International Journal of Pharmaceutics 465 (2014) 317–324

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Bench to bedside development of GMP grade Rhenium-188-HEDP, a radiopharmaceutical for targeted treatment of painful bone metastases

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ARTICLE INFO

Article history:
Received 17 December 2013
Accepted 27 January 2014
Available online 19 February 2014

Keywords:
Rhenium-188
Rhenium-188-HEDP
Radionuclide therapy
Bone metastases
Prostate cancer
GMP

ABSTRACT

Bone-targeting therapeutic radiopharmaceuticals are effective agents for treatment of painful bone metastases. Rhenium-188-HEDP is such a therapeutic radiopharmaceutical and has advantages over commercially available alternatives in terms of efficacy, safety and the ability to be produced on-site, allowing rapid treatment upon presentation of patients with pain. Unlike many other radiopharmaceuticals, there are no standardized preparation methods for Rhenium-188-HEDP. It is known, however, that drug composition may not only affect stability of the final drug product, but it may also influence bone affinity and, thus, efficacy. Furthermore, for support of clinical studies with Rhenium-188-HEDP as an investigational medicinal product, preparation of this radiopharmaceutical has to be performed under GMP conditions. To our knowledge, no group has reported on the preparation of Rhenium-188-HEDP under GMP conditions or on stock production of sterile non-radioactive starting materials.

We present the production of GMP grade Rhenium-188-HEDP for application of this therapeutic radiopharmaceutical in routine clinical practice and for support of clinical studies. In addition, bio-distribution data of Rhenium-188-HEDP in mice and in patients with bone metastases originating from prostate cancer are presented.

1. Introduction

Bone metastases are a major complication in the end stage of different cancers, including prostate cancer and lead to serious complications like pain, fractures and hypocalcaemia resulting in a decreased quality of life and poor survival (Suva et al., 2011). Often, in this phase of the disease, palliative treatment with chemotherapy and opioids do not alleviate these symptoms. Radionuclide therapy with bone-targeting radiopharmaceuticals is then a proven effective and safe treatment modality with a response rate of approximately 80% (Lam et al., 2007). Bone-targeting therapeutic radiopharmaceuticals exert their action by specific accumulation at osteoblastic bone metastases, whereupon the local and specific delivery of a high β-radiation dose results in destruction of malignant cells. The dose-limiting toxicity of bone-targeting therapeutic radiopharmaceuticals is also explained by this mechanism, since the radiation that reaches the bone marrow may result in a mild and reversible thrombocytopenia (Lam et al., 2007).

Rhenium-188-HEDP is a bone-targeting therapeutic radiopharmaceutical being a complex of the metallic radionuclide ^{188}Rhenium with the bone-targeting agent hydroxyethylidene diphosphonic acid (HEDP, also known as etidronic acid), which belongs to the class of bisphosphonates. The high bone affinity of the bisphosphonate is retained when ^{188}Rhenium is complexed with HEDP (Hsieh et al., 1999). ^{188}Rhenium is a radionuclide with a half-life of 17 h and decays to stable ^{188}Osmium by β^- (2.2 MeV)

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http://dx.doi.org/10.1016/j.ijpharm.2014.01.034

and γ (155 keV) decay (Pillai et al., 2012). Rhenium-188-HEDP is synthesized by heating a mixture of radioactive ^{188}Rhenium (as sodium perrhenate), non-radioactive Rhenium (as ammonium perrhenate), stannous chloride, HEDP (as disodium etidronate) and gentisic acid in sodium chloride 0.9% (Hsieh et al., 1999; Lin et al., 1999, 1997; Maxon et al., 1998). In this mixture the stannous ion is a reductor for the perrhenate ion, needed for complexation of ^{188}Rhenium with HEDP. Gentisic acid acts as an anti-oxidant and is used as a stabilizer in radiopharmaceutical preparations (Tofe et al., 1980). The sodium chloride 0.9% for injections is used for elution of the generator. After the labeling reaction, the pH of the acidic mixture has to be adjusted to an acceptable pH for injection, for example with a sodium acetate solution (Lin et al., 1997; Maxon et al., 1998).

^{188}Rhenium (as perrhenate salt) can be eluted from commercially available ^{188}Tungsten/^{188}Rhenium generators that, due to the long half-life of mother radionuclide ^{188}Tungsten of 69 days, can be used up to 6 months. Rhenium, like Technetium, is a member of group 7 (manganese group) of the periodic table and, therefore, the radiochemistry of ^{188}Rhenium-bisphosphonate complexes is similar to that of ^{99m}Technetium-bisphosphonate complexes that are used in daily clinical practice for bone scintigraphy (Deutsch et al., 1986).

