium-medronate and ^{153}Samarium-lexidronam. These bi- and tetra-phosphonate-based radiopharmaceuticals were chosen, because their in-vivo distribution is similar to that of ^{188}Rhenium-HEDP and because they are licensed drugs that are commercially available and widely used for diagnostic and therapeutic purposes. Their hydroxyapatite affinity can therefore be considered a reference standard for our newly developed ^{188}Rhenium-HEDP. Also, the hydroxyapatite binding of ^{188}Rhenium-HEDP prepared without carrier and with amounts of 0.01 μmol, 0.1 μmol, 1 μmol, 10 μmol, 20 μmol, 50 μmol and 100 μmol of carrier in the reaction vial during labeling was investigated. Lastly, the affinity of unbound ^{188}Rhenium (as perrhenate, the main degradation product of ^{188}Rhenium-HEDP) was determined, to ensure that the results of the hydroxyapatite binding experiments were not obscured by degradation of the complex or presence of perrhenate due to incomplete labeling. Significance in differences between hydroxyapatite binding results were calculated with a double sided Student's t-test. Correlation between variables was determined with simple linear regression.

2.4. In-vivo bone accumulation experiments

To explore the in-vivo bone affinity, ^{188}Rhenium-HEDP was prepared in three different formulations, based on the results of the in-vitro hydroxyapatite binding experiments. Each formulation of ^{188}Rhenium-HEDP was administered to 3 male C57Bl6/J mice with an average body weight of 34 gram in a dose of 37 MBq. Four hours after tail vein injection, a single photon emission computed tomography (SPECT) image was acquired during 60 minutes with a VECTor/CT (MILabs B.V., Utrecht, The Netherlands) [16] equipped with a high energy ultra high resolution mouse (HE-UHR-M) collimator. During image acquisition, mice were anesthetized by isofluorane (2.5% flow rate) and kept under anesthesia via a nose-cone setup. The animal experiments were carried out in compliance with Dutch laws relating to the conduct of animal experimentation.

Table 1
Hydroxyapatite binding experiments results.

Radiopharmaceutical	Hydroxyapatite binding (mean ± SD)	Radiochemical purity
^{188}Rhenium-HEDP prepared without carrier	43.3% ± 3.3	35.2%
^{188}Rhenium-HEDP prepared with 0.01 μmol carrier	81.0% ± 0.2	96.1%
^{188}Rhenium-HEDP prepared with 0.1 μmol carrier	95.4% ± 0.8	98.4%
^{188}Rhenium-HEDP prepared with 1 μmol carrier	97.1% ± 0.3	98.7%
^{188}Rhenium-HEDP prepared with 10 μmol carrier	95.6% ± 1.4	98.3%
^{188}Rhenium-HEDP prepared with 20 μmol carrier	96.3% ± 0.2	100%
^{188}Rhenium-HEDP prepared with 50 μmol carrier	66.5% ± 1.9	68.1%
^{188}Rhenium-HEDP prepared with 100 μmol carrier	37.6% ± 0.3	43.6%
^{99m}Technetium-oxidronate	99.0% ± 0.1	≥95%*
^{99m}Technetium-medronate	99.8% ± 0.1	≥95%*
^{153}Samarium-lexidronam	100.0% ± 0	≥99%*

* As specified by the manufacturer.

From the acquired scintigraphic data 3D SPECT images were reconstructed with pixel based ordered subset expectation maximization (POSEM) with 4 subsets and 25 iterations [17,18] followed by 1.2 mm wide 3D Gaussian post filter. Thereafter, the healthy-bone-to-soft-tissue ratio was investigated by drawing a volume of interest (VOI) around three lumbar vertebrae and a background VOI around surrounding soft tissue. The healthy-bone-to-soft-tissue ratio was calculated by the uptake in the vertebrae divided by the background uptake.

3. Results and discussion

3.1. Hydroxyapatite binding experiments

We found that the hydroxyapatite binding reached a maximum for all radiopharmaceuticals at hydroxyapatite quantities of at least 200 mg suspended in 3 mL of PBS (data not shown). Therefore, an amount of 300 mg hydroxyapatite was chosen for all subsequent experiments. We also found that unbound ^{188}Rhenium (as perrhenate) did not bind to hydroxyapatite (data not shown).

