Development and biodistribution of ^{188}Re-SSS lipiodol following injection into the hepatic artery of healthy pigs

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Abstract. Although intra-arterial radiotherapy with ^{131}I-labelled lipiodol is a useful therapeutic approach in the treatment of hepatocellular carcinomas, various disadvantages limit its use. Here we describe the development of ^{188}Re-SSS lipiodol, as well as its biodistribution in the healthy pig after injection into the hepatic artery. The ^{188}Re-SSS lipiodol was obtained after dissolving a chelating agent, previously labelled with ^{188}Re, in cold lipiodol. The radiochemical purity (RCP) of the labelling was checked immediately and at 24 and 48 h. The ^{188}Re-SSS lipiodol was injected into the hepatic artery of six healthy pigs. They were killed 1, 24 and 48 h post injection, for ex vivo counting. An autoradiographic study was performed in three cases. ^{188}Re-SSS lipiodol was obtained with a yield of 87%±9.1%. The immediate RCP was 93%±3.4%. This radiolabelling was reproducible and stable at 48 h in plasma: 90.6%±1.5% of the activity remained in the lipiodol with an RCP of 91%±4%. Ex vivo counting confirmed the predominantly hepatic uptake and revealed weak lung and intestinal uptake. There was very weak urinary elimination (2.3%±0.5% at 48 h) and a slightly higher level of intestinal elimination (4.8%±1.9% at 48 h). The autoradiographic studies showed ^{188}Re-SSS lipiodol to be located mainly in sinusoids, like ^{131}I-lipiodol. By using the method described here, ^{188}Re-SSS lipiodol can be obtained with a very high yield and a satisfactory RCP. Its biodistribution in the healthy pig is in agreement with data published elsewhere concerning other types of radiolabelling used for lipiodol, except for the very weak urinary and intestinal elimination, which probably indicates better stability of ^{188}Re-SSS labelling.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent cancers, being ranked fifth in importance worldwide [1]. While its incidence is low in Caucasian populations of industrialised countries (<5/100,000), it is very high in some countries, e.g. in eastern Asia or western Africa (27–48/100,000), owing to the high prevalence of hepatitis B and C [1]. The prognosis for this cancer is extremely poor, and a curative treatment (surgery, local ablative procedures) can only be carried out in 20–30% of cases. Currently there is no standardised treatment for patients who are not candidates for a radical procedure [2], but palliative treatment, principally arterial chemo-embolisation and intra-arterial radiotherapy, can be proposed for those who do not present with an excessively advanced form. At present, ^{131}I-labelled lipiodol is the most widely used therapeutic agent in intra-arterial radiotherapy of HCC [3]. Randomised studies have shown that this treatment (a) is at least as effective as but less toxic than chemo-embolisation [4], (b) significantly increases the survival of patients presenting with HCC with portal thrombosis [5], and (c) leads to a 50% increase in the survival rate 3 years after resection [6].

Nevertheless, the administration of ^{131}I-lipiodol poses some major problems with respect to radiation protection that limit its use. Indeed, it is necessary to hospitalise the patient in a shielded room for a duration of at least 8 days (to obtain a dose rate at 1 m from the patient of be-

low 20 μSv/h, the French recommendation based on adaptation of ICRP 60) because of the intense gamma-ray emission of ^{131}I and its long half-life. There have consequently been various attempts to label lipiodol with other isotopes that are less restrictive to its use, in particular ^{188}Re [7, 8, 9]; however, certain problems persist such as a relatively weak labelling yield [8, 9].

The aim of this study was to test the feasibility of radiolabelling lipiodol with ^{188}Re using a new lipophilic complex that may contain either technetium or rhenium [10, 11].

