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Research Article

A kit formulation for the labelling of lipiodol with generator-produced ^{188}Re

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Summary

A lyophilised kit formulation for the efficient labelling of lipiodol with generator-produced ^{188}Re is described. This method involves the reaction of $[^{188}Re^{VII}O_4]^-$ (37–370 MBq) with $SnCl_2$ as a reducing agent, potassium oxalate as a reduction promoter, ascorbic acid as antioxidant and sodium gluconate as a weak chelate. The intermediate compound $Na[^{188}Re^{V}O(gluc)_2]$ reacts with the sodium salt of a dithiobenzoate ligand to give the neutral complex $[^{188}Re^{III}(PhCS_3)_2(PhCS_2)]$. This complex is then quantitatively extracted with lipiodol to afford a stable solution. Radiochemical purity (RCP) was greater than 90% and the yield of extraction was about 88%. The role of the different kit components has been studied in detail to find the most efficient formulation (amount of reducing agent, antioxidant). The use of 0.8 mg of stannous chloride, with 40 mg of potassium oxalate and 30 mg of ascorbic acid, was found necessary. The stability of the ^{188}Re-radiolabelled lipiodol has been investigated, in the presence of plasma. The radiolabelled lipiodol (^{188}Re-SSS lipiodol) is stable at least 48 h (RCP = 91.0 ± 4.0%). Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: lipiodol; ^{188}Re; dithiobenzoate ligands; SSS

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumours in the world. Due to generally late detection, curative treatments,

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such as surgical resection, are usually not possible. In these cases, palliative treatments, that use carriers of therapeutic molecules, such as chemotherapeutic drugs or radioactive isotopes,[1–3] can be attempted. One such carrier is lipiodol, an iodinated ester of poppyseed oil, containing 38% iodine by weight. Due to its high lipophilicity and viscosity, it has been used as an embolic agent for the detection of liver cancer.[4] Liver tumours are mainly perfused by the hepatic artery, while the blood supply of the normal hepatic cells is obtained from the portal vein. Consequently, embolic agents, such as lipiodol, accumulate in the tumour by embolization if administered through the hepatic artery. Moreover, lipiodol injected through the hepatic artery has shown a selective and prolonged retention within the tumour.[5]

There have been many attempts to label lipiodol with therapeutic radioisotopes, including ^{131}I, ^{90}Y, ^{186}Re and ^{188}Re.[6–12] ^{131}I-labelled lipiodol ($E_{\beta max} = 0.81$ MeV; $E\gamma = 364$ keV (81%); $t_{1/2} = 8.02$ day) is commercially available and is currently used in many countries. Nonetheless, despite encouraging preliminary results, there are some problems with ^{131}I, particularly in the low-energy beta and high-energy gamma emissions. It has been suggested by some authors that ^{90}Y is better suited.[7] Although ^{90}Y-lipiodol has been found to localise and retain in the tumour, it has also been found to accumulate in non-target organs, especially in the skeletal system. The radiation burden to the bone marrow, highly sensitive to radiation, may limit the clinical use of ^{90}Y-lipiodol.

^{188}Re is an attractive alternative for therapy, since it has similar high beta energy ($E_{\beta max} = 2.1$ MeV), with a shorter physical half-life ($t_{1/2} = 16.9$ h). In addition, ^{188}Re emits a 155 keV γ-ray in 15% abundance suitable to monitor biodistribution and to calculate dosimetry. A further advantage is that ^{188}Re is now conveniently produced through a ^{188}W/^{188}Re generator system, that enables the on site production of ^{188}Re-radiopharmaceuticals, from the obtained sodium perrhenate.[13] However, no ^{188}W/^{188}Re generator is currently produced with pharmaceutical grade, which may appear as a limitation.