The results of the hydroxyapatite binding experiments are summarized in Table 1 and depicted in Fig. 1. The hydroxyapatite binding of the commercially available bisphosphonate-based radiopharmaceuticals ^{99m}Technetium-oxidronate, ^{99m}Technetium-medronate and ^{153}Samarium-lexidronam was high (>99%) and comparable to hydroxyapatite binding percentages for bisphosphonate-based radiopharmaceuticals as established by other groups, being 80–100% [14,15,19–22].

As expected, ^{188}Rhenium-HEDP without carrier showed a relative low hydroxyapatite binding of 43.3% ± 3.3% (SD). The hydroxyapatite binding was > 80% at a carrier amount of 0.01 μmol, increased to a plateau of approximately 95% at carrier amounts between 0.1 and 20 μmol (see Fig. 1), and this was comparable to the hydroxyapatite affinity of the commercially available phosphonate-based radiopharmaceuticals ^{99m}Technetium-oxidronate, ^{99m}Technetium-medronate and ^{153}Samarium-lexidronam. At amounts of more than 20 μmol of carrier, the hydroxyapatite binding of ^{188}Rhenium-HEDP decreased again. Our recently developed formulation of ^{188}Rhenium-HEDP for clinical use contains 10 μmol of carrier. The mean hydroxyapatite binding of this formulation was found to be 96% and thus well within the range wherein hydroxyapatite binding of ^{188}Rhenium-HEDP reached its maximum.

The radiochemical purity showed a similar trend as the hydroxyapatite binding. Without carrier, the radiochemical purity was low and did not meet the specification of > 93% for release for clinical use [3]. The radiochemical purity of the complex came within specification (>93%) at carrier amounts of 0.01 μmol and more and decreased to < 70% at carrier amounts of 50 μmol and above. As shown in Fig. 2, the mean hydroxyapatite binding strongly correlated with the radiochemical purity of ^{188}Rhenium-HEDP ($R^2 = 0.9435$), which indicated that the decreased hydroxyapatite binding was mainly a result of presence of free perrhenate in the solution. We hypothesize that the observed apparent maximum in hydroxyapatite binding of ^{188}Rhenium-HEDP is the result of a slow reaction rate at low carrier concentrations as well as a result of an incomplete labeling due to relatively insufficient amounts of HEDP, stannous chloride or gentisic acid at high carrier concentrations. However, this hypothesis needs further confirmation by further experimentation. Since the radiochemical purity of the formulation that has been chosen for clinical use was well above 93%, further purification of the drug product to improve radiochemical purity was not deemed necessary.

3.2. In-vivo bone accumulation experiments

Although the influence of an extensive range of carrier amounts (0–100 μmol) on the hydroxyapatite binding of Rhenium-188-HEDP was investigated *in vitro*, we did not find it ethical to study such a large range in mice. We therefore chose to study a limited set of ^{188}Rhenium-HEDP formulations, containing amounts of 0 μmol, 0.01 μmol and