However, unlike for the preparation of ^{99m}Technetium-bisphosphonate complexes, there are neither standardized preparation methods for Rhenium-188-HEDP, nor ^{188}Tungsten/^{188}Rhenium generators and HEDP coldkits available as approved drugs. Therefore, each institute that produces Rhenium-188-HEDP separately develops this radiopharmaceutical resulting in varying preparation methods and different compositions of the final drug product. It is known, however, that composition and preparation method may not only affect stability of the final drug product, but it may also influence bone affinity and bio-distribution, and thus efficacy (Hsieh et al., 1999; Lin et al., 1999, 1997). For example, it has been found that the presence of non-radioactive perrhenate as a carrier in the radiopharmaceutical is a prerequisite for the bone affinity of the Rhenium-188-HEDP complex. Also, besides the amount of carrier, the stannous concentration in the reaction mixture, as well as reaction time, reaction temperature and pH during the labeling procedure have been shown to influence the radiochemical purity and stability of the drug product (Hashimoto, 2013; Hsieh et al., 1999; Lin et al., 1999, 1997; Verdera et al., 1997). Furthermore, due to the low specific activity of the eluate of some generators, some groups have introduced an undesirable and laborious concentration procedure of the eluate before labeling (Guhlke et al., 2000). Also, for routine clinical application of Rhenium-188-HEDP, ideally all necessary non-radioactive starting materials, like an HEDP coldkit, ammonium perrhenate and sodium acetate solutions are available as sterile stock products. Although various groups have reported the labeling methods for Rhenium-188-HEDP (Hashimoto, 2013; Hsieh et al., 1999; Li et al., 2001; Lin et al., 1997; Maxon et al., 1998; Palmedo et al., 2000), to the best of our knowledge no one has reported the pharmaceutical development and sterile stock production of these indispensable starting materials. Lastly, for support of clinical studies with Rhenium-188-HEDP as an investigational medicinal product, preparation of this radiopharmaceutical has to be performed under Good Manufacturing Practices (GMP) conditions. Thus far, no group has described the small-scale preparation of GMP grade Rhenium-188-HEDP.

In this manuscript we describe the development and validation of the small-scale preparation of GMP grade Rhenium-188-HEDP, including sterile stock production of all necessary starting materials, for application of this radiopharmaceutical in routine clinical care and the support of clinical studies. Furthermore, we discuss our experience with the first year of routine Rhenium-188-HEDP preparation.

2. Materials and methods

2.1. General

All chemicals were commercially available and used as obtained. All materials and excipients were approved on the basis of in-house quality controls and their suppliers were qualified as reliable. Furthermore, the used equipment the production processes and analytical methods for production of starting materials for Rhenium-188-HEDP were validated and all pharmacy technicians were qualified for aseptic preparation of pharmaceuticals.

Stock production of non-radioactive sterile starting materials was performed in a GMP class C cleanroom. Generator elution and radiopharmaceutical labeling was performed in a GMP grade A workstation in a grade C environment. All cleanrooms were subjected to a monitoring program for viable and non-viable particles at resting and operating state in concordance with the current European Good Manufacturing Practices.

2.2. Radiation safety

All radiopharmacy technicians were trained in radiation protection. Personal thermoluminescence dosimeters (TLD's) (NRG, Petten, The Netherlands) were worn on their body and fingers. For adequate shielding of the high energetic β/γ-radiation vials and syringes were shielded with acrylic glass (PMMA) and/or tungsten vial containers. Furthermore, the labeling procedure was carried out in a shielded laminar flow bench, equipped with a sliding lead glass window with a 1 cm layer of acrylic glass on the inside. Also, the walls of the bench were covered with acrylic glass. An aluminum worktop was installed to reduce backscatter as a result of the highly energetic β-radiation.

2.3. Production of starting materials

2.3.1. Ammonium perrhenate and sodium acetate solutions

Sterile water for injections was obtained from Baxter (Utrecht, The Netherlands). The 10 mL sterile polypropylene injection vials and polytetrafluoroethylene (Teflon) caps were obtained from Brocacef Supplies and Services (Maarssen, The Netherlands).

Ammonium perrhenate (>99% pure) was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). An amount of 5.36 g ammonium perrhenate was dissolved in 400 mL water for injections, resulting in a 0.05 M concentration. This solution was then purged with nitrogen gas until the relative oxygen concentration of the solution was below 5%. After filtration through a 0.2 μm membrane filter (Baxter, Utrecht, The Netherlands), 2 mL aliquots of the solution were subsequently transferred to the sterile polypropylene injection vials with a Baxa repeater pump (Baxter, Utrecht, The Netherlands) and after closing, the vials were sterilized at 121 °C in a superheated water sterilizer during 15 min. For production of the 1.5 M sodium acetate solution, sodium acetate trihydrate (Ph. Eur.) was obtained from Spruyt-Hillen (IJsselstein, The Netherlands). A total of 102.1 g sodium acetate trihydrate was dissolved in 500 mL water for injections and, after dissolution, 5 mL aliquots were directly transferred to injection vials through a sterile 0.2 μm in line-filter (Baxter, Utrecht, The Netherlands) with a repeater pump. The vials were subsequently sterilized at 121 °C during 15 min in a superheated water sterilizer. Both the ammonium perrhenate and sodium acetate solutions were stored at ambient temperature until further handling.

2.3.2. HEDP coldkit

Kits for labeling are usually available as lyophilized products. Because lyophilization equipment often is not available in healthcare establishments, we aimed to develop a liquid kit, containing HEDP (as 50 mg disodiumetidronate), 18.4 mg of stannous chloride dihydrate and 15 mg of gentisic acid. Gentisic acid, HEDP and stannous chloride were obtained from Ofichem (Ter Apel, The Netherlands), water for injections was obtained from Baxter (Utrecht, The Netherlands).