Several attempts have been made to label lipiodol with ^{188}Re. Two strategies have been studied which are the covalent bonding between lipiodol and the ^{188}Re-chelate[8,14] and the solubilization of a lipophilic ^{188}Re-complex into cold lipiodol, as a consequence of the strong hydrophobic interaction between the lipophilic metal complex and the fatty oil.[10–12,15,16] This second approach, easier to perform, seems to be the most promising. However, the methods described lack a satisfactory yield and reproducibility.[12,17]

We have previously described the synthesis, at the macroscopic scale, of a neutral and lipophilic complex [M(PhCS$_3$)$_2$(PhCS$_2$)], M-SSS (SSS = Super Six Sulphur, M = Re, Tc), both with rhenium[18] and with technetium.[19] The analogous ^{99m}Tc radiotracer has also been successfully prepared[20] and has been used to label lipiodol (RCP = 92.5 $\pm$ 2.6%, yield = 96.2 $\pm$ 2.8%),[21] to

study the optimal labelling conditions. Due to differences between technetium and rhenium, especially concerning their redox behaviour and the kinetic of exchange reactions,[22] the reaction conditions must be modified to obtain the ^{188}Re complex with a satisfactory yield (> 90%).

In this study, a kit formulation for the preparation of a neutral and lipophilic rhenium complex [^{188}Re(PhCS$_3$)$_2$(PhCS$_2$)] (^{188}Re-SSS), suitable for the labelling of lipiodol, in view of HCC treatment, was investigated.