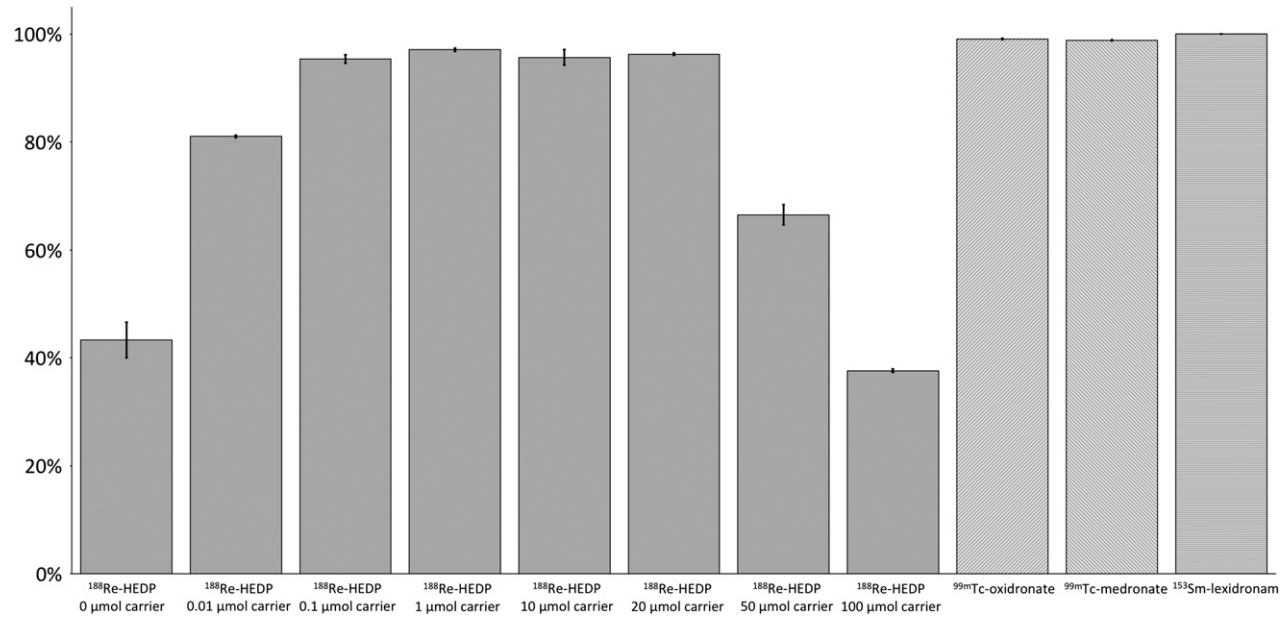

Fig. 1. Hydroxyapatite binding (%) for the various tested radiopharmaceuticals with vertical error bars depicting the standard deviation.

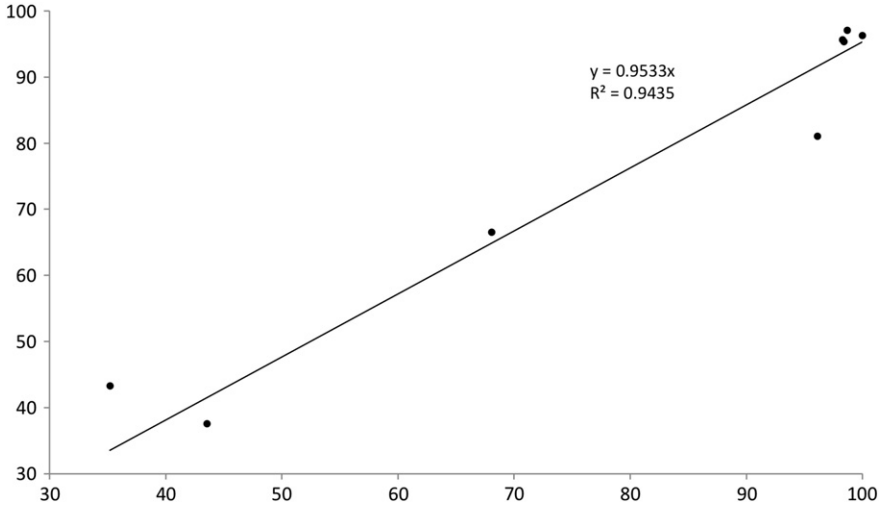

Fig. 2. Mean hydroxyapatite binding (%, Y-axis) of ^{188}Rhenium-HEDP versus radiochemical purity (%, X-axis).

10 μmol carrier. This choice was based on the rationale that we would like to compare the in-vivo bone affinity of our clinical ^{188}Rhenium-HEDP formulation (with 10 μmol carrier) with the formulation that still showed adequate hydroxyapatite affinity (>80% binding) and radiochemical purity (>93%) (formulation with 0.01 μmol carrier) and with the formulation without carrier.

