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PREPARATION AND IN VITRO STABILITY STUDY OF ^{188}Re-HEDP AS A BONE SEEKING RADIOPHARMACEUTICAL

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Abstract

The majority of patients with bone metastases will require some kind of therapy for bone pain palliation with the objective of improving the quality of live.

Bone seeking radiopharmaceuticals emitting beta particles have been used for palliation of bone metastases. Recently, scientists propose a slightly different radionuclide, ^{188}Re that shows such promise for treating primary and metastatic tumors as a radiopharmaceutical ^{188}Re-HEDP. ^{188}Re-HEDP is a chemical complex of a bisphosphonate ligand with an incorporated atom of radioactive ^{188}Re.

Bisphosphonates are ligands that contain the P-C-P bond which makes the molecules resistant to breakdown by enzymatic hydrolysis and are chemically very stable. In this study, we showed the conditions for the labeling of HEDP with ^{188}Re, and the in vitro stability of the radiopharmaceutical complex. ^{188}Re-HEDP is intended for the treatment of patients whose condition often requires for the therapeutic dose to be transported to the institution where the patient is hospitalized.

Our stability study is performed in order to determine the optimal storage conditions of the radiopharmaceutical if it is not applied to a patient immediately after preparation.

The results of radiochemical purity of ^{188}Re HEDP using ITLC quality control technique was above 98%. The stability study for ^{188}Re HEDP stored at different ambiental conditions showed that the complex is most stable in the first 3 hours after preparation, if it is stored at +4^{o}C in the dark.

It can be concluded that the prescribed preparation conditions (composition and concentration of chemical components, temperature and incubation time) are optimal for the formation of high percentage and stable initial chelating complex of ^{188}Re-HEDP.

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

Introduction

^{188}Re-HEDP is an osteotropic radiopharmaceutical used in palliative treatment of primary and metastatic bone tumors as an adjunct to external radiation therapy [1].

Similar to ^{99m}Tc complexes with MDP (methylene diphosphonate) for bone scintigraphy, ^{188}Re-HEDP is a complex compound composed of a bisphosphonate ligand molecule that incorporates an atom of the radioactive isotope rhenium-188 [2].

Bisphosphonates are ligands that contain the chemical bond P-C-P, which make these molecules resistant to the hydrolytic action of polyphosphatases and hence more stable in vivo [2].

The choice of the biphosphonate ligand was based on literature data, according to which ^{188}Re-HEDP has a higher affinity for bone tissue compared to ^{188}Re-MDP and ^{188}Re-HDP which tend to accumulate in soft tissues and especially in the liver [3,4].

^{188}Re is a beta-emitting radionuclide with very suitable physical characteristics for its therapeutic use. Its physical half-life is 16.9 hours and the high-energy beta emission reaches maximum 2.1 MeV. This high-

energy beta emission of ^{188}Re allows maximum penetration of the electrons into soft tissues of 11 mm and average penetration depth of 3.8 mm, characteristics suitable for the palliative treatment of bone metastases and primary bone tumors.

Another important feature is the 25% available gamma emission with 155 KeV energy. It enables simultaneous imaging of patients using a gamma camera, monitoring of radiopharmaceutical accumulation in bone tumor lesions, dosimetric measurements, as well as in vivo biodistribution studies [5- 7).

This is especially important for internal dosimetry methods that obtain values for the absorbed dose in both target tumor lesions and non-target surrounding healthy tissues and organs. Another significant feature of ^{188}Re is that it is a generator product obtained from ^{188}W/^{188}Re generator without the presence of a "carrier" which facilitates its widespread use [8].

In addition, ^{188}Re is a generator product that can be obtained from a ^{188}W/^{188}Re generator, suitable for various types of nuclear medicine centers and hospitals, similar to the ^{99}Mo/^{99m}Tc generator, which is used to prepare ^{99m}Tc-labeled diagnostic radiopharmaceuticals [9].

During preclinical studies, as well as in their clinical application, it is of essential importance that the radiopharmaceuticals maintain their properties, such as radiochemical purity and the affinity for the target tissues and organs. Under the influence of various environmental factors, radiopharmaceuticals can decompose, aggregate, undergo radiolysis or otherwise change their physico-chemical and biological properties during storage or transport to the site of application to patients.

Any change in radiochemical stability of the radiopharmaceuticals would seriously compromise scientific findings and/or clinical efficacy, as well as patient safety [10].