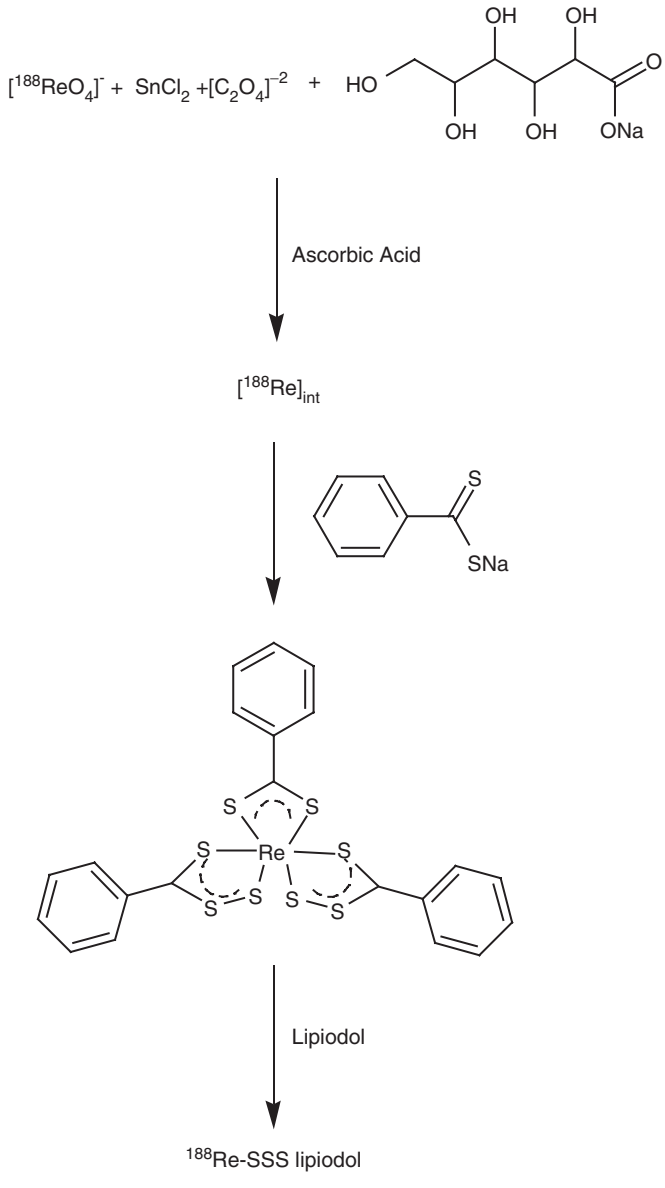
Results and discussion

This study was designed to develop a simple and reliable kit formulation for the labelling of lipiodol with ^{188}Re, suitable for the treatment of hepatocellular carcinoma. The labelling approach involved the selective extraction and retention of the [^{188}Re(PhCS$_3$)$_2$(PhCS$_2$)] (^{188}Re-SSS) complex into lipiodol owing to the strong hydrophobic interaction between this lipophilic compound and the fatty oil. The complex was synthesised with the same scheme applied for the analogous technetium complex, i.e. formation of an intermediate complex with gluconate (Na[^{188}Re^{V}O(C$_6$H$_{12}$O$_7$)$_2$]) in a first step, and addition of the dithiobenzoate ligand to the reaction medium in a second step (Scheme 1). Due to the differences of reactivity between rhenium and technetium, the combined action of the different components of the kit (reducing agent, ligand, additives) has been studied in detail to find the most efficient kit formulation. The reaction conditions have also been investigated, as well as the *in vitro* stability of the final solution of ^{188}Re-SSS lipiodol.

Reducing agent

Several reducing agents have been described in the literature, such as stannous chloride,[14] stannous fluoride,[23] stannous tartrate,[24] sodium dithionite,[25] or phosphines with hydrochloric acid.[26] Stannous chloride is the most commonly used and is found in most of the commercial kits to reduce pertechnetate. This is the reducing agent we used to reduce perrhenate, which is more difficult to reduce than pertechnetate. Reduction of perrhenate requires larger amounts of tin. For example, for the preparation of ^{188}Re(V)-DMSA, the commercial kit for ^{99m}Tc(III)-DMSA is used, which implies a 10-fold amount of tin chloride, in comparison to technetium. Pirmettis *et al.* have proposed a kit formulation for the ^{188}Re(V)-DMSA, with 100 mg of tin chloride.[27] A very big amount of tin chloride can have a deleterious effect on the nervous system,[28] and its use is thus limited by its toxicity (LD_{50} (rats) = 2000 mg/kg orally and 43 mg/kg i.v.). The suitable amount of stannous chloride for the reduction of the perrhenate has been investigated and the results are summarised in Table 1.

The reduction of perrhenate is closely related to the amount of tin. Nonetheless, even with 16 mg of tin chloride (i.e. 200-fold molar excess compared to ^{99m}Tc), and 75 mg of sodium gluconate, the reduction and



Scheme 1. Preparation of ^{188}Re-SSS lipiodol

Table 1. Influence of the amount of $SnCl_2$ on the RCP of [^{188}ReO(gluc)$_2$]

$SnCl_2$ (mg)	0.75	4	8	16
RCP (%)	<1	22	40	65

(Eluent: EtOH/toluene/$CHCl_3$/$AcONH_4$, H_2O 0.5 M 6/3/3/1 v/v, $R_f = 0$).

formation of the Na[^{188}ReO(gluc)$_2$] intermediate is not quantitative. Moreover, when the dithiobenzoate is added, precipitation occurs. Most of the radioactivity appears to be in the precipitate, as shown by extraction with an

organic solvent (dichloromethane). TLC analysis showed formation of the expected complex. The M-SSS complex (M = ^{99m}Tc, ^{188}Re) is highly lipophilic and, thus, is insoluble in saline. In the case of ^{99m}Tc, the complex is in suspension in saline. In the case of ^{188}Re, this suspension is removed from the solution by the precipitation of the ligand.

Additives

The use of different chelating agents has been attempted, to prevent precipitation of tin salts. Among the most commonly used agents are citrate and PDTA (1,2-propylene diamine tetraacetic acid). Both proved inefficient for our kit, since precipitation still occurred. For these reasons, the amount of reducing agent must be reduced to prevent precipitation.