Stannous chloride dissolved faster in an aqueous solution containing hydrochloric acid and the disodiumetidronate dissolved faster in the presence of gentisic acid. Hence, for preparation of the liquid HEDP coldkit two initial solutions were made. Solution 1 was prepared by dissolving 3.0 g gentisic acid in 352 mL of water for injections. Thereafter, 10 g of HEDP (as disodium etidronate) was added and the mixture was ultrasonicated during 15 min to accelerate dissolution. Solution 2 was prepared by mixing 31.4 g of hydrochloric acid (3.5 M) with 18.6 g water for injections and subsequent addition and dissolution of 4.4 g stannous chloride dihydrate. The hydrochloric acid was added to prevent precipitation of an insoluble tin salt due to hydrolysis. Thereafter, 43.5 g of solution 2 was added to solution 1 to obtain the final solution. The final solution was purged with nitrogen to prevent oxidation of the stannous chloride and to reduce the formation of hydrogen peroxide as a result of radiolysis in the drug product, which may affect Rhenium-188-HEDP stability (Sailerova and Billinghurst, 2013).

Then, 2 mL aliquots were transferred to 10 mL injection vials through a 0.2 μm membrane filter (Baxter, Utrecht, The Netherlands) and after closing and labeling, the vials were transferred to a container with dry ice (−80 °C) for immediate freezing. Lastly, the frozen vials in a container with dry ice were then sterilized overnight by gamma irradiation with an overkill radiation dose (>25 kGy radiation dose) by Synergy Health (Ede, The Netherlands). After sterilization, the vials were kept frozen at −20 °C until further handling. Frozen storage was considered necessary to prevent hydrolysis of stannous chloride in the solution to the insoluble salt Sn(OH)Cl.

2.4. Generator elution and labeling procedure

A sterile 37 GBq ^{188}Tungsten/^{188}Rhenium generator was obtained from ITG (Munich, Germany). Sodium chloride 0.9% for injection was obtained from B. Braun Medical (Oss, The Netherlands), sterile vacuum injection vials were obtained from GE Healthcare (Eindhoven, The Netherlands) and sodium acetate and ammonium perrhenate solutions and the HEDP coldkit were produced in-house as described in section 2.3.

Within 30 min before generator elution, an HEDP coldkit was thawed at room temperature. Then, the generator was eluted with 2 mL NaCl 0.9% for injections (Fig. 1A). This first fraction was stored for quality control of the eluate. Subsequently, 0.2 mL of the 0.05 M ammonium perrhenate solution was added to the vial containing the HEDP coldkit. Then, using syringe 1, the generator was directly eluted with 6 mL of 0.9% NaCl. This fraction of the eluate was entirely transferred to the vial with HEDP coldkit through a 0.2 μm membrane filter (Fig. 1A). The vial containing the HEDP coldkit, the ammonium perrhenate solution and the generator eluate was then gently mixed for 5 s and subsequently incubated for 30 min at 90 °C in a dry block heater (VWR, Amsterdam, The Netherlands). After incubation, the pH of the mixture was adjusted to pH 4–6 by adding 0.5 mL sodium acetate solution to the vial. Then, the final drug product was filtered through a 0.2 μm syringe filter (Sartorius Stedim, Nieuwegein, The Netherlands) by means of a vacuum vial, to assure minimal radiation exposure for the radiopharmacy technician (Fig. 1B). The drug product was stored in the vial until its dispensing.

2.5. Quality control and specifications

2.5.1. Quality control of ammonium perrhenate and sodium acetate solutions and of the HEDP coldkit

Ammonium perrhenate concentrations were determined in the first and in a random sample of each produced batch by means of a titration with 0.1 M sodium hydroxide using phenolphthalein as indicator. Sodium acetate concentrations were also determined in a first and random sample by determination of the sodium concentration by means of a measurement with a Metrohm ISE sodium ion selective electrode (Metrohm Applikon, Schiedam, The Netherlands). For both solutions, a concentration between 95% and 105% of specification was required. Quality control of the HEDP coldkit was carried out by performing a labeling with ^{188}Rhenium as described in Section 2.4 and subsequent quality control of the drug product as described in Section 2.5.3. For release of these products, also tests for sterility and a bacterial endotoxin content were performed, in accordance with the European Pharmacopoeia (European Pharmacopoeia Commission 2013).