The stability studies of radiopharmaceuticals aim to investigate the influence of the preparation conditions, as well as the various conditions under which the radiopharmaceuticals are stored until their application to patients. Also, the objective of the stability studies is to predict the likelihood that the radiopharmaceuticals will retain their chemical integrity when administered into the bloodstream of the subject (patient or experimental animal). In this study we evaluated the preparation conditions, initial radiochemical purity and in vitro stability in different storage conditions of ^{188}Re-HEDP before its application to patients in therapeutic nuclear medicine departments [10, 11].

Materials and methods

Obtaining ^{188}Re

The radionuclide ^{188}Re is a generator product obtained from a ^{188}W/^{188}Re generator (Oak Ridge National Laboratories, USA and MAP Medical, Finland), which is one of the biggest advantages over the other therapeutic radionuclides. The principle on which the ^{188}W/^{188}Re generator works is similar to a ^{99}Mo/^{99m}Tc generator (148), where the parent isotope (^{188}W) is chemically adsorbed on the chromatographic carrier - Al_2O_3, and the daughter isotope (^{188}Re) is separated by elution with physiological solution (0.9% NaCl).

^{188}W is a reactor product, produced in a nuclear reactor (Oak Ridge National Laboratories ORNL, USA), and it is obtained from "enriched" ^{186}W according to the following scheme of radioactive decay:

(*"enriched"*)^{186}**W(n,γ) →^{187}W (n,γ) →^{188}W (β,γ) →^{188}Re(β,γ) →^{188}Os** (*stable*)

Preparation of HEDP kit:

^{188}Re-HEDP was prepared by in-house developed HEDP solution. The HEDP kit contains: Na_2HEDP, gentisic acid, as well as $SnCl_2$ dissolved in 0.9% NaCl. The whole procedure is performed under aseptic conditions in a laminar. First, Na_2HDP (166 mg) and gentinic acid (60 mg) are dissolved in 10 mL of nitrogenated 0.9% NaCl. During constant stirring of the previous solution, $SnCl_2$ (77 mg) was added. This solution, which has a pH of approximately 1, is filtered through a 0.22μm Millipore filter which performs mechanical sterilization. The sterilized solution is dispersed into multidose sterile vials, 1 mL in each vial. The kit vials are stored at a temperature of -20ºC to -70ºC [3].

Radioactive labeling of HEDP with ^{188}Re:

^{188}Re-HEDP is prepared by adding 1-3 mL of $^{188}ReO_4^-$ with an activity of 1-4 GBq to the vial containing 1 mL HEDP cold kit, and 20 mL of "carrier"**[1]** (Lepareur N. et al.,2019), which is a solution of stable Re in the form of NH_4ReO_4. After mixing intensively for 1-5 minutes, the mixture is placed in a water bath at 100ºC for 30 minutes. The solution is then cooled to room temperature, or under running cold water, and 2 mL of Na-acetate buffer**[2]** is added, adjusting the pH to about 5-6 [3].

[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°C.

[2]Preparation of Na-acetate buffer: 390 mg of Na-acetate (x $3H_2O$) are dissolved in 10 mL of dd H_2O and then 20 μL of 30% NaOH are added. The solution was filtered through a 0.22 μm Millipore filter.

Determination of 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 [3]. In these methods, a small drop of the radiopharmaceutical is put onto the bottom of a strip of support medium (silica gel coated sheets) and the strip is put into a tank containing a small amount of solvent (95% acetone).

The solvent migrates up the strip due to the capillarity effect. The components of the radiopharmaceutical are separated according to the solubility in the solvent and adsorption to the support medium.

The detection of the radioactivity in the strip was carried out by cutting the strip in two halves and counting the activity of two sections in a well scintillation counter.

After determination of the number of counts, calculations were performed to determine the percentage of radioactivity bound to ^{188}Re-HEDP complex and the percentage of radiochemical impurities.

In this chromatographic system, the ^{188}Re -HEDP complex remains at the application site (Rf≈0.0-0.1), while the free perrenate migrates with the solvent (Rf≈0.9-1.0). The ITLC analysis of radiochemical purity was determined on three chromatographic strips, and the average value of the three samples was calculated [12].

Stability study of ^{188}Re-HEDP

For the aims of the stability study, the radiopharmaceutical was exposed to different environmental conditions, namely: at 20°C and 4°C in the dark, as well as at 20°C in the light. The stability in 0.9% NaCl was investigated because saline is used for diluting radiopharmaceuticals in single dose doses. In order to assess the stability of ^{188}Re-HEDP complex in the bloodstream after its intravenous administration, the stability of the radiopharmaceutical mixed with serum at 37°C was examined.

