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BIODISTRIBUTION STUDY OF ^{188}Re-HEDP AS A RADIOPHARMACEUTICAL FOR BONE PAIN PALLIATION

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Abstract

Bone seeking radiopharmaceuticals like Rhenium-188 hydroxy ethylidene diphosphonate (^{188}Re-HEDP) are used for palliative treatment of bone metastases, and as target radionuclide therapy in patients with inoperable osteosarcoma in addition to the external radiation therapy.

In this study we evaluated the preparation, quality control and biodistribution of ^{188}Re-HEDP in mice to determine the in vivo affinity to the target bone tissue and to the other non-target tissues and organs. ^{188}Re-HEDP was prepared with radiochemical purity of 98.87%.

The biodistribution data showed high uptake and durability in the skeletal tissues without significant uptake in other tissues and organs. In conclusion, whit chosen ^{188}Re-HEDP kit formulation and the prescribed conditions in our study we obtained a radiopharmaceutical with promising therapeutic efficiency in palliative treatment of primary bone tumors as well as secondary osseous metastatic lesions.

Keywords: bone pain palliation, radionuclide therapy, radiopharmaceuticals, biodistribution, Rhenium-188.

Introduction

Nuclear medicine is a medical specialty where radionuclides are used for diagnostic or therapeutic purpose in the form of radiopharmaceuticals.

Apart from the radiochemical purity, the in vivo behavior is also of great importance for the prepared radiopharmaceuticals, particularly for obtaining a high-quality and reliable image in the case of diagnostic radiopharmaceuticals, and local delivery of radiation dose in pathological tissues concerning therapeutic radiopharmaceuticals. Animal biodistribution studies are generally used to estimate the in vivo stability, behavior and the affinity to the target tissues and organs[1].

In recent years, therapeutic radiopharmaceuticals are re-emerging as attractive anticancer agents, with specific, very selective, and deliverable characteristics for given, molecularly defined cancers for which they are intended to treat [2].^{188}Re-HEDP is a relatively new osteotropic radiopharmaceutical used for palliative treatment of bone metastases, as well as for targeted radionuclide therapy in patients with inoperable osteosarcoma, as an adjunct to radiation therapy.

The physical characteristics, the method of obtaining and the lower cost make ^{188}Re suitable for use in nuclear medicine centers of different types, which is why ^{188}Re-HEDP is proposed as an alternative to ^{186}Re-HEDP.

One of the problems with osteotropic radiopharmaceuticals for diagnostic and therapeutic purposes is that increased soft tissue accumulation sometimes occurs, which does not correlate with the percentage of unbound radioactivity obtained through in vitro radiochemical chromatographic analysis.

This biodistribution study was performed in order to determine the affinity of ^{188}Re-HEDP to bone tissue as a target, but also its affinity to other non-target tissues and organs in in vivo conditions [3].

Material and Methods

Radioactive labeling of HEDP with ^{188}Re:

^{188}Re-HEDP is prepared by adding 1-3mL of $^{188}ReO_4$- to a kit vial containing 1 mL of HEDP with an activity of 1-4 GBq, and 20 mL of "carrier"[1], which is a solution of stable Re in the chemical form of NH_4ReO_4. After mixing intensively for 1-5 minutes, the mixture is placed in a water bath at 100^{o}C for 30 minutes.

The solution is then left to cool to room temperature or under running cold water, and 2 ml of Na-acetate buffer is added, adjusting the pH to about 5-6 (4).

[1]Preparation of the "carrier" solution: 20g NH_4ReO_4 (Aldrich Chemical Co.) are dissolved in 2 mL of previously nitrogenated 0.9% NaCl. This solution is stored at + 4^{o}C.

Radiochemical purity of ^{188}Re-HEDP

The radiochemical purity of ^{188}Re-HEDP was performed by instant thin layer chromatography (ITLC) technique, using ITLC-Silicagel strips developed in 95% acetone [4].