The difficulty of perrhenate reduction lies partly in the geometry change between the tetrahedral perrhenate and the octahedral hexacoordinated reduced species, that reduces redox potentials.[29] Thus, an intermediate octahedral species with an oxidation number of VII, obtained by substitution of oxo groups with a suitable ligand, must limit this negative effect. This phenomenon is called the coordination sphere expansion, and is easier for rhenium than for technetium.[22] Oxalate and citrate permit this coordination sphere expansion by chelating to the rhenium.[30] Boschi *et al.* showed a significant effect of oxalate on perrhenate reduction,[29] enabling it with softer conditions. We have thus studied the influence of oxalate on perrhenate reduction for the formation of the gluconate intermediate (Table 2). Use of 40 mg of potassium oxalate enabled the formation of the intermediate complex with a radiochemical purity (RCP) of 60% with only 0.8 mg of tin chloride. In contrast, 16 mg of tin chloride was required for the same results without oxalate.

Since rhenium has a tendency to reoxidize easily to perrhenate, an antioxidant is thus generally required. Some investigators have described the use of ascorbic acid, gentisic acid or α-tocopherol.[31–33] The most commonly used antioxidant is ascorbic acid. A combination of ascorbic acid and gentisic acid could also be used for example. The greater the activity level, the greater the requirement for the presence of an antioxidant, since the extent of radiolysis is proportional to activity. The influence of the antioxidant on perrhenate reduction has thus been determined. The two antioxidants that

Table 2. Influence of the potassium oxalate amount on the RCP of [^{188}ReO(gluc)$_2$]

Oxalate (mg)	0[a]	31[b]	40[a]	60[a]
RCP (%)	16	30	65	67

[a] 0.8 mg $SnCl_2$, 7.5 mg sodium gluconate, 30 mg ascorbic acid;
[b] 15 mg $SnCl_2$, 75 mg sodium gluconate, 30 mg ascorbic acid.

Table 3. Influence of the antioxidant on the RCP of [^{188}ReO(gluc)$_2$]

Antioxidant	Ascorbic acid[a]	Ascorbic acid[a]	Ascorbic acid[b]	α-Tocopherol[b]
Amount (mg)	28	56	30	30
RCP (%)	35	16	65	2

[a] 4 mg $SnCl_2$, 75 mg sodium gluconate;
[b] 0.8 mg $SnCl_2$, 7.5 mg sodium gluconate, 40 mg potassium oxalate.

have been studied are ascorbic acid and α-tocopherol. The results are summarised in Table 3.

Since α-tocopherol is insoluble in water, it cannot play its role towards rhenium. In contrast, ascorbic acid is efficient.[34] However, larger amounts decrease the reduction yield, as has already been shown with ^{188}Re-HEDP.[13]

Reaction conditions

Perrhenate reduction requires more drastic conditions than pertechnetate reduction, and optimal reaction temperature are usually much higher. For example, the preparation of ^{99m}Tc-DMSA can be performed at room temperature, whereas, for the analogous ^{188}Re-DMSA, it is necessary to heat at 100°C.[27] In our case, it is not necessary to heat the solution to reduce the perrhenate. Heating does not increase the yield nor the kinetic of the reaction.

Reaction time is one key point in the preparation of radiopharmaceuticals, due to the short half-life of most of the radioisotopes used in nuclear medicine. Thus, maximal RCP must be attained in the shortest possible time. Reduction of perrhenate with our kit formulation has been followed over a time period of one hour (Table 4), and the RCP of the ^{188}Re-gluconate intermediate does not increase significantly after 15 min.

The pH is another important factor, since radiopharmaceuticals are often very pH-sensitive. For most of ^{188}Re-based radiopharmaceuticals, the preparation conditions are very drastic, and usually require strongly acidic conditions. Indeed, tin chloride is more efficient at low pH, and is usually dissolved in acidic medium such as hydrochloric or acetic acid. In the case of two-step reactions, it is thus necessary to increase the pH before addition of the ligand, as is the case for the preparation of ^{188}Re-nitrido complexes.[29] With our kit formulation, the solution pH was 3 after addition of the perrhenate. The influence of the pH increase on the yield of the second step was investigated and is summarised in Table 5. Our results show that the pH rise has a negative effect on the RCP of the ^{188}Re-SSS complex.