Chromatographic analyses for the radiochemical purity were performed 1 hour, 3, 6, 24, 48 and 72 hours after the preparation by standard ITLC procedure. ITLC-Silicagel tapes as stationary phase and 95% acetone as mobile phase were used [13].

As in the initial determination of radiochemical purity, the chromatographic analyses were performed in three samples and their average value was calculated.

Statistical analysis

The statistical processing of the data was performed using the software systems STATISTICA for Njindonjs JP Professional and STATISTICA 6.0 StatSoft. Group results were expressed through average and standard deviation. The variance of intergroup variability in the ^{188}Re-HEDP stability study (F-distribution) was determined by analysis of variance (ANOVA) methods. The Tukey's Honest Significant Difference (HSD) test was applied to evaluate the significantly larger F-values (F>1.00), and to determine which of the groups has a significantly pronounced difference.

Results

Radiochemical purity of ^{188}Re-HEDP

Following the prescribed protocol for radioactive labeling of HEDP with ^{188}Re, quality control was performed on the prepared ^{188}Re-HEDP. First, by visual inspection, it was concluded that the preparation was clear, without turbidity and visible particles, while the pH was approximately 6.5.

The result of the initial radiochemical purity, with the used chromatographic-ITLC technique, for the percentage of formed ^{188}Re HEDP complex (as an average value of the three samples) was 98.87% (SD = 0.03) as shown in table 1:

Table 1: Initial radiochemical purity of ^{188}Re HEDP using ITLC in saline

Rf	Sample 1		Sample 2		Sample 3		AVERAGE		***SD***
	0,00	%	0,01	%	0,00	%	0,00	%	**0,0047**
0,000	**98,91**	%	**98,83**	%	**98,89**	%	**98,87**	%	**0,0340**
0,125	0,03	%	0,05	%	0,04	%	0,04	%	**0,0082**
0,250	0,02	%	0,02	%	0,02	%	0,02	%	**0,0000**
0,375	0,02	%	0,02	%	0,02	%	0,02	%	**0,0000**
0,500	0,02	%	0,02	%	0,02	%	0,02	%	**0,0000**
0,625	0,02	%	0,02	%	0,02	%	0,02	%	**0,0000**
0,750	0,03	%	0,06	%	0,04	%	0,04	%	**0,0125**
0,875	0,25	%	0,39	%	0,30	%	0,31	%	**0,0579**
1,000	0,70	%	0,58	%	0,65	%	0,64	%	**0,0492**
Σ	100,00	%	100,00	%	100,00	%	100	%	**0,0000**

Stability study of ^{188}Re-HEDP

As shown in Table 2, the conducted stability study enabled us to collect a lot of information about the stability of the radiopharmaceutical depending on time, temperature and light exposure, in sera and saline respectively.

Table 2: Numerical values of the results of the stability study of ^{188}Re-HEDP

Hours after radioactive labeling	+37ºC in sera in dark place	+20ºC in 0.9%NaCl in dark place	+4ºC in 0.9%NaCl in dark place	+20ºC in 0.9%NaCl in light place
0	98,87 %	98,87 %	98,87 %	98,87 %
1	96,85 %	92,96 %	95,05 %	93,97 %
3	96,1 %	90,73 %	94,16 %	92,84 %
6	95,17 %	88,76 %	92,24 %	91,51 %
24	52,81 %	50,70 %	71,89 %	56,25 %
48	47,86 %	34,22 %	65,26 %	37,65 %
72	41,97 %	26,02 %	58,14 %	32,76 %

The performed stability study has shown the greatest stability when ^{188}Re- HEDP complex was dissovled in physolgical solution (0.9% NaCl) and stored at +4ºC in the dark.

The decreasing stability rate of this complex was the slowest when preapared and kept under these conditions, showing stability of 71.89% (SD= 4.9) after 24 hours, 65.26% (SD=0.71) measured after 48 hours

and 58.14% (SD=1.28) after 72 hours. Following this, the ^{188}Re-HEDP complex dissolved in saline and stored in a dark place at +4ºC give higher percentage of stability compared to other conditions after 72 hours.

Moreover, all of the sample preparations have shown satisfactory percenatage of stability after 6 hours of disolving the preparation regardless of light, temperature and dissolution medium (mean= 91.92% , SD= 2.64). Unfortunately, all of the preparations showed rapid decrease in stability after 24 hours and on, despite the complex mixed with saline, when stored at +4ºC in a dark place.

