Journal of Radioanalytical and Nuclear Chemistry, Vol. 260, No. 2 (2004) 363–368

Preparation and evaluation of two ^{188}Re-radiopharmaceuticals for endovascular irradiation*

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(Received July 29, 2003)

Procedures for complexing DTPA with ^{188}Re from a ready kit and by conventional manipulation were elaborated and the study of the stability and biodistribution of ^{188}Re perrhenate and ^{188}Re-DTPA were performed. Best labeling was achieved using DTPA (38 mM) with 2 mg/ml of $SnCl_2 \cdot 2H_2O$. The radiochemical purity was 95.9±2.9%. The complex was stable for 24 hours when ascorbic acid was used. Greatest uptake of ^{188}Re-DTPA was by kidneys (19.3±2.1%ID/g) and for ^{188}Re-perrhenate by stomach (21.3±2.8%ID/g). In conclusion, a kit of freeze-dried DTPA was developed. Organ damage is unlikely by virtue of its rapid urinary excretion.

Introduction

The incidence of atherosclerotic coronary artery disease is high, and one of the accepted modes of treatment is balloon angioplasty. Restenosis occurs within 6 months in a high percentage (30–50%) of all cases who undergo this treatment,[1,2] and it results in recurrent angina as well as the need for additional procedures. However, restenosis is rarely followed by acute myocardial infarction or death. Successful therapy for restenosis, preventing clinical recurrence, may also have economic consequences.[3] The major causes of restenosis are the elastic recoil of the arterial walls, intimal proliferation resulting in growth of new tissues inside the vessel and wound healing process.[4]

Administration of anticoagulants, antiplatelet agents, steroids, certain antispamodics and lipid lowering agents, as well as the mechanical approach of implantation of a stent in the previously dilated artery have been used to reduce the incidence of restenosis. However, these treatments showed some disadvantages such as high dose of the drugs with increased risk of side effects.[2] Local irradiation is a new approach for reduction of stenotic recurrence.

It is already well established that proliferative cells are radiosensitive and suitable doses of ionizing radiation can retard their growth.[5]

The radioisotope ^{188}Re has been proposed as a suitable candidate for the preparation of radiopharmaceuticals for therapeutic applications, particularly because of its favorable nuclear properties. Carrier-free ^{188}Re ($T_{1/2}$ = 17 h) can be obtained from an in-house ^{188}W/^{188}Re generator cost-effectively and on-demand.[6] The parent radionuclide ^{188}W is produced in a reactor by double neutron capture of enriched ^{186}W targets with high neutron flux of $5 \cdot 10^{14}$ n·cm^{-2}·s^{-1}.[7] ^{188}Re decays by emission of a relatively high energy beta-particle (E_{max} = 2.11 MeV) which is suitable for radiotherapy, followed by emission of 155 keV gamma-photons in 10% abundance. The gamma-photon can be used to monitor the biodistribution and for estimate dosimetry with standard scintigraphic equipment. Its low abundance minimizes exposure to medical personnel.

Diethylenetriaminepentaacetic acid (DTPA) has been used as a radiopharmaceutical labeled with ^{99m}Tc for brain imaging, but its main application is for renal imaging. It has also been used as an intermediary chelating agent for labeling biomolecules with ^{111}In, ^{90}Y, ^{153}Sm and ^{177}Lu.

The aim of the present study was to evaluate the conditions for labeling DTPA with ^{188}Re from ready kits for ^{99m}Tc and from DTPA salt, and to study the optimization of labeling, stability and radiochemical yield, and biodistribution, compared to perrhenate. The findings are considered within the context of clinical use, to fill balloons for endovascular treatment of coronary artery obstructions.