In this chromatographic system, the ^{188}Re-HEDP complex remains at the application site (Rf ≈0.0-0.1), while the free perrhenate migrates with the solvent (Rf ≈0.9-1.0). Radiochemical purity was determined on three chromatographic strips, and the mean value of the three samples was calculated.

Biodistribution study

A biodistribution study was performed using adult laboratory mice of the balb/c type, weighing 17-18 grams. The radiopharmaceutical was injected in the tail vein, 30-50 μCi, in a volume of 0.2-0.3 ml.

The animals were sacrificed at different intervals: 10 min, 30 min, 1 hour, 3, 6, 24, 48 and 72 hours after application of the radiopharmaceutical by excessive dosage with chloroform, according to the prescribed regulations ("Principles of laboratory animal care" - NIH publication no. 86-23, revised 1985). After the sacrifice, the organs of interest were removed, weighed, placed in previously labeled test tubes and stored at -20^{o}C in a refrigerator. The following organs were taken as organs of interest: bones, muscles, heart, thyroid gland, lungs, liver, spleen, kidneys, stomach, small and large intestine.

The weight of the stomach and intestines was measured after removing the contents. Radioactivity was measured simultaneously in all samples to avoid differences in radioactivity due to physical decay of the radioisotope that would occur if they were measured at different time points after application. Finally, the mean of the three animals for the respective time intervals was calculated [5].

The study included 3 animals for each time interval, or a total of 24 animals. Due to the small size of the animals, it was possible to measure the weight and radioactivity of whole organs, with the exception of bones, muscles and blood, for which an additional correction was made, taking blood volume as 7%, bones as 6% and muscles as 10.5% of body weight.

Measurements of radioactivity in the organs were performed in a well-type NaI scintillation counter (Etsko Electronics). The distribution of the radioactivity by organ was calculated as a percentage of the total injected radioactivity per gram of tissue (%ID/g).

The total activity injected was calculated from the net activity of the syringe (minus the activity at the injection site in the tail).

Results

Radiochemical purity of ^{188}Re-HEDP

The labeling efficiencies with the used chromatographic-ITLC technique showed that the mean value of the percentages of formed ^{188}Re HEDP complex for the three samples was 98.87% (SD = 0.21). The results are shown in Fig. 1.