The stability of this sample preparation was even 65.25% after 48 hours compared with 34.22% as lowest in the sample prepared with the same dissolution medium, kept also in a dark place but stored at +20ºC. The results after 72 hours for these two preparations gave the same discrepance in decrease of stability 58.14% (SD=1.28) and 26.02% (SD=0.48), respectively.

The following data suggest that temperature has meaningful role in matter of stability.

Statistical analysis of stability study

- *1 hour after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of ^{188}Re-HEDP complex measured after 1 hour under four different conditions - F = 20.321 p = 0.00042. The Tukey's Honest Significant Difference (HSD) test shows the differences of the average values of the percentage of ^{188}Re-HEDP complex measured under the four conditions separately after 1 hour. (Table 3.)

Table 3: Tukey Honest Significant Difference (HSD) test after 1 hour

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.000516
37ºC sera & 4ºC saline	p = 0.0349
37ºC sera & 20ºC saline/light	p = 0.00256
20ºC saline &i 4ºC saline	p = 0.0168
20ºC saline & 20ºC saline/light	p = 0.2864
4ºC saline & 20ºC saline/light	p = 0.2449

- *3 hours after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of ^{188}Re-HEDP complex measured after 3 hours under four different conditions - F = 20.808 p = 0.00039.The results of the Tukey's Honest Significant Difference (HSD) test are shown in table 4.

Table 4: Tukey Honest Significant Difference (HSD) test after 3 hours

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.000453
37ºC sera & 4ºC saline	p = 0.0911
37ºC sera & 20ºC saline/light	p = 0.00717
20ºC saline &i 4ºC saline	p = 0.00539
20ºC saline & 20ºC saline/light	p = 0.0656
4ºC saline & 20ºC saline/light	p = 0.3064

- *6 hours after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of ^{188}Re-HEDP complex measured after 6 hours under four different conditions

- F = 44.018 p = 0.000026. The results of Tukey's Honest Significant Difference (HSD) test are shown in table 5.

Table 5: Tukey Honest Significant Difference (HSD) test after 6 hours

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.000234
37ºC sera & 4ºC saline	p = 0.00373
37ºC sera & 20ºC saline/light	p = 0.00102
20ºC saline &i 4ºC saline	p = 0.00132
20ºC saline & 20ºC saline/light	p = 0.00521
4ºC saline & 20ºC saline/light	p = 0.5943

- *24 hours after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of ^{188}Re-HEDP complex measured after 24 hours under four different conditions - F = 34.799 p = 0.00006. The results of the Tukey's Honest Significant Difference (HSD) test are shown in table 6.

Table 6: Tukey Honest Significant Difference (HSD) test after 24 hours

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.7960
37ºC sera & 4ºC saline	**p = 0.000349**
37ºC sera & 20ºC saline/light	p = 0.4824
20ºC saline &i 4ºC saline	**p = 0.000273**
20ºC saline & 20ºC saline/light	p = 0.1514
4ºC saline & 20ºC saline/light	**p = 0.000801**

- *48 hours after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of ^{188}Re-HEDP complex measured after 48 hours under four different conditions- F = 84.872 p = 0.000002. The results of the Tukey's Honest Significant Difference (HSD) test are shown in 7.

Table 7: Tukey Honest Significant Difference (HSD) test after 48 hours

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.00113
37ºC sera & 4ºC saline	**p = 0.000370**
37ºC sera & 20ºC saline/light	p = 0.00625
20ºC saline &i 4ºC saline	**p = 0.000231**
20ºC saline & 20ºC saline/light	p = 0.4269
4ºC saline & 20ºC saline/light	**p = 0.000231**

- *72 hours after preparation of ^{188}Re-HEDP*

Analysis of variance (ANOVA) shows that there are statistically significant differences between the mean values of the percentage of 188Re-HEDP complex measured after 72 hours under four different conditions- F = 169.33 p = 0.000001. The results of Tukey's Honest Significant Difference (HSD) test are shown in table 8.

Table 8: Tukey Honest Significant Difference (HSD) test after 72 hours

Compared groups	Tukey (HSD) test
37ºC sera & 20ºC saline	p = 0.000241
37ºC sera & 4ºC saline	**p = 0.000239**
37ºC sera & 20ºC saline/light	p = 0.00147
20ºC saline &i 4ºC saline	**p = 0.000231**
20ºC saline & 20ºC saline/light	p = 0.00927
4ºC saline & 20ºC saline/light	**p = 0.000231**

Discussion

Stability studies help to estimate the stability of the radiopharmaceutical under typical storage conditions over a specified time (shelf life). Our stability study was performed in order to determine the optimal storage conditions of the radiopharmaceutical if it is not applied to a patient immediately after the preparation.