Experimental

Materials

Rhenium-188 was obtained from an alumina-based ^{188}W/^{188}Re generator. Tungsten-188 (^{188}W), produced by neutron irradiation in high flux nuclear reactor, was fixed into an aluminum oxide (Al_2O_3) column in quartz glass. ^{188}Re, which is formed by decay from ^{188}W, was obtained from the column by elution with 3 ml of sodium chloride for injection (9 mg/ml). The generator was supplied by MAP Medical Technologies Oy (Tikkakoski, Finland) with a yield of 70%, under a grant of IAEA.

* This study was presented in part at the European Association of Nuclear Medicine Annual Congress, Vienna, Austria, August 31-September 4, 2002.

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0236–5731/2004/USD 20.00
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Diethylenetriaminepentaacetic acid (DTPA), stannous chloride dihydrate, sodium para-aminobenzoic acid (PABA) and ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). Chromatography stationary phase (ITLC-SG) was provided by Gelman Sciences Inc., USA.

DTPA kit for labeling with ^{99m}Tc was produced in house by the Institute of Energetic and Nuclear Research/National Commission of Nuclear Energy (IPEN/CNEN-São Paulo, Brazil).

Preparation and labeling of ^{188}Re-DTPA

The studies were first done with a kit of lyophilized DTPA produced by IPEN/CNEN that contains: 10 mg DTPA, 1 mg $SnCl_2 \cdot 2H_2O$, and 2 mg PABA. To the freeze-dried vial 1 ml of nitrogenated water was added and the vial was heated to dissolve the powder. Because additional stannous ions are required for reduction of perrhenate, a greater amount of $SnCl_2 \cdot 2H_2O$ in nitrogenated 0.1N HCl was added, followed by the addition of $Na^{188}ReO_4$ eluted from the $^{188}W/^{188}Re$ generator in 0.9% saline. The mixture was heated for 30 minutes in a water bath at 100 °C. The solution was then cooled to room temperature prior to use.

Other studies were performed using the same method described above, but starting the preparation from DTPA salt. In all cases ten consecutive experiments ($n = 10$) were conducted, and results are presented as mean values.

Radiochemical control

Radiochemical analysis of ^{188}Re-DTPA was performed by instant thin-layer chromatography (ITLC) to determine the labeling efficiency and colloid formation. ITLC-SG chromatography paper was cut into 1.5×13 cm^2 strips and activated by heating for 30 minutes, at 110 °C, according to manufacturer's instructions.

Labeling efficiency was determined using 0.9% saline and acetone as the mobile phase. Radioactivity of the strips was analyzed by cutting the strips into 1 cm segments and introducing in a well-type gamma-counter (Packard Cobra II, Meridien, CT USA).

When using the solvent 0.9% saline, free perrhenate ($^{188}ReO_4^-$) and ^{188}Re-DTPA migrated with the solvent front ($R_f = 1$), whereas radiocolloid ($^{188}ReO_2$) remained at the origin ($R_f = 0$). Using acetone ^{188}Re-DTPA and the radiocolloid stayed at the origin ($R_f = 0$) and $^{188}ReO_4^-$ moved to the solvent front ($R_f = 1$).

Radiochemical stability

Radiochemical purity of the labeled product was analyzed during 24 hours after preparation both with or without the stabilizing agent ascorbic acid.

Optimization of radiolabeling

Optimization was achieved by varying relevant parameters including DTPA mass (10 to 30 mg, $1.27 \cdot 10^{-2}$–$3.8 \cdot 10^{-2}$M), quantity of stannous chloride from 2 to 4 mg/ml, pH between 3 and 5, and reaction time (15 minutes to 1.0 hour) in the preparation starting from DTPA salt. For the ready kits optimization studies, only the amount of stannous chloride, from 0.5 to 4 mg/ml was changed. In all cases, ten experiments ($n = 10$) were performed for each procedure and results are presented as mean values.

Biodistribution studies

Experiments were carried out in compliance with the guidelines for conduct in animal experimentation, by the Scientific Ethics Committee, IPEN/CNEN-SP.