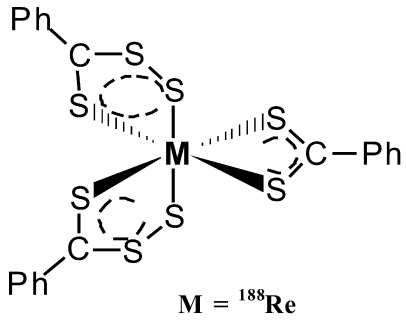

Fig. 1. Structure of the ^{188}Re-SSS complex

Materials and methods

Radiochemistry. ^{188}Re-labelled lipiodol was obtained after dissolving in cold lipiodol a ^{188}Re-labelled lipophilic chelating agent forming the ^{188}Re-$(S_2CPh)(S_3CPh)_2$ complex (^{188}Re-SSS complex). ^{188}Re was obtained from a $^{188}W/^{188}Re$ generator (Oak Ridge National Laboratory, Tennessee). ^{188}Re-SSS complex was obtained by reaction of the ligand sodium dithiobenzoate [10, 11] with a freeze-dried formulation containing 7.5 mg sodium gluconate, 30 mg ascorbic acid, 40 mg potassium oxalate and 0.8 mg $SnCl_2 \cdot 2H_2O$ reconstituted in 0.5 ml of physiological serum. The required perrhenate activity (in 0.5 ml) was added and the solution mixed for 15 min at ambient temperature. Twenty milligrams of sodium dithiobenzoate (in 0.5 ml, pH=7) was added before heating at 100°C for 30 min, thus enabling formation of the ^{188}Re-SSS complex. The structure of the ^{188}Re-SSS complex is presented in Fig. 1.

Three millilitres of lipiodol (Lipiodol ultra-fluide, Guerbet, France) was added to the ^{188}Re-SSS complex. This solution was centrifuged at 2,200 *g* (3,500 rpm) for 10 min to separate lipiodol from the aqueous phase. The aqueous phase was removed with a syringe. The lipophilic phase containing the lipiodol and the ^{188}Re-SSS complex was homogenised for 1 min with a rotary stirrer and carefully collected.

The radiochemical purity of the ^{188}Re-SSS lipiodol was determined by chromatography as the ratio of migrated radioactivity to total radioactivity (R_f 0.6). The chromatography was carried out on silica/aluminium 60 F_{254} gel plates (Merck), using a solution of petrol ether/dichloromethane (6/4) as eluant.

The stability of the ^{188}Re-SSS lipiodol was checked after 24 and 48 h at room temperature in plasma. The reproducibility of the radiolabelling was assessed on ten tests. The stability at 24 and 48 h was checked on five occasions.

Animal experimentation. Animal experiments were carried out in compliance with French regulations (law 0189.4 of January 24, 1990) on a group of six pigs each weighing 40 kg (Large White crossbred with Landrace and Pietrin).

The injection of ^{188}Re-SSS lipiodol was carried out surgically directly via the hepatic artery. Administered activities of ^{188}Re-SSS lipiodol were 30, 92 or 130 MBq for biodistribution studies at 1, 24 or 48 h, respectively (*n*=2 in each case). Scintigraphic acquisitions were performed in anterior and posterior projections (150–300 kcp, 256×256 matrix), 1 (*n*=6), 24 (*n*=4) and 48 h (*n*=2) post injection using an Elscint SP6 gamma camera (Haifa, Israel).

The animals were killed 1, 24 or 48 h after injection, organs were removed and homogenised, and an aliquot was analysed using a gamma counter (Auto gamma cobra II, Packard). A blood sample was also taken from each pig, while urine and faeces were collected from the four pigs that were killed 24 or 48 h post injection.

An autoradiographic study of the liver was carried out on three pigs, using samples taken 1 h (*n*=2) and 48 h (*n*=1) post injection. The procedure used has been described elsewhere [11]; in the present study there was just a reduction in the incubation time (6 h).

Results

Radiochemistry

For the ten radiolabelling experiments, ^{188}Re-SSS lipiodol was obtained with a final yield of 87%±9.1% (mean ± 1 standard deviation). The radiochemical purity of the ^{188}Re-SSS lipiodol was 93%±3.4%.

In vitro, the ^{188}Re-SSS lipiodol remained stable for 24 h in plasma. In fact, when ^{188}Re-SSS lipiodol was placed in contact with plasma, 90.6%±1.5% of the ^{188}Re-SSS complex remained in the lipiodol after 48 h, with a radiochemical purity of 91%±4%.

Animal experiments

The scintigraphic images revealed exclusive and homogeneous hepatic uptake of ^{188}Re-SSS lipiodol at 1 h and also at 24 and 48 h post injection.

The ex vivo counting carried out 1, 24 and 48 h post injection showed almost exclusive hepatic uptake, in addition to weak lung uptake and the appearance of weak intestinal uptake from 24 h onwards. The results of these counting experiments are reported in Tables 1, 2 and 3.