Another key parameter is the volume, and Boschi *et al.* showed the volume, for the preparation of [^{188}ReN(DEDC)$_2$], must not exceed 1 ml for the formation of the $^{188}\text{Re} \equiv \text{N}$ group, to maintain an optimal RCP.[29] We thus

Table 4. Influence of reaction time on the RCP of [^{188}ReO(gluc)$_2$]

t (min)	15	30	45	60
RCP (%)	43	42	45	45

8 mg $SnCl_2$, 75 mg sodium gluconate, 30 mg ascorbic acid, RT.

Table 5. Influence of pH on the RCP of [^{188}Re(PhCS$_3$)$_2$(PhCS$_2$)]

pH	3	4.5	5	5.5	6
pH_{final}	4–4.5	5.5	6	6–6.5	6.5
RCP (%)	69	29	26	16	31

0.8 mg $SnCl_2$, 7.5 mg sodium gluconate, 30 mg ascorbic acid, 40 mg potassium oxalate, 100°C, 30 min.

Table 6. Influence of perrhenate volume on the RCP of the different complexes

Volume (ml)	0.5	1.5	2	3
RCP (%)				
[^{188}ReO(gluc)$_2$]$^-$	65	39	21	21
[^{188}Re(PhCS$_3$)$_2$(PhCS$_2$)]	40–70	59	33	3.5
^{188}Re-SSS lipiodol	93.0 ± 3.4	94	91	86
Extraction (%)	87.0 ± 9.1	86	81	66

0.8 mg $SnCl_2$, 7.5 mg sodium gluconate, 30 mg ascorbic acid, 40 mg potassium oxalate.

have investigated the influence of the perrhenate volume on the RCP of the compounds obtained at the different steps. The results are summarised in Table 6. As shown by other authors, raising the volume has a negative effect on the formation of the final compound. The volume must thus be maintained minimal to obtain the complex with a maximal RCP.

Based on our studies, the optimal kit formulation is: 0.8 mg $SnCl_2 2H_2O$ (dissolved in 0.1 ml HCl 1 M), 7.5 mg sodium gluconate, 30 mg ascorbic acid, 40 mg potassium oxalate. This kit is reconstituted in 0.5 ml saline and the perrhenate (0.5 ml of saline) is then added. After 15 min at room temperature, 20 mg of sodium dithiobenzoate are added, and the solution is heated 30 min at 100°C, to provide the ^{188}Re-SSS complex, as a precipitate.

Lipiodol labelling

After preparation of the ^{188}Re-SSS complex, the complete reaction mixture and precipitate are mixed with lipiodol and the mixture then centrifuged to allow a good separation of the two phases. After separation of the two phases, 87.0 ± 9.1% of the activity was found in the lipid phase, as a result of the lipophilic character of the ^{188}Re-SSS complex. The radiochemical purity of the

radiolabelled lipiodol, determined by TLC, was 93 ± 3.4%. Unfortunately, no HPLC was available to corroborate these results.

Stability

The stability of the ^{188}Re-labelled lipiodol was investigated over a period of 48 h, in the presence of human plasma. At the different time points (24 and 48 h), the two phases were separated and the RCP of the labelled lipiodol then checked by TLC. The results are summarised in Table 7 and show no degradation of the complex and a good stability of the labelling, even if there is no covalent bonding between the chelate and the lipiodol.