Biodistribution studies of ^{188}Re-perrhenate and ^{188}Re-DTPA were performed in Swiss mice weighing 25–30 mg. Intravenous injection of 0.1 ml (3.7 MBq) of each radiopharmaceutical was done in the tail vein. The animals were sacrificed 0.25, 0.5, 1,2 and 4 hours post injection in groups of 6 animals ($n = 6$). The tissues and organs were excised, weighed and the activity measured in a well-type gamma-counter (Packard Cobra II, Meridien, CT USA). Tissue concentrations were calculated and expressed as percent of injected dose per gram or per milliliter.

Results

Labeling yield of ^{188}Re-DTPA obtained from freeze-dried kit (the same used for labeling with ^{99m}Tc), and varying concentration of reducing agent can be seen in Fig. 1. Using stannous chloride up to 2 mg/ml, the percentage of radiochemical purity achieved was under 80%. The best result corresponded to 3.25 mg/ml of stannous chloride (86.3±2.9%). It must be emphasized that in the lyophilized kit there was already 1 mg of $SnCl_2 \cdot 2H_2O$ (0.5 mg/ml). For concentrations between 3.25 and 4 mg/ml no improvement was observed in the complexation yield.

The effect of concentration of DTPA on the complexation yield starting from DTPA salt is shown in Fig. 2. In this case 95.9±2.9% purity was achieved with 38 mM of DTPA. The percentage of radiocolloid and free rhenium under these conditions are given in Fig. 3.

Reactions were carried out at pH 3. When pH was progressively increased to 5, a proportional decrease in yield was observed.

Radiochemical stability was observed for 24 hours. When the stabilizing agent ascorbic acid was used in the formulation, ^{188}Re-DTPA stayed stable for the entire period of time. When ascorbic acid was not used, stability decreased to about 11% (Fig. 4).

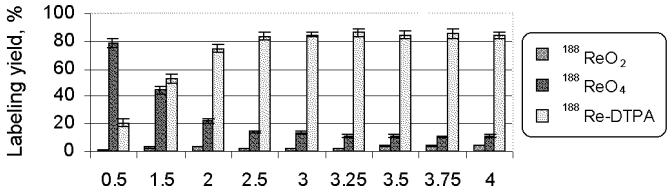

Fig. 1. Labeling yield of ^{188}Re-DTPA using the kit for labeling with ^{99m}Tc (DTPA: 10 mg; $n = 10$)

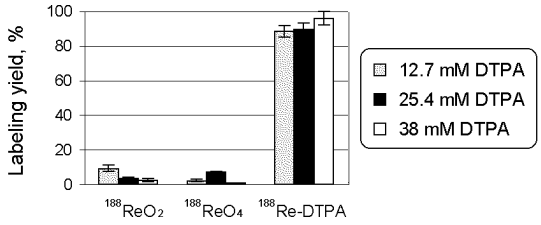

Fig. 2. Labeling yield vs. molarity of DTPA ($SnCl_2 \cdot 2H_2O$: 1 mg/ml; $n = 10$)

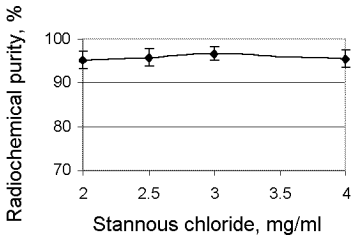

Fig. 5. Radiochemical yield of ^{188}Re-DTPA vs. amount of reducing agent ($n = 10$)

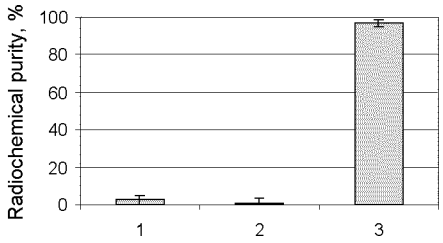

Fig. 3. Radiochemical purity of ^{188}Re-DTPA (DTPA: 38 mM; $SnCl_2 \cdot 2H_2O$: 1 mg/ml; 1 – $^{188}ReO_2$, 2 – $^{188}ReO_4$, 3 – ^{188}Re-DTPA, $n = 10$)