Representative scintigrams are presented in Fig. 3 for each studied ^{188}Rhenium-HEDP formulation. Generally, the healthy-bone-to-soft-tissue ratio of bisphosphonate-based radiopharmaceuticals is approximately 10–25 [23–25]. As shown in Fig. 3A, no bone accumulation could be observed for the ^{188}Rhenium-HEDP formulation without carrier, and uptake of activity in the bladder, stomach and thyroid gland of the mice could be observed. With a carrier amount of 0.01 μmol the mean healthy-bone-to-soft-tissue ratio increased to 3.4 ± 1.1 (SD), but still uptake in the bladder, stomach and thyroid gland could be observed. We attribute the uptake of activity in the bladder, stomach and thyroid gland to the accumulation of free perrhenate in these organs, analogous to accumulation of ^{99m}Technetium-pertechnetate, by uptake of these chemically similar ions by the sodiumiodide transporter [26]. The uptake ratio increased further to 12.3 ± 2.3 (SD) with a carrier quantity of 10 μmol in the formulation, and unwanted uptake of activity in other organs with this formulation was not observed.

The formulation without carrier showed a hydroxyapatite binding of 43.3%, but to our surprise no uptake of activity in bone could be observed for this formulation in mice. Furthermore, the formulation

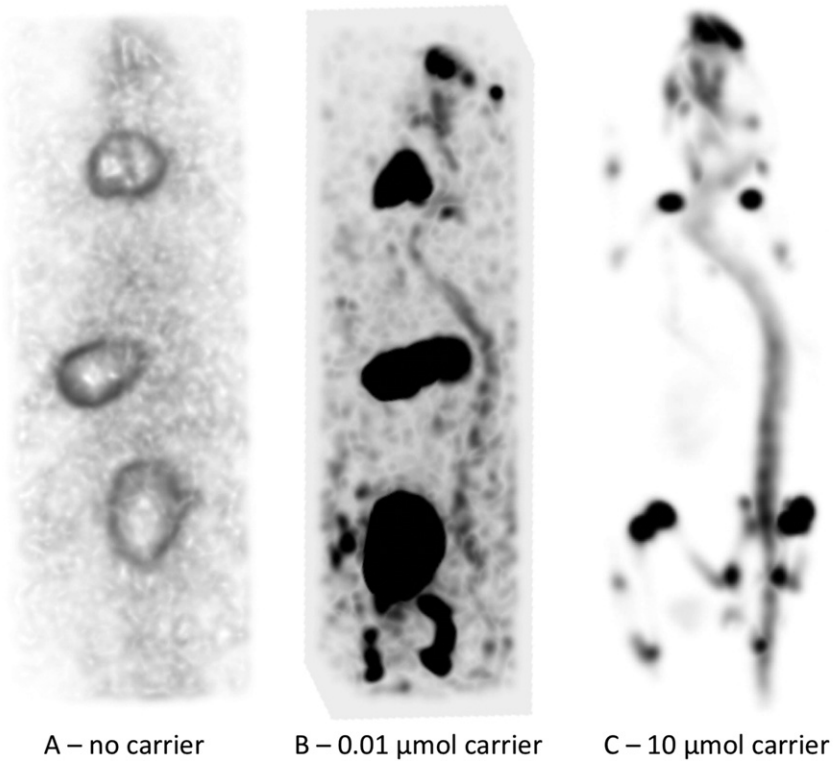

Fig. 3. Typical scintigrams showing the bone-uptake with varying amounts of carrier in the formulation. Four hours after tail vein injection of 37 MBq of the radiopharmaceutical, a single photon emission computed tomography (SPECT) image was acquired during 60 minutes. During acquisition, mice were anesthetized by isofluorane (2.5% flow rate) and kept under anesthesia via a nose-cone setup for imaging.

with 0.01 μmol carrier that demonstrated adequate radiochemical purity (>93%) and hydroxyapatite binding (>80%) only exhibited marginal bone uptake *in vivo*, combined with unwanted uptake of activity in other organs. The cause for this phenomenon remains to be investigated, but we postulate that the ^{188}Rhenium-HEDP complex is less stable when labeled without carrier or with low carrier amounts, resulting in *in vivo* degradation and release of free perrhenate from the complex in the body. The observation of significant uptake of activity in the stomach, thyroid gland and bladder, which is typical for perrhenate [26], supports this hypothesis.