2.5.2. Quality control of the ^{188}Tungsten/^{188}Rhenium generator

The radionuclidic purity of the eluate was determined by measurement of eluate activity decay. A maximum of 0.1% ^{188}Tungsten breakthrough was allowed, analogous to 0.1% ^{99}Molybdenum breakthrough for the ^{99}Molybdenum/^{99m}Technetium generator eluate monograph in European Pharmacopoeia (European Pharmacopoeia Commission 2013). Therefore, after elution the activity of the eluate was measured in a VIK-202 ionization chamber (Veenstra, Joure, The Netherlands). The ^{188}Tungsten breakthrough was less than 0.1% if the activity 7–10 days after the first measurement was less than 0.1% of the initial activity. The chemical purity of the eluate was assessed by determination of the aluminum content in the eluate with a Microquant chromazurol S color reaction kit (Merck Millipore, Amsterdam, The Netherlands) and a maximum of 2 parts per million (ppm) aluminum was allowed in the generator eluate, also by analogy to the specifications for chemical purity of ^{99}Molybdenum/^{99m}Technetium generator eluate in (European Pharmacopoeia Commission 2013). The radiochemical purity of the eluate was determined by means of Instant Thin Layer Chromatography (ITLC) with a glass microfiber chromatography paper impregnated with a silica gel stationary phase (Agilent Technologies, Amsterdam, The Netherlands) and sodium chloride 0.9% as a mobile phase. On this system, the reduced Rhenium (ReO_2) remains at the application spot and the perrhenate ion (ReO_4^-) migrates with the mobile phase. Radiochromatogram scanning was performed with a VCS-101 chromatography scanner (Veenstra, Joure, The Netherlands). The specification for radiochemical purity of the eluate was >98%, defined as the relative ^{188}Rhenium content in the chemical form of perrhenate. For continuous microbiological monitoring of the generator eluate, in each week of elution a sterility test and determination of the endotoxin content were performed. The sterility test of the eluate was performed by direct inoculation of Tryptic Soy Broth (TSB) and Fluid Thioglycollate Medium (FTM) and subsequent incubation during 14 days at ambient temperature and 32.5 °C, respectively. The endotoxin content of the eluate was determined by means of a kinetic chromogenic LAL assay (European Pharmacopoeia Commission, 2013). A flowchart of the manufacturing process and the process controls of the complete labeling process is schematically depicted in Fig. 2.

2.5.3. Quality control of Rhenium-188-HEDP

The pH of the drug product was checked with a pH indicator strip (Merck Millipore, Amsterdam, The Netherlands) and accepted

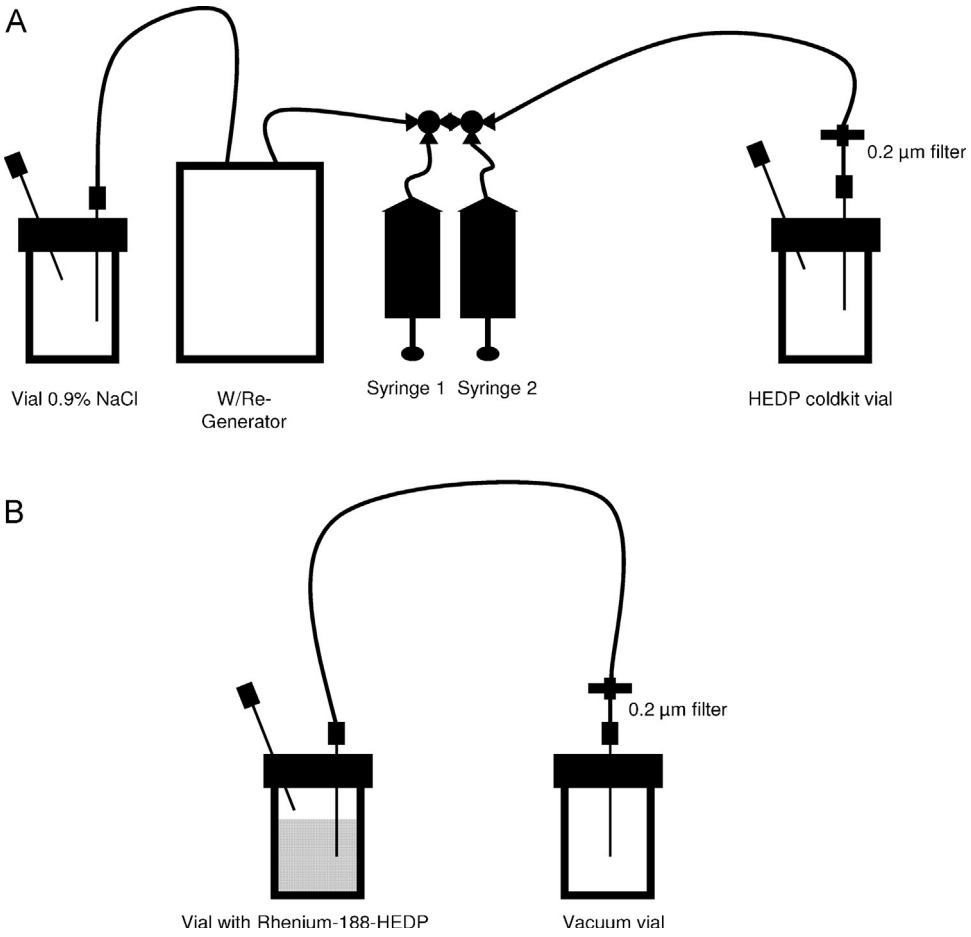

Fig. 1. (A) Elution setup. (B) Drug product filtration setup.

if the pH was 4–6, based on the assumptions that this pH range is feasible to obtain after pH correction with sodium acetate after labeling and that this pH range is acceptable for injection. The radiochemical purity was determined with thin layer chromatography analogous to Technetium-bisphosphonate complexes (European Pharmacopoeia Commission, 2013). The stationary phase was a cellulose strip (Whatman 3MM, Whatman Nederland, Den Bosch, The Netherlands). For determination of free perrhenate (ReO_4^-), the mobile phase was acetone (Merck Millipore, Amsterdam, The Netherlands). In this chromatographic system the Rhenium-188-HEDP complex and reduced rhenium (ReO_2) remain at the application spot and the free perrhenate migrates with the mobile phase. For determination of free ReO_2 a 0.01 M disodium etidronate solution in sodium chloride 0.9% was used. In this system, ReO_2 remains at the application spot and the Rhenium-188-HEDP complex and free ReO_4^- migrate with the mobile phase. The radiochemical purity was then calculated with Eq. (1).

$$\text{Radiochemical purity}(\%) = 100 - [\text{amount of free } ReO_2(\%)] - [\text{amount of free } ReO_4^-(\%)] \quad (1)$$

The specification for radiochemical purity of the drug product was set at >93%, based on labeling efficiencies in other institutes during routine clinical application (personal communication) defined as relative ^{188}Rhenium content in the chemical form of Rhenium-188-HEDP.