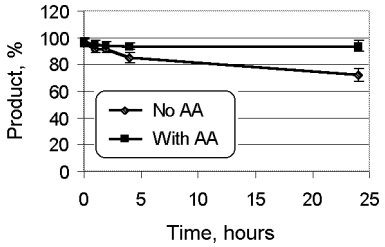

Fig. 4. Stability of ^{188}Re-DTPA for 24 hours with and without ascorbic acid ($n = 10$)

The amount of 2 mg/ml of $SnCl_2 \cdot 2H_2O$ was sufficient to reduce perrhenate. No variation in complexation yield was observed when elevating the proportion of stannous chloride from 2 to 4 mg/ml (Fig. 5). It is not recommended to use higher concentrations of stannous chloride due to its toxicity and to the risk of increasing radiocolloid impurity. The effect of ^{188}Re activity (1 to 20 mCi) on complexation yield was not very marked.

The results of biodistribution experiments carried out with ^{188}Re-DTPA are displayed in Fig. 6 and Table 1, and for $Na^{188}ReO_4$ in Fig. 7 and Table 2, respectively. We can see that the activity of ^{188}Re-DTPA is cleared through the renal pathway. Greatest uptake of ^{188}Re-DTPA was by the kidneys (19.3±2.1%ID/g) and for ^{188}Re-perrhenate by the stomach (21.3±2.8%ID/g), 15-minute post injection. Uptake of ^{188}Re-perrhenate by the stomach increased at 4 hours post injection (Fig. 7).

Discussion

Endovascular radiation therapy for prevention of restenosis provides a new opportunity for patients suffering from severe atherosclerotic lesions of the vascular system.

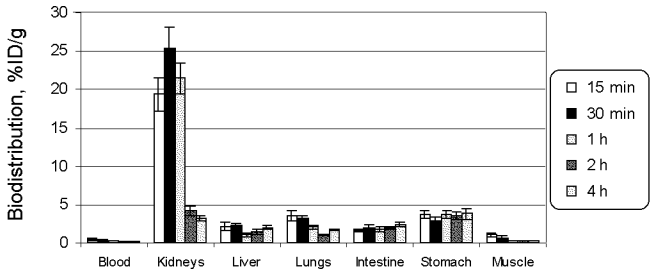

Fig. 6. Biodistribution of ^{188}Re-DTPA ($n = 6$)

Table 1. Biodistribution of ^{188}Re-DTPA in Swiss mice (in %ID/g ± SD)

Time	Blood	Kidneys	Liver	Lungs	Intestine	Stomach	Muscle
15 min	0.5 ± 0.1	19.3 ± 2.1	2.12 ± 0.5	3.6 ± 0.6	1.6 ± 0.2	3.7 ± 0.5	1.0 ± 0.3
30 min	0.34 ± 0.0	25.4 ± 2.6	2.2 ± 0.3	3.3 ± 0.3	2.0 ± 0.4	3.0 ± 0.4	0.7 ± 0.2
1 h	0.3 ± 0.0	21.4 ± 2.0	1.1 ± 0.2	2.0 ± 0.2	1.8 ± 0.3	3.7 ± 0.5	0.4 ± 0.0
2 h	0.1 ± 0.0	4.1 ± 0.6	1.4 ± 0.3	1.1 ± 0.1	1.9 ± 0.1	3.5 ± 0.5	0.3 ± 0.0
4 h	0.2 ± 0.0	3.2 ± 0.3	2.0 ± 0.3	1.7 ± 0.1	2.4 ± 0.3	3.8 ± 0.7	0.3 ± 0.0

$n = 6$.
SD: Standard deviation.