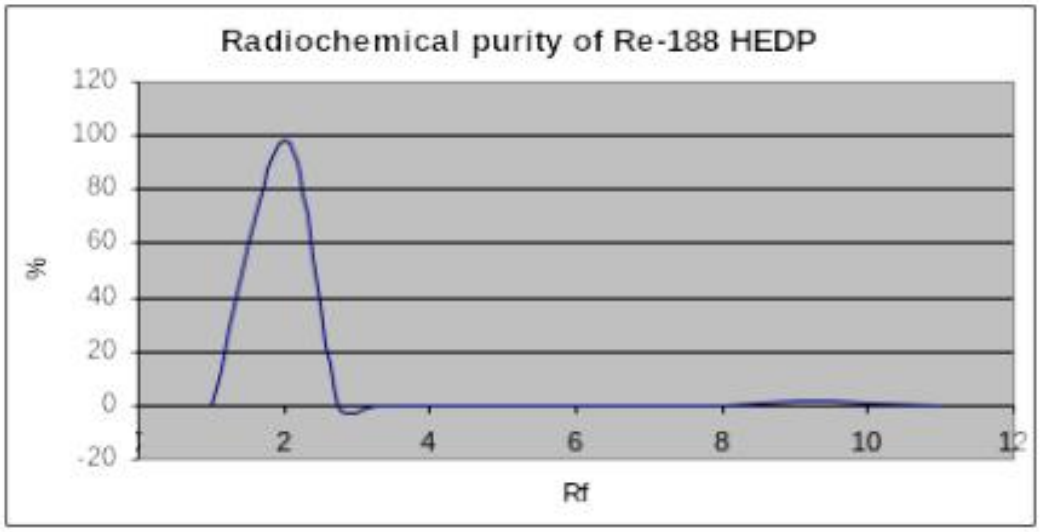
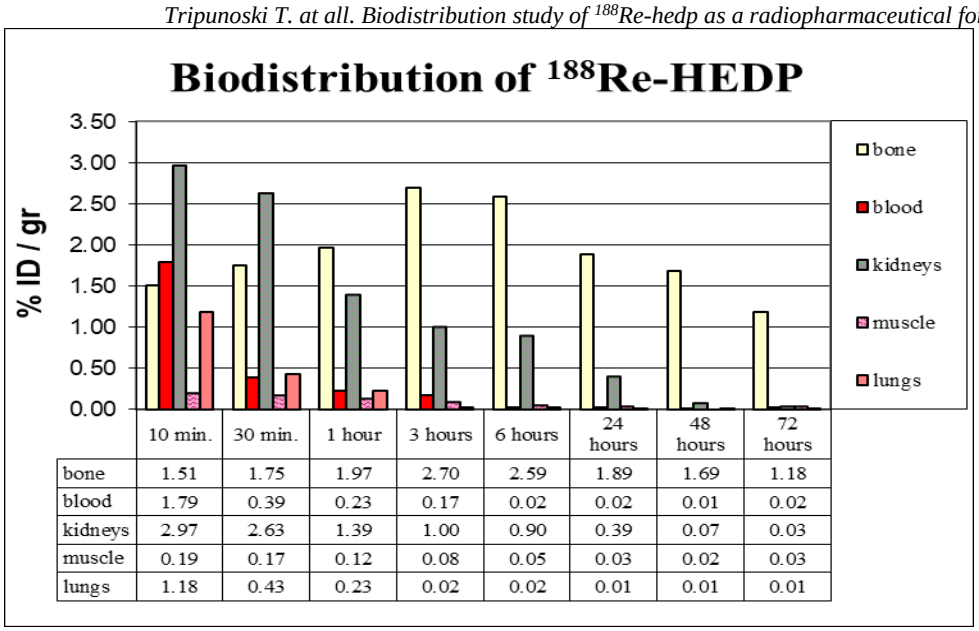


Figure 1. Radiochemical purity of ^{188}Re HEDP using ITLC in saline

Biodistribution study

The results of the biodistribution study are shown numerically in Fig. 2. Selective accumulation of radioactivity in the bone tissue was registered 1 hour after the application of the radiopharmaceutical, 1.97 % ID/g (SD = 0.15). The uptake of ^{188}Re-HEDP in bone tissue reaches its peak with a value of 2.7 % ID/g (SD = 0.09) 3 hours after the application.

The radioactivity in the kidneys and blood shows the highest values 10 minutes after the application of 2.97% ID/g (SD = 0.1) and 1.79 % ID/g(SD = 0.09) respectively, while at later intervals it shows a trend of rapid decline. One hour after the application, the values for the uptake of the radiopharmaceutical in the thyroid gland, liver, lungs and other organs are generally less than 0.5 % ID/g with a trend of significant decrease at later intervals.



	10 min.	30 min.	1 hour	3 hours	6 hours	24 hours	48 hours	72 hours
bone	1.51	1.75	1.97	2.70	2.59	1.89	1.69	1.18
blood	1.79	0.39	0.23	0.17	0.02	0.02	0.01	0.02
kidneys	2.97	2.63	1.39	1.00	0.90	0.39	0.07	0.03
muscle	0.19	0.17	0.12	0.08	0.05	0.03	0.02	0.03
lungs	1.18	0.43	0.23	0.02	0.02	0.01	0.01	0.01

Figure 2. Biodistribution pattern of ^{188}Re-HEDP in mice

The results are presented as the percentage of injected dose per gram of tissue (%ID/gr)

Discussion

Similar to ^{99m}Tc complexes with MDP (methylene diphosphonate) for bone scintigraphy, another 1,1-bisphosphonate analogue, HEDP (hydroxy ethylidene diphosphonate) with ^{188}Re as ^{188}Re-HEDP, is used in palliative treatment of primary and metastatic bone tumors in addition to external beam therapy.