As a part of continuous environmental monitoring and prospective validation of aseptic working conditions, after each labeling a media fill was performed. In short, sodium chloride 0.9% for injections was introduced into the used labeling setup (Fig. 1A), and transferred to the TSB and FTM media. These were subsequently incubated during 14 days at ambient temperature and 32.5 °C, respectively. The results of the continuous microbial monitoring program were not a part of product release.

2.5.4. Hydroxyapatite binding assay

The hydroxyapatite binding assay was performed as previously described for various bone-targeting radiopharmaceuticals (Ogawa et al., 2006a,b). Shortly, Bio-Gel HTP hydroxyapatite crystals were obtained from Bio-Rad (Veenendaal, The Netherlands) and phosphate buffered saline (containing 19.4 mM potassium phosphate, 27 mM potassium chloride and 1.37 M sodium chloride) was obtained from VWR (Amsterdam, The Netherlands). After preparation of the drug product, 0.10 mL of Rhenium-188-HEDP was added to 3.0 mL of phosphate buffered saline with 300 mg hydroxyapatite in a 5 mL polypropylene test tube and the tube was subsequently closed. The tube was then vortex-mixed for 3 s and incubated at 37 °C for 150 min with 10 s vortex mixing every 30 min. Thereafter, the radioactivity was measured in each test tube and the content of the test tube was filtered through a 0.2 µm syringe filter. Then, of 1.0 mL of the filtrate the activity was measured and the hydroxyapatite binding was calculated as described in Eq. (2).

$$\text{Hydroxyapatite binding}(\%) = 100 - \frac{100 \times \text{activity of 1.0 mL filtrate}}{[\text{activity of tube after incubation}/3.1]} \quad (2)$$

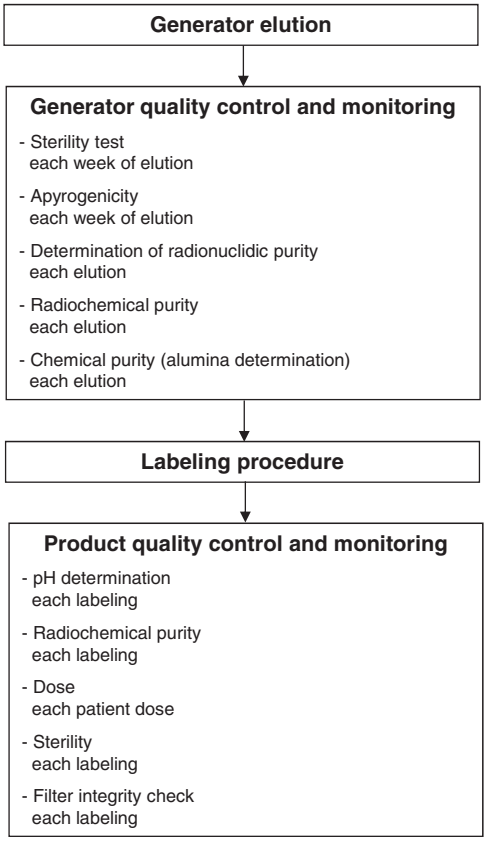

Fig. 2. Flow chart of the manufacturing process.

2.5.5. Determination of bio-distribution of Rhenium-188-HEDP in mice

Bio-distribution of Rhenium-188-HEDP was determined in 3 male C57Bl6/J mice with an average body weight of 34 g. Four hours after tail vein injection of 37 MBq Rhenium-188-HEDP in a volume of approximately 0.2 mL, SPECT-CT imaging where acquired with a VECTor/CT (MILabs B.V., Utrecht, The Netherlands) with a clustered pinhole collimator with 162 pinholes of 0.7 mm diameter (Goorden et al., 2013). Images were reconstructed with 25 iterations Pixel-based Ordered Subset with 4 subsets (Branderhorst et al., 2010) which includes iterative resolution recovery (van der Have et al., 2008).

The bone to soft tissue ratio was then calculated by drawing a region of interest around three lumbar vertebrae and a background region of interest around surrounding soft tissue. The uptake ratio was defined by the uptake in the vertebrae divided by the background uptake.

2.6. Validation experiments

2.6.1. Stability of ammonium perrhenate and sodium acetate solutions and of the HEDP coldkit

Prospective stability testing was carried out for these products for routinely produced batches at 6, 12, 18 and 24 months after storage at their relevant conditions (ambient temperature for the ammonium perrhenate and sodium acetate solutions and −20 °C for the HEDP coldkit), by performing a quality control of these products as described and specified in Section 2.5.1.