4. Conclusion

We have shown that the amount of carrier (as ammonium perrhenate) that is present in the reaction mixture during labeling, influences hydroxyapatite binding *in vitro* as well as bone accumulation *in vivo*. With regard to hydroxyapatite binding, the variation in binding seemed to be mainly driven by variation in radiochemical purity. Furthermore, we observed an optimum in hydroxyapatite binding and radiochemical purity depending on the carrier concentration. The *in vivo* bone accumulation appeared to be a more complex process, where satisfactory radiochemical purity and hydroxyapatite affinity were not necessarily predictive for acceptable bio-distribution. This finding warrants further investigations, for example of the *in vivo* stability of the ^{188}Rhenium-HEDP complex in various formulations.

We conclude that for development of new bisphosphonate-based radiopharmaceuticals for clinical use, human administration should not be performed without animal bio-distribution experiments. Furthermore, our clinical formulation of ^{188}Rhenium-HEDP, containing 10 μmol of carrier, showed excellent bone accumulation that was comparable to other bisphosphonate-based radiopharmaceuticals and with no apparent uptake in other organs, supporting our assumption that a proper formulation was chosen for clinical application. We are now thoroughly assessing the benefit-to-risk ratio of the application of ^{188}Rhenium-HEDP for treatment of painful osteoblastic bone metastases of different types of cancer in routine clinical practice in ongoing clinical studies.

Acknowledgements

R.G. Rummenie and A.M. Wakker of the Radiopharmacy department of the Meander Medical Center are greatly acknowledged for their help with the experiments.