The radio labeling of HEDP carried out using ^{186}Re as a reactor produced radioisotope from neutron radiative capture (n,γ) reaction on enriched ^{185}Re or natural rhenium (37.4% enriched in ^{185}Re). Compared with ^{186}Re, ^{188}Re is characterized by more suitable physical characteristics and appropriate way of obtaining. ^{188}Re is a beta-emitting radionuclide with gamma energy radiation very similar to the gamma irradiation of ^{99m}Tc, which allows monitoring its biodistribution with conventional gamma cameras.

Another important feature of ^{188}Re is that it is a generator, "carrier" free product, obtained from ^{188}W/^{188}Re generator which facilitates its widespread use [6,7].

^{188}Re-HEDP is a chemical complex of bisphosphonate ligand with an incorporated atom of radioactive ^{188}Re. The connection between the ligand and the metal atom (^{188}Re) is through coordinative chemical bonds. In this type of chemical binding, the metal atom is the so-called central atom with an unfilled electron layer, while the ligands possess atoms (N or O) with free electron pairs, so-called donor groups, in the peripheral electron layer [8].

Bisphosphonates are ligands that contain the P-C-P bond which makes the molecules resistant to breakdown by enzymatic hydrolysis and chemically very stable. Similar to pyrophosphate, the bisphosphonates bind to the hydroxyapatite crystals of bone and prevent both their growth and their dissolution. By this mechanism of biodistribution, bisphosphonates inhibit osteoclast bone resorption and play a major role in the palliative treatment of bone tumors, as well as in the treatment of some benign conditions, such as osteoporosis [9].

Following the prescribed protocol for radioactive labeling of HEDP with ^{188}Re, in our study [4], the quality control was performed on the prepared ^{188}Re-HEDP. First, by visual inspection, it was concluded that the radiopharmaceutical is clear, without turbidity and visible particles, while the pH was approximately 6.5. In terms of radiochemical purity, with the used chromatographic-ITLC technique, the percentage of formed ^{188}Re HEDP complex was 98.87% (SD = 0.21). As the chemical properties of ^{188}Re (VII) perrhenate and ^{99m}Tc (VII) pertechnetate are very similar, the choice of ^{188}Re labeling ligands is based on the experience gained from ^{99m}Tc-labeled diagnostic radiopharmaceuticals [10, 11].

The radiopharmaceuticals used in nuclear medicine show great diversity in terms of physical and chemical characteristics [12,13].

Biodistribution mechanisms depend on their physical properties (size and charge of the molecule), their biochemical properties (e.g., radioactive ^{131}I) [14] or specific biological affinities, such as antibodies and receptors ligands [15].

In some radiopharmaceuticals, the mechanism of biodistribution is not completely known [16].

The biodistribution of radiopharmaceuticals cannot often be explained by only one mechanism, but by the interaction of several mechanisms such as: initial dilution, passive and active transmembrane transport, protein binding, metabolic incorporation, as well as elimination and excretion. For larger molecules, radioactive labeling generally does not cause changes in their biodistribution, but for smaller chemical complexes, radioactive labeling (e.g., with ^{99m}Tc, ^{186}Re and ^{188}Re) can lead to changed characteristics.

In addition, degradation of the radiopharmaceutical may occur in vivo, whereby the distribution of radioactivity is due to the free radionuclide but not to the intact molecule of the previously formed radioactive complex [16, 17].

Compared to other studies, our biodistribution study showed similar or higher values for the uptake of ^{188}Re-HEDP in bone tissue [4,18,19].

The maximum uptake of ^{188}Re-HEDP in bone tissue in our study was reached 3 hours after application (2.70 %ID/g) and it remained almost constant until 6 hours after application (2.59 %ID/g).

Compared to the biodistribution study by WY Lin et al. [20], ^{188}Re-HEDP in our study shows a slightly better affinity for bone tissue, which can also be seen from the %ID/g values. In this study, the uptake of ^{188}Re-HEDP in the bone tissue was as high as 1.877% ID/g 1 hour after the application and climbed to 2.017% ID/g 4 hours after the application.