2.6.2. Qualification of the aseptic labeling process of Rhenium-188-HEDP

For the initial qualification of the aseptic labeling process, three media fills of the aseptic labeling process were performed on three separate days, as recently proposed by the FDA for validation of aseptic production of radiopharmaceuticals (US Department of Health and Human Services, 2011), by direct inoculation of TSB and FTM media. ^{188}Rhenium in the eluate emits highly energetic β-radiation, which obscures interpretation of the results due to its bactericidal properties. Therefore, validation of the aseptic labeling process was carried out with sterile sodium chloride 0.9% for injections instead of generator eluate. The validation was carried out using the same setup as described in Section 2.4 and depicted in Fig. 1. After heating and subsequent filtration through a 0.2 μm membrane filter, the solution was added to TSB and FTM media and incubated at ambient temperature and 32.5 °C, respectively, for two weeks.

2.6.3. Retrospective evaluation of the continuous microbiological monitoring program

As previously stated, sterility and endotoxin testing could not be part of the product release due to radiation safety requirements and undesirable time consuming procedures. Hence, a continuous microbiological monitoring program for the generator and drug product was initiated, as described in Sections 2.5.2 and 2.5.3. The results of 1 year microbiological monitoring of the Rhenium-188-HEDP labeling procedure were evaluated and the aseptic working conditions were considered appropriate if no sign of microbial contamination of the eluate or Rhenium-188-HEDP was detected.

2.6.4. Initial qualification of labeling procedure and determination of radiochemical stability

For initial qualification of the labeling process and drug product stability, three validation batches of Rhenium-188-HEDP were produced, with each batch produced on a separate day. Quality control of the drug product was performed directly after labeling and after 24 h of storage at ambient temperature in either the glass injection vial or a polypropylene syringe. The radiochemical stability was considered suitable if all the quality control results of Rhenium-188-HEDP at these time points and storage conditions were within specifications. Furthermore, an hydroxyapatite binding assay was performed in triplicate directly after labeling as described in Section 2.5.4. Since no in vitro hydroxyapatite affinity results have yet been reported for Rhenium-188-HEDP, the specification for hydroxyapatite binding was set at >60%, as usually observed other with technetium- and rhenium-bisphosphonate complexes (Ogawa et al., 2006a,b; Uehara et al., 2007).

2.6.5. Product quality review

After the initial qualification of the labeling process and evaluation of the bio-distribution in mice, Rhenium-188-HEDP was introduced in the clinic. As a part of product release, a quality control procedure and monitoring program was set up (Fig. 2). We retrospectively evaluated the results of the routine quality control on the drug product, as obtained during 1 year of routine production. Also, the process capability index (C_p) was calculated with Eq. (3) for the radiochemical purity of the drug product, representing the robustness of the labeling process (Stafford, 1999). When the C_p was >1, the labeling process of Rhenium-188-HEDP was considered robust.

$$C_p = \frac{USL - LSL}{6\sigma} \quad (3)$$

In this equation USL and LSL represent the respective upper and lower limits of specification for radiochemical purity (93% and 100%) and σ represents the observed standard deviation of the radiochemical purity.

2.6.6. Bio-distribution in mice

The in vivo bone affinity in mice was considered suitable if the healthy-bone-to-soft-tissue-ratio of Rhenium-188-HEDP was comparable (approximately 10–25) to bone uptake ratio's as reported for ^{99m}Technetium-bisphosphonate complexes and Rhenium-188-HEDP (Bergqvist et al., 1984; Fruhling et al., 1986; Hsieh et al., 1999; Pauwels et al., 1983).

2.7. Bio-distribution in a patient

As a part of routine clinical care, prior to Rhenium-188-HEDP therapy, a diagnostic bone scintigram was acquired using an injection activity of 555 MBq of ^{99m}Tc-HDP. Rhenium-188-HEDP was intravenously administered in a dose of 40 MBq/kg. A routine post-therapy Rhenium-188-HEDP scintigram was then acquired 3 h post injection.

3. Results and discussion

3.1. Radiation safety

Personal dosimetry did not reveal an increase in radiation exposure to the body, but a significant increase in finger dose, as a result of the highly energetic beta-radiation was observed. The observed finger dose during a labeling experiment was variable and ranged from 5 to 50 mSv, depending on the worker and the remaining activity in the generator. This finger dose was acceptable, considering the once to twice weekly production scheme and involvement of four pharmacy technicians. However, due to the expected expansion of application of this radiopharmaceutical, we are currently investigating additional radiation safety measures like automate production to further decrease the extremity dose as a result of the beta radiation. This may involve further shielding of the used tubing and syringe filters with acrylic glass and the use of under-pressure to transfer radioactive solutions to vials, resulting in decreased proximity of the hands to the radioactivity.

3.2. Stability of ammonium perrhenate and sodium acetate solutions and of the HEDP coldkit

At time of writing of this manuscript, prospective testing showed 6 months stability for the HEDP coldkit stored at −20 °C, and 18 months for the sodium acetate and 24 months for the ammonium perrhenate solutions stored at ambient temperature. Long term stability testing (up to 3 years) for all products at these storage conditions is currently ongoing.