Table 2. Biodistribution of $Na^{188}ReO_4$ in Swiss mice (in %ID/g±SD)

Time	Blood	Kidneys	Liver	Lungs	Intestine	Stomach	Muscle
15 min	0.6 ± 0.0	2.4 ± 0.3	2.7 ± 0.2	2.9 ± 0.3	1.4 ± 0.2	21.3 ± 2.8	1.4 ± 0.0
30 min	0.5 ± 0.0	2.7 ± 0.3	2.7 ± 0.3	3.3 ± 0.3	1.5 ± 0.4	36.3 ± 1.4	0.6 ± 0.0
1 h	0.4 ± 0.0	1.8 ± 0.3	1.5 ± 0.1	3.9 ± 0.3	1.5 ± 0.3	38.4 ± 2.2	0.8 ± 0.0
2 h	0.3 ± 0.0	1.3 ± 0.2	1.8 ± 0.1	2.5 ± 0.2	1.0 ± 0.0	42.3 ± 2.5	0.5 ± 0.0
4 h	0.4 ± 0.0	1.7 ± 0.1	2.2 ± 0.2	3.0 ± 0.2	1.3 ± 0.1	96.6 ± 2.9	0.5 ± 0.0

$n = 6$.
SD: Standard deviation.

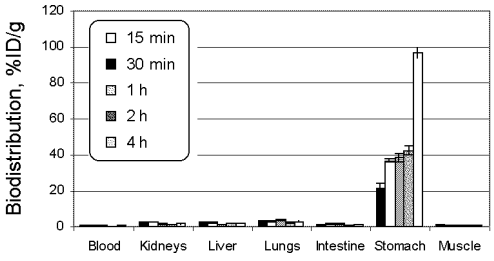

Fig. 7. Biodistribution of $Na^{188}ReO_4$ ($n = 6$)

The earliest observation that blood vessels in irradiated tissues show specific changes was made 100 years ago.[8] Part of the mechanism of restenosis appears to be the proliferation of smooth muscle cells, triggered to divide by the damage caused by angioplasty, and this proliferation can be inhibited by irradiation with γ-rays or β-rays.[9–11]

Some techniques of endovascular radiation treatment of coronary artery walls have been used. In the past, first radioactivity coated stents containing ^{32}P were tried, and then sealed sources of ^{192}Ir in the form of tiny spheres or wires. ^{32}P, ^{89}Sr and ^{90}Y isotopes were also used as a coated thin wire to be attached to a retrievable catheter wire for insertion into the artery under treatment for a pre-determined time.[12]

A new concept for preventing restenosis is the use of unsealed radioactivity in the form of a liquid-filled balloon with radioactive liquid at low pressure.[13,14]

Holmium-166, ^{188}Re and ^{90}Y radiopharmaceuticals, such as ^{166}Ho-EC , ^{166}Ho-DTPA, ^{188}Re-EC, ^{188}Re-DTPA, ^{188}Re-MAG_3, ^{90}Y-DOTA, ^{90}Y-DTPA, have been studied for endovascular radionuclide therapy.[15–20]

Most of the used salts are renal agents. ^{166}Ho has gamma- and beta-rays similar to ^{188}Re, but although it features easier production and lower costs than ^{188}Re. Possible presence of the long-lived radioactive contaminant ^{166m}Ho ($T_{1/2} = 1200$ y) is relevant.[21,22]

An early feasibility study utilizing beta-radiation in heart disease was performed in Geneva, Switzerland. In this study, ^{90}Y wire was delivered after conventional

percutaneous coronary angioplasty (PTCA) through a closed-end lumen catheter centered by a segmented balloon.[23]

The use of radioactive solutions[24] for balloon inflation to deliver arterial wall radiation after PCTA is one approach that offers the important advantage of uniform vessel irradiation.[25]

Low pressure balloon inflation may be rarely associated with balloon rupture (<1 per 10,000).[17] If this occurs, radioisotope release could potentially result in significant dose to radiation-sensitive organs. Therefore, it is advised to give preference to molecules that have better biological performance in the case of a balloon rupture.