Our results are an indication that the kit formulation allows obtaining a radiopharmaceutical with a high degree of osteotropy, which is the main imperative.

There are several factors that determine the distribution of osteotropic radiopharmaceuticals in living organisms that are not related to the unbound radioactivity in the complex.

These can be divided into those related to the kit formulation of radiopharmaceuticals and to factors that depend on the biological characteristics of living organisms.

Comparative studies showed that the amount of non-radioactive rhenium ("carrier") and the choice of stabilizing agent in the formulation of the kit are the factors that can affect the biodistribution of osteotropic radiopharmaceuticals. HPLC analyses showed that ^{186}Re-HEDP and ^{188}Re-HEDP are a complex mixture of still undefined components. The ionic character of each of the components is a significant factor in their affinity to bone tissue. Comparative studies with the rhenium analogue ^{188}Re-SEDP (2-sulfonatoethzlene-1,1-diphosphonate), which is more basic than ^{188}Re-HEDP, showed that it has a greater affinity to bone tissue [21,22]. This leads to the conclusion that ^{188}Re-HEDP kit formulation, in which complexes with a more basic molecular character prevail, will have a greater uptake in bone tissue.

The affinity of osteotropic radiopharmaceuticals to bind to plasma proteins is a factor that affects their biokinetics and biodistribution. In the study by Schumitschen et al., it was determined that the binding of osteotropic complexes to plasma proteins reduces their uptake in the bone tissue, that is, it slows down the clearance from the blood. This can be proven by the linear correlation between the percentage of the unbound fraction and the bone uptake of ^{99m}Tc-MDP studied in rats [23].

Conclusion

Based on the results obtained from the study, it can be concluded that the prescribed preparation conditions (composition and concentration of chemical components, temperature and incubation time) are optimal for the initial formation of the chelating complex ^{188}Re-HEDP with high purity.

The results of ex vivo biodistribution indicate that ^{188}Re-HEDP kit formulation and preparation conditions in our study allow obtaining a radiopharmaceutical with uptake in bone tissue, without significant accumulation in other non-target tissues and organs.

This radiopharmaceutical can be a useful agent with high therapeutic efficiency in palliative treatment of primary bone tumors as well as secondary osseous metastatic lesions. The physical properties, the method of obtaining and the relatively lower cost make ^{188}Re-HEDP suitable for use in nuclear medical centers of different types.