3.3. Initial qualification of the aseptic labeling process of Rhenium-188-HEDP and evaluation of the prospective microbial monitoring program

During initial qualification of the aseptic labeling process, no bacterial growth was observed after incubation of the media in any of the three simulation experiments. Evaluation of our prospective microbiological monitoring program showed that during 1 year of follow-up (the lifespan of 2 generators) no microbial contamination was detected during prospective sterility testing of the eluate. Also, the endotoxin content met the requirements at any occasion. This indicated that the microbial quality of the generators could be assured during their 6 month lifespan. Furthermore, in total 40 prospective media fills were performed and none of the media showed microbial growth after incubation, also indicating adequate microbial quality. We concluded that the aseptic conditions could be maintained throughout the lifespan of generator and throughout the production process. We postulate that, besides an adequate production environment, the highly energetic beta radiation in the generator, the eluate and the drug product, the double sterile filtration and the heating during the labeling process all contribute to the adequate microbiological quality of the drug product.

Table 1
Results of process validation and stability experiments.

	Directly after labeling ($n=3$)	After 24 h storage in a glass injection vial ($n=3$)	After 24 h storage in a polypropylene syringe ($n=3$)
Appearance	Clear lightbrown solution	Clear lightbrown solution	Clear lightbrown solution
Mean pH (range)	4.8 (4.5–5)	4.8 (4.5–5)	4.8 (4.5–5)
Mean radiochemical purity (%) ± SD	96.7 ± 1.5	97.3 ± 0.8	97.1 ± 0.2
Hydroxyapatite binding (%) ± SD	80.8 ± 0.5	–	–

SD: standard deviation.

3.4. Rhenium-188-HEDP labeling process validation and stability experiments

It was observed that during heating of the reacting mixture, the colorless clear solution in the vial turned light brown, as a result of the complexation of the stannous, perrhenate and etidronate ions, as previously described (Elder et al., 1997). The pH in the reaction vial after heating was approximately 2 and increased to 4.5 after addition of the sodium acetate solution, indicating the necessity of this pH adjustment before administration of the drug product to a patient.

The results of the process validation and stability experiments are shown in Table 1. In short, the hydroxyapatite affinity was high with a reproducible binding percentage of 80.8 ± 0.5% (SD). Hitherto, no reference value for hydroxyapatite affinity has been reported for Rhenium-188-HEDP. Nonetheless, we showed that the hydroxyapatite was affinity was high and well within the range of hydroxyapatite binding percentages observed in bisphosphonate-based diagnostic and therapeutic radiopharmaceuticals (Ogawa et al., 2006a,b; Uehara et al., 2007). Interestingly, it was observed that upon introduction and vortex-mixing of the Rhenium-188-HEDP in the hydroxyapatite suspension, the brown color of Rhenium-188-HEDP immediately disappeared, indicating an almost instantaneous binding of Rhenium-188-HEDP complex to hydroxyapatite.

Approximately 20% of the drug product did not bind to hydroxyapatite. We ruled out that this was the result of saturation of the hydroxyapatite by Rhenium-188-HEDP, because increasing the amount of hydroxyapatite during the experiments did not influence the binding percentage (data not shown). Since it is known that Rhenium-bisphosphonate complexes consist of complicated mixtures of oligomers that may have varying bone affinity (Elder et al., 1997), the unbound radioactivity may be explained by formation of Rhenium-HEDP complexes without sufficient hydroxyapatite affinity and, to a lesser extent, by unbound perrhenate and rhenium oxide in the solution.

The radiochemical purity of the drug product was >95% at any occasion and the Rhenium-188-HEDP complex was shown to be stable for at least 24 h at ambient temperature in the injection vial as well as in the polypropylene syringe. This stability was in concordance with the stability as previously reported by Lin et al. (1999, 1997). Our results demonstrated that Rhenium-188-HEDP was stable for storage for at least 24 h at ambient temperature in both containers. These stability results prove that preparation of the radiopharmaceutical may be performed up to 24 h in advance

of administration, making shipment of the radiopharmaceutical to other healthcare establishments feasible.

3.5. Bio-distribution in mice

These experiments showed that Rhenium-188-HEDP had a high bone affinity with a mean uptake ratio of 12.3 (range 9.6–14). This was comparable to uptake ratio's reported previously for various Technetium- and Rhenium-bisphosphonate complexes (Bergqvist et al., 1984; Fruhling et al., 1986; Hsieh et al., 1999; Pauwels et al., 1983). A representative skeletal scintigram after injection of 37 MBq Rhenium-188-HEDP in a mouse is depicted in Fig. 3. The soft-tissue accumulation of Rhenium-188-HEDP was negligible and most of the radiopharmaceutical accumulated in bone tissue.

3.6. Rhenium-188-HEDP product quality review

During the first year of routine production (the lifespan of two generators), the generator eluate was subjected to quality control 60 times. The median radiochemical purity was 98.6% (range 97.3–99.4%) and was within specification (>98%) 58 out of 60 times. When the eluate quality was out of specification, this was due to the presence of an increased amount of rheniumoxide, a reduced form of the perrhenate ion. During product development, we found that it was of utmost importance to use the eluate within 60 min after elution of the generator. This prevented the formation of excess rheniumoxide, although the clinical relevance of minimal amounts may be subject to discussion, considering the specification of >93% radiochemical purity for the drug product. The median aluminum content of the generator eluate was 0.2 ppm (range 0–0.75 ppm) and always within specification (<2 ppm), indicating that the column in the generator could withstand at least a half year of use. This was confirmed by our results of the radionuclidic purity:

Fig. 3. Bone scintigram of a mouse, obtained 3 h post injection of 37 MBq Rhenium-188-HEDP.