References

[1] Elder RC, Yuan J, Helmer B, Pipes D, Deutsch K, Deutsch E. Studies of the structure and composition of Rhenium-1,1-Hydroxyethylidenediphosphonate (HEDP) analogues of the radiotherapeutic agent (186)ReHEDP. Inorg Chem 1997;36(14):3055–63.
[2] Maxon III HR, Schroder LE, Washburn LC, Thomas SR, Samaratunga RC, Biniakiewicz D, et al. Rhenium-188(Sn)HEDP for treatment of osseous metastases. J Nucl Med 1998;39(4):659–63.
[3] ter Heine R, Lange R, Breukels OB, Bloemendal HJ, Rummenie RG, Wakker AM, et al. Bench to bedside development of GMP grade Rhenium-188-HEDP, a radiopharmaceutical for targeted treatment of painful bone metastases. Int J Pharm 2014;465(1–2):317–24.
[4] Deutsch E, Libson K, Vanderheyden JL, Ketring AR, Maxon HR. The chemistry of rhenium and technetium as related to the use of isotopes of these elements in therapeutic and diagnostic nuclear medicine. Int J Rad Appl Instrum B 1986;13(4):465–77.
[5] Tisato F, Bolzati C, Porchia M, Refosco F. Contribution of electrospray mass spectrometry for the characterization, design, and development of nitrido technetium and rhenium heterocomplexes as potential radiopharmaceuticals. Mass Spectrom Rev 2004;23(5):309–32.
[6] Lam MG, de Klerk JM, van Rijk PP, Zonnenberg BA. Bone seeking radiopharmaceuticals for palliation of pain in cancer patients with osseous metastases. Anticancer Agents Med Chem 2007;7(4):381–97.
[7] Lin WY, Hsieh JF, Lin CP, Hsieh BT, Ting G, Wang SJ, et al. Effect of reaction conditions on preparations of rhenium-188 hydroxyethylidene diphosphonate complexes. Nucl Med Biol 1999;26(4):455–9.
[8] Liepe K, Kropp J, Runge R, Kotzerke J. Therapeutic efficiency of rhenium-188-HEDP in human prostate cancer skeletal metastases. Br J Cancer 2003;89(4):625–9.
[9] Palmedo H, Guhlke S, Bender H, Sartor J, Schoeneich G, Risse J, et al. Dose escalation study with rhenium-188 hydroxyethylidene diphosphonate in prostate cancer patients with osseous metastases. Eur J Nucl Med 2000;27(2):123–30.
[10] Lin WY, Lin CP, Yeh SJ, Hsieh BT, Tsai ZT, Ting G, et al. Rhenium-188 hydroxyethylidene diphosphonate: a new generator-produced radiotherapeutic drug of potential value for the treatment of bone metastases. Eur J Nucl Med 1997;24(6):590–5.
[11] Technescan HDP - Summary of Product Characteristics. http://db.cbg-meb.nl/IB-teksten/h16133.pdf; 2013. [7-17-2014.].
[12] Medronaat DRAXIMAGE - Summary of Product Characteristics. http://db.cbg-meb.nl/IB-teksten/h57717.pdf; 2013. [7-17-2014.].
[13] Quadramet - EMEA Scientific Discussion. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000150/WC500041752.pdf; 2004. [7-17-2014.].
[14] Ogawa K, Mukai T, Inoue Y, Ono M, Saji H. Development of a novel 99mTc-chelate-conjugated bisphosphonate with high affinity for bone as a bone scintigraphic agent. J Nucl Med 2006;47(12):2042–7.
[15] Uehara T, Jin ZL, Ogawa K, Akizawa H, Hashimoto K, Nakayama M, et al. Assessment of 186Re chelate-conjugated bisphosphonate for the development of new radiopharmaceuticals for bones. Nucl Med Biol 2007;34(1):79–87.
[16] Goorden MC, van der Have F, Kreuger R, Ramakers RM, Vastenhouw B, Burbach JP, et al. VECTor: a preclinical imaging system for simultaneous submillimeter SPECT and PET. J Nucl Med 2013;54(2):306–12.
[17] Branderhorst W, Vastenhouw B, Beekman FJ. Pixel-based subsets for rapid multi-pinhole SPECT reconstruction. Phys Med Biol 2010;55(7):2023–34.
[18] van der Have F, Vastenhouw B, Rentmeester M, Beekman FJ. System calibration and statistical image reconstruction for ultra-high resolution stationary pinhole SPECT. IEEE Trans Med Imaging 2008;27(7):960–71.
[19] Okamoto Y. Accumulation of technetium-99 m methylene diphosphonate. Conditions affecting adsorption to hydroxyapatite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;80(1):115–9.
[20] Francis MD, Ferguson DL, Tofe AJ, Bevan JA, Michaels SE. Comparative evaluation of three diphosphonates: in vitro adsorption (C- 14 labeled) and in vivo osteogenic uptake (Tc-99 m complexed). J Nucl Med 1980;21(12):1185–9.
[21] Chirby D, Franck S, Troutner DE. Adsorption of 153Sm-EDTMP on calcium hydroxyapatite. Appl Radiat Isot 1988;39(6):495–9.
[22] Hashimoto K, Matsuoka H, Izumo M. Adsorption of 188 Re complexes with aminomethylenephosphonate on hydroxyapatite. J Nucl Radiochem Sci 2001;2(1–2):5–9.
[23] Bergqvist L, Brismar J, Cederquist E, Darte L, Naversten Y, Palmer J. Clinical comparison of bone scintigraphy with 99Tcm-DPD, 99Tcm-HDP and 99Tcm-MDP. Acta Radiol Diagn (Stockh) 1984;25(3):217–23.
[24] Fruhling J, Verbist A, Balikdjian D. Which diphosphonate for routine bone scintigraphy (MDP, HDP or DPD)? Nucl Med Commun 1986;7(6):415–25.
[25] Pauwels EK, Blom J, Camps JA, Hermans J, Rijke AM. A comparison between the diagnostic efficacy of 99mTc-MDP, 99mTc-DPD and 99mTc-HDP for the detection of bone metastases. Eur J Nucl Med 1983;8(3):118–22.
[26] Zuckier LS, Dohan O, Li Y, Chang CJ, Carrasco N, Dadachova E. Kinetics of perrhenate uptake and comparative biodistribution of perrhenate, pertechnetate, and iodide by NaI symporter-expressing tissues in vivo. J Nucl Med 2004;45(3):500–7.