The radiopharmaceutical is intended for palliative treatment of patients with malignant diseases whose condition often does not allow transport to nuclear medicine centers and as an alternative there is a need for the therapeutic dose to be transported to the institution where the patient is hospitalized [14].

In this study we intended to examine the conditions in which the transported radiopharmaceutical will remain stable for the longest time, after its dispensing in syringes as individual doses. From literature, it is known that the environmental factors that can affect the stability of the rhenium-diphosphonate complexes are temperature and light [3].

The stability of the radiopharmaceuticals is most appropriately assessed by performing QC (radiochemical purity) multiple times during the proposed shelf life of the radiopharmaceutical. If the stability of the radiopharmaceutical is shorter than the assumed shelf life, its formulation should be further investigated and optimized.

The results of our stability study showed that the radiopharmaceutical ^{188}Re-HEDP prepared according to the standard prescription is less stable if after the preparation the volume is adjusted in a syringe with saline. According to the stability study by Lin WY. et al., 1999 [3], the percentage of the ^{188}Re-HEDP complex was 92.3% for the preparation undissolved in saline and kept at +25ºC after 24 hours.

After 72 hours, the percentage of ^{188}Re-HEDP complex in this study declined to 89.6% [3].

In our study, the initial value of the complex was 98.87%, which is higher than the initial percentage of the complex obtained in the cited study (>95%). 24 hours after the preparation, the percentage of the complex has dropped below 60% with the exception of the sample kept at +4ºC in 0.9% NaCl in the dark with value of 71.89%. After 72 hours of the preparation, the highest stability of the complex was shown by the sample kept at +4ºC in 0.9% NaCl in the dark.

The value of that sample was 58.14%, which is a significantly lower percentage compared to the values from the previously cited study (89.6%).

The results of our stability study indicate the fact that the volume reconstitution of ^{188}Re-HEDP with saline makes the formed radioactive complex unstable. The reason for the destabilization of the ^{188}Re-HEDP complex is the reoxidation of ^{188}Re-HEDP into $^{188}ReO_4^-$ under the influence of oxygen inserted into the radiopharmaceutical.

In the case of radiopharmaceutical preparations, the largest amount of oxygen is introduced when the contents of the bulk preparations are drawn into the syringe for dispensing into individual doses, while the oxygen dissolved in the saline with which the volume reconstitution is performed, also has a significant share [15].

The results of our stability study that the formed complex remains stable for the longest time if the preparation is kept at a low temperature (+4ºC), are explained by the fact that the chemical reactivity between

oxygen and the rhenium complex is slowed down when the temperature of the reaction medium is low. Our stability study showed that light has no effect on the stability of the ^{188}Re-HEDP complex.

The stability study in sera is an important method to assess suitability of a radiopharmaceutical. This study shows the stability of the radiopharmaceutical in the blood, into which the radiopharmaceutical is most often administered.

The objective is to predict the likelihood that the tracer will retain its integrity when administered into the bloodstream of the test subject (experimental animal or human subject/patient).

It is important to ensure that the tested fluid remains sterile to avoid microbial degradation of the tracer. In our study, the results for the stability of the complex in serum showed that in the first 6 hours the preparation mixed with serum has higher stability of the complex compared to the preparation kept in other conditions.

However, after 24 hours, the percentage of the complex is lower compared to that part of the preparation that was kept at +4^{o}C in 0.9% NaCl in the dark, but still higher compared to the preparation kept at +20^{o}C in 0.9% NaCl in the dark and light.

These results can be explained by the existence of biological antioxidants in the serum that reduce the reoxidation of the ^{188}Re-HEDP complex. Since their amount in the serum is limited, their concentration decreases after 24 hours, which reduces their protective effect.

Conclusion

Based on the results obtained from our study, it can be concluded that the prescribed preparation conditions (composition and concentration of chemical components, temperature and incubation time) are optimal for the formation a high percentage (>98%) of the initial chelating complex ^{188}Re-HEDP.

The initially formed complex of ^{188}Re-HEDP loses the radiochemical stability with dispensing of individual doses and volume adjustment with saline.

The ^{188}Re-HEDP complex in a single dose is the most stable for 1-3 hours (≈ 95%) if the preparation is stored at +4^{o}C. The light has no effect on the stability of the initially formed ^{188}Re-HEDP complex. Single patient doses of this product for the radionuclide treatment of bone metastases should be transported refrigerated within 3 hours of preparation.

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