^{188}Tungsten breakthrough was always <0.01% and thus always within specification of <0.1%. Furthermore, during 1 year of routine production, 48 labeling procedures were performed for administration of Rhenium-188-HEDP to approximately 70 patients. The pH of the drug product was always within specifications (pH 4–6). In only one occasion the drug product quality was not sufficient due to a low radiochemical purity of 87.1%, due to a human fault. The median radiochemical purity was 97.3% (range 87.1–98.9%). Overall, the labeling process showed very reproducible results, which

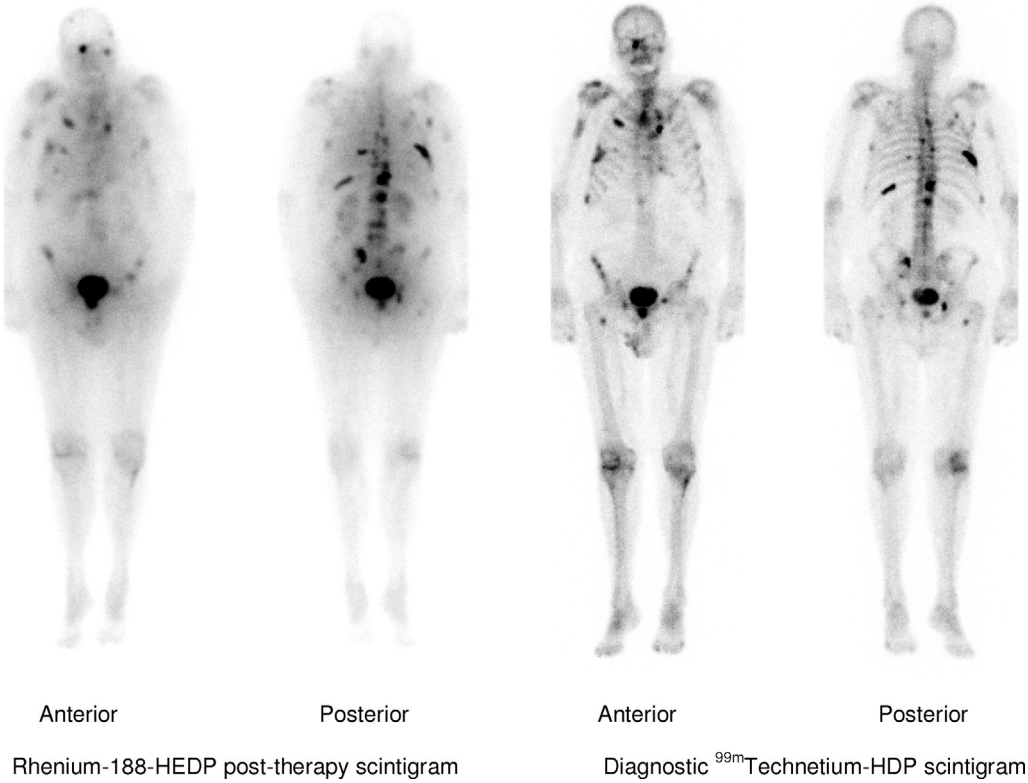

Fig. 4. Rhenium-188-HEDP post-therapy skeletal scintigram, 3 h post injection of 40 MBq/kg Rhenium-188-HEDP (left) and recent diagnostic skeletal scintigram, 3 h post injection of 555 MBq 99mTechnetium-HDP (right).

was confirmed with the process capability index (C_p) of 1.16. Therefore, the labeling process was considered robust.

3.7. Bio-distribution of Rhenium-188-HEDP in a patient

Diagnostic Tc-99m-HDP and post-therapy Rhenium-188-HEDP scintigrams of an 84-year-old patient with prostate cancer metastatic to bone are shown in Fig. 4. The post therapy Rhenium-188-HEDP scintigram showed no uptake in other organs than the skeleton and kidneys. The Rhenium-188-HEDP images were identical to the Tc-99m-HDP scintigram, showing the same number and localization of metastases. This indicated that the two radiopharmaceuticals concentrate in metastatic bone lesions by a similar mechanism and that Rhenium-188-HEDP shows similar bone uptake as previously shown by other groups.

4. Conclusion

In conclusion, we clearly demonstrated the feasibility of preparing GMP grade Rhenium-188-HEDP. The production process was validated and proven to be robust. Rhenium-188-HEDP showed a high affinity for bone tissue in vitro as well as in vivo. To the best of our knowledge, we are the first to describe the production of all necessary starting materials and the drug product under GMP conditions and this method can now be transferred to other sites. Rhenium-188-HEDP is currently being applied at our institute for routine treatment of painful bone metastases. Clinical studies investigating the therapeutic efficiency of radionuclide therapy combined with chemotherapy in prostate cancer metastatic to bone are ongoing.

Acknowledgements

Fred van het Schip, Remmert de Roos and Willem Meulenhoff of the University Medical Center in Utrecht (The Netherlands) as well as Stefan Guhlke at the University of Bonn (Germany) are kindly acknowledged for their useful suggestions for the pharmaceutical development of Rhenium-188-HEDP. Furthermore, Ruud Ramakers of MILabs in Utrecht (The Netherlands) is kindly acknowledged for his help with the animal experiments.

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