Experimental section

Materials and methods

^{188}Re as carrier-free Na[^{188}ReO$_4$] in physiologic solution was obtained by saline elution of a ^{188}W/^{188}Re generator (Oak Ridge National Laboratory, USA). An Alumina A Sep-Pak cartridge (Waters, Milford Massachusetts, USA) was used for trapping ^{188}W contaminations. $SnCl_2 2H_2O$ was generously provided by CIS bio International/Schering SA (Gif-sur-Yvette, France). All other compounds were commercially available (Aldrich, Saint Quentin Fallavier, France) and used as purchased, except the dithiobenzoate sodium salt, that was prepared according to the literature.[35]

Radiochemical purity (RCP) of ^{188}Re compounds was measured by thin-layer chromatography (TLC) on aluminium-backed silica-gel plates (F_{254}, Merck) using, as mobile phases, butanone for the perrhenate ($R_f = 1$), a mixture of ethanol/toluene/chloroform/aqueous ammonium acetate 0.5 M (6/3/3/1) to differentiate perrhenate ($R_f = 0.5$) and the intermediate gluconate complex ($R_f = 0$) and a mixture of petroleum ether and dichloromethane ($\frac{6}{4}$) for the final complex ($R_f = 0.7$). Quantitative evaluation of radioactivity profiles was obtained after development of the chromatograms. The plates were dried under an air stream and protected with an adhesive tape to avoid contamination. The chromatograms were then placed in close contact with a Fuji imaging plate (BAS-IIIS), in a dark box, for 5 min. Location and quantification of the radioactivity as a dark spot were accomplished with a Fujix Bas 1000 bio-imaging analyser. The results were confirmed by counting

Table 7. Stability of ^{188}Re-SSS lipiodol

	t_0	24 h	48 h
Extraction (%)	87.0 ± 9.1	92.6 ± 1.5	98.3 ± 0.6
RCP (%)	93.0 ± 3.4	93.8 ± 2.3	91.0 ± 4.0

of the plates, cut in correspondence of each spot, with a CAPINTEC CR 120 γ-counter.

Preparation of [$^{188}Re(PhCS_3)_2(PhCS_2)$] and labelling of lipiodol

The optimised formulation and reaction conditions obtained for the labelling of lipiodol with ^{188}Re-SSS are as follows: A lyophilised kit, containing 0.8 mg of $SnCl_2 2H_2O$, 7.5 mg of sodium gluconate, 40 mg of potassium oxalate and 30 mg of ascorbic acid, is reconstituted in 0.5 ml of 0.9%-saline. Na[$^{188}ReO_4$] (37–370 MBq), in 0.5 ml saline (RCP >99%), is added and the vial is agitated at room temperature for 15 min. Then, 20 mg of sodium dithiobenzoate, dissolved in 0.5 ml of saline, are added and the solution is heated at 100°C for 30 min. The mixture is then stirred by use of a vortex and 2–3 ml of Lipiodol Ultra-Fluide (Guerbet, France) are added. After 10 min centrifugation (3500 rpm), the two phases are separated and the lower phase, containing the radioactivity is homogenised with a rotary stirrer.

Stability

The *in vitro* stability of the ^{188}Re-SSS lipiodol was determined by measuring RCP at 48 h after preparation, in the presence of human plasma. No significant decrease of RCP was found under these experimental conditions.

Summary and conclusions

A new method for the ^{188}Re-labelling of lipiodol has been demonstrated. This simple method leads to a stable labelling *in vitro* and good reproducible yield. These properties are particularly important in the case of the preparation of therapeutic doses. The precipitation problem is of minor importance, since the complex is then dissolved in the lipid phase. Compared with the ^{188}Re-HDD method, a sufficient labelling yield is easily obtained (87 vs 65%) and the synthesis is reproducible. It also compares well with the ^{188}Re-DEDC described by Boschi *et al.* to which it could come as an alternative. Their relative biodistribution and stability remain to be compared. Lipiodol labelling with stronger activities (therapeutic doses) of ^{188}Re is also envisaged, and potential problems with autoradiolysis must be evaluated. Larger amounts of antioxidant will be probably required, to protect the metal in the lipid phase.

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