References

1. Kunos CA, Capala J, Kohn EC, Ivy SP. Radiopharmaceuticals for persistent or recurrent uterine cervix cancer. Front Oncol. 2019; 9:560. doi: 10.3389/fonc.2019.00560 - DOI - PMC – PubMed.
2. Kunos CA, Howells R, Chauhan A, Myint ZW, Bernard ME, El Khouli R, et al. Radiopharmaceutical validation for clinical use. Front Oncol.2021; 11:630827 doi: 10.3389/fonc.2021.630827.
3. Lepareur, N.; Lacœuille, F.; Bouvry, C.; Hindré, F.; Garcion, E.; Chérel, M.; Noiret, N.; Garin, E.; Knapp, F.F.R. Rhenium-188 labeled radiopharmaceuticals: Current clinical applications in oncology and promising perspectives. Front. Med. 2019; 6, 132.
4. Lin WY, Hsieh JF, Lin CP, Hsieh BT, Ting G, Wang SJ, Knapp FF JR. Effect of reaction conditions on preparations of rhenium-188 hydroxy ethylidene diphosphonate complexes. Nucl Med Biol. 1999; 26(4):455-9. doi: 10.1016/s0969-8051(99)00007-4. PMID: 10382850
5. Mani RS. Radiopharmaceuticals, Biodistribution studies-general aspects: Handbook of Nuclear Medicine Practice in Developing Countries. Viena: IAEA, 1991; 213-217.
6. Kleynhans J, Duatti A, Bolzati C. Fundamentals of Rhenium-188 Radiopharmaceutical Chemistry. Molecules. 2023; 28(3):1487. https://doi.org/10.3390/molecules28031487
7. Liepe K. 188Re-HEDP therapy in the therapy of painful bone metastases. World J Nucl Med. 2018; 17(3):133-138. doi: 10.4103/wjnm.WJNM_85_17
8. Pourhabib, Z., Ranjbar, H., Bahrami Samani, A., Azizkalantari, B. Preparation and biological evaluation of 186/188Re-HEDP as a new cocktail radiopharmaceutical for palliative treatment of osseous metastases in wild type rat. Iranian Journal of Nuclear Medicine, 2021; 29(1): 8-14.
9. Drake MT, Clarke BL, Khosla S. Bisphosphonates: Mechanism of action and role in clinical practice. Mayo Clinic proceedings. 2008;83(9):1032-1045. doi: 10.4065/83.9.1032
10. Deutsch E, Libson K, Vanderheyden J, Alan R, Maxon RH. The Chemistry of Rhenium and Techenetium as Related to the Use of Isotopes of these Elements in Therapeutic and Diagnostic Nuclear Medicine, Nucl. Med. Biol. 1986;13(4): 465-477.
11. Tripunoski T. and Mathe D., AndocsG., Balogh L.,Polyak A., Ugrinska A., Chaudhari P.R., JanokiGy.A., Gorgoski I., Vaskova O. Prediction of ^{188}Re-HEDP tumor uptake based on ^{99m}Tc-MDP skeletal scintigraphy using spontaneous osteosarcoma bearing dogs as pathological animal model, Acta Morphologica. 2006; 3(1):38-42.
12. Hosain F, Spencer RP. Radiopharmaceuticals for Palliation of Metastatic Osseus Lesions: Biologic and Physical Background, Seminars in Nuclear Medicine. 1992; 22(2): 11-16.
13. Lewington VJ. Targeted radionuclide therapy for bone metastases, EJNM. 1993; 20:66-74
14. Klain M. et all. Radioiodine therapy for papillary and follicular thyroid carcinoma, EJNM. 2002; 29(2): S479-S485.
15. Pakula RJ, Scott PJH. Applications of radiolabeled antibodies in neuroscience and neuro-oncology. J Labelled Comp Radiopharm. 2023;66(9):269-285. doi: 10.1002/jlcr.4049. Epub 2023 Jun 15. PMID: 37322805
16. Nowotnik DP. Physico-Chemical Concepts in The Preparation of Technetium Radiopharmaceuticals: Textbook of Radiopharmacy. Cambridge: Gordon & Breach Science Publishers. 1994; 29-51.
17. Winter DE et al. Visualisation of the Stomach on Renium-186 HEDP Imaging After Therapy for Metastasized Prostate Carcinoma, Clin.Nuc.Med.1999; 24(11):898-899.
18. Maxon RH et al. Re-188(Sn)HEDP for treatment of osseous metastases, JNM. 1998; 39(4): 659-663.
19. Hsieh BT et al. Comparison of various rhenium-188-labeled diphosphonates for the treatment of bone metastases, Nuclear medicine and Biology. 1999; 26(8):973-976.
20. Lin WJ et al. Rhenium-188 hydroxyethylidene diphosphonate: a new generator produced radiotherapeutic drug of potential value for the treatment of bone metastases, EJNM and Molecular Imagin.1997; 24(6):590-595.
21. Vallabhajosula S, Killeen RP, Osborne JR. Altered biodistribution of radiopharmaceuticals: role of radiochemical/pharmaceutical purity, physiological, and pharmacologic factors. Semin Nucl Med. 2010; 40(4):220-41. doi: 10.1053/j.semnuclmed.2010.02.004. PMID: 20513446

22. Lisic EC et all., Synthesis of a new bisphosphonic acid ligand (SEDP) and preparation of a 188Re-(Sn) SEDP bone seeking radiotracer, Nuclear medicine and Biology. 2001; 28:419-424.
23. Schumichen C. et all, Binding of Technetium-99m to Plasma Proteins on the Distribution of Tc-99m Phosphate Agents, JNM. 1980; 21: 1080-1085.