^{90}Y is an avid bone seeker and balloon emptying would result in significant bone marrow doses by beta-radiation even with rapid urinary bladder excretion, or when complexed with DOTA or DTPA. This complexation must be carried out with excess of ligand to ensure that no free yttrium is present which could accumulate in bone.

^{166}Ho can have the long-lived ^{166m}Ho ($T_{1/2}$ = 1200 y) radioactive contaminant, whereas ^{188}Re can be obtained from a ^{188}W/^{188}Re generator, that is ready to use and exhibits a long shelf-life. Gaseous ^{133}Xe, might be an alternative to the liquid-filled balloon and carries only a low risk of tissue incorporation. However, it is much more expensive than ^{188}Re.[25]

Rhenium-188 is located in the same group (Group VII) as ^{99m}Tc in the periodic table, so it shows similar chemical properties. Some variables differ such as redox potential (for ^{188}Re it is 200 mV lower than for ^{99m}Tc). The perrhenate ion ($^{188}ReO_4^-$) is thermodynamically more stable and more difficult to reduce to a lower oxidation state. Quantities of reducing agent ($SnCl_2 \cdot 2H_2O$) for this purpose must be high and other labeling conditions should be proportionally stronger, including heating, acidic conditions and reaction time.

Any labeling procedure must comprise an appropriate reduction step required to change the oxidation state of sodium perrhenate eluted from the generator from +7 to lower values, and to allow the formation of complexes between the metal and the selected ligand. Labeling with perrhenate in solution leads to potential contaminants: incompletely reduced and re-oxidated perrhenate, as well as colloidal rhenium, considered to be in the form of ReO_2. The use of large amount of reducing agent can lead to an increased production of ^{188}Re colloid and occasional formation of precipitates. The two by-products (perrhenate and colloidal rhenium) must be kept minimal so that no further purification of the resulting product is required.

The use of the same kit of DTPA available for labeling with ^{99m}Tc, in the labeling procedure with ^{188}Re, did not show much advantage. The final amount of stannous chloride was higher (3.75 mg/ml) than for DTPA salt (2 mg/ml). The highest complexation yield was just 86.3±2.9% and the required time was the same. It seems better to prepare all solutions fresh or to provide a specific freeze-dried kit for labeling with ^{188}Re only. The kit recommendation is 38 mM of DTPA, 2 mg/ml of $SnCl_2 \cdot 2H_2O$, and 2 mg/ml of ascorbic acid. Complexation yield with ^{188}Re thus obtained was higher than 95%. Due to oxidation of ^{188}Re-DTPA, and also to limiting factors such as radiolysis in vitro, it is necessary to store the product with an appropriate stabilizer. Ascorbic acid was selected for this study as it has good efficacy and low toxicity.

HSIEH et al.[18] labeled DTPA with ^{188}Re using a concentration of stannous chloride of 7.5 mg/ml, much more than recommended in the literature[17] or in commercials kits (Mallinckrodt).

^{188}Re-perrhenate, analogously to ^{99m}Tc-pertechnetate, accumulated in the stomach. Although perrhenate is excreted via the urinary system, ^{188}Re-DTPA exhibited much faster renal clearance with low uptake in vital organs. Although these findings were here confirmed in Swiss mice only, they are conventionally accepted for humans as well.[12,14,17,25] Faster excretion of the radiopharmaceuticals by the organism is an advantage, with lower accumulation of radiation in non-target organs in case of accidental release of activity due to rupture of the endovascular balloon.[12,14,25] Similar results were reported by HSIEH et al.[18]

Conclusions

A kit of freeze-dried DTPA could be developed for labeling with ^{188}Re, by means of an easy and rapid method, according to the parameters of this investigation. It appears to be a better option than labeling a commercial technetium kit and just adding more reducing agent. Protection for main organs with this molecule, in the case of balloon damage during angioplasty, would be considerably higher than with other radionuclides by virtue of rapid excretion via the urinary system.

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The valuable support of the International Atomic Energy Agency is appreciated.

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