Autoradiography

On autoradiography 1 h post injection, the radioactivity was localised mainly in the sinusoid capillaries. In certain zones, there was also a small amount of activity in Kierman's space and the perilobular connective trabeculae (veins and arteries) and, to a lesser degree, in the centrolobular veins as well.

Table 1. Mean activities (±1SD) present in various organs 1 h post injection in two pigs

	Liver	Lungs	Stomach	Duodenum	Spleen	Kidney	Heart	Blood
% IA/g	$7.74\ 10^{-2}\pm 1.51\ 10^{-2}$	$1.81\ 10^{-2}\pm 2.4\ 10^{-2}$	$5.14\ 10^{-4}\pm 9.9\ 10^{-5}$	$7.12\ 10^{-4}\pm 7.2\ 10^{-5}$	$2.23\ 10^{-3}\pm^{3} 1.9\ 10^{-}$	$7.24\ 10^{-5}\pm 7.5\ 10^{-5}$	$5.3\ 10^{-5}\pm 5.6\ 10^{-5}$	$8.9\ 10^{-5}\pm 9.2\ 10^{-5}$
% IA	97.3±3.6	6.4±8.3	$1.3\ 10^{-1}\pm 1.2\ 10^{-1}$	$2.1\ 10^{-2}\pm 2.5\ 10^{-3}$	$1.6\ 10^{-1}\pm 2\ 10^{-1}$	$1.4\ 10^{-2}\pm 1.3\ 10^{-2}$	$1.5\ 10^{-2}\pm 1.2\ 10^{-2}$	ND

% IA/g, Percentage injected activity per gram of tissue; % IA, percentage injected activity present in the organ; ND, not determined

Table 2. Mean activities (±1SD) present in various organs 24 h post injection in two pigs

	Liver	Lung	Stomach	Intestine[a]	Spleen	Kidney	Urine	Heart	Blood
% IA/g	$6.17\ 10^{-2}\pm 2.5\ 10^{-3}$	$4.84\ 10^{-3}\pm 8.6\ 10^{-4}$	$1.69\ 10^{-3}\pm 8.9\ 10^{-4}$	$3.97\ 10^{-4}\pm 1.3\ 10^{-5}$	$6.02\ 10^{-4}\pm 1.3\ 10^{-4}$	$3.53\ 10^{-4}\pm 8.3\ 10^{-5}$	$1.13\ 10^{-3}\pm 2.8\ 10^{-5}$	$1.24\ 10^{-4}\pm 3.3\ 10^{-5}$	$2.25\ 10^{-4}\pm 7.6\ 10^{-4}$
% IA	98±5.8	2.15±0.6	$5.4\ 10^{-1}\pm 4\ 10^{-1}$	$1.2\pm6\ 10^{-1}$	$8.1\ 10^{-2}\pm 9.8\ 10^{-2}$	$6.6\ 10^{-2}\pm 8.5\ 10^{-3}$	$7.9\ 10^{-1}\pm 1.2\ 10^{-1}$	$6.5\ 10^{-2}\pm 3\ 10^{-2}$	ND

% IA/g, Percentage injected activity per gram of tissue; % IA, percentage injected activity present in the organ; ND, not determined
[a] Intestine plus faecal matter

Table 3. Mean activities (±1SD) present in various organs 48 h post injection in two pigs

	Liver	Lung	Stomac	Intestine[a]	Spleen	Kidney	Urine	Heart	Blood
% IA/g	$5.51\ 10^{-2}\pm 8.2\ 10^{-3}$	$1.09\ 10^{-2}\pm 1.5\ 10^{-3}$	$1.15\ 10^{-3}\pm 3.7\ 10^{-4}$	$1.68\ 10^{-3}\pm 6.1\ 10^{-4}$	$4.31\ 10^{-3}\pm 3.8\ 10^{-3}$	$6.73\ 10^{-4}\pm 6.3\ 10^{-5}$	$1.65\ 10^{-3}\pm 8.4\ 10^{-5}$	$1.78\ 10^{-4}\pm 2.7\ 10^{-5}$	$3.66\ 10^{-4}\pm 2.8\ 10^{-4}$
% IA	80.3±3.5	4.6±1.5	$4.3\ 10^{-1}\pm 5.6\ 10^{-2}$	4.8±1.9	$6.8\ 10^{-1}\pm 2.7\ 10^{-1}$	$1.2\ 10^{-2}\pm 1.4\ 10^{-3}$	$2.3\pm 5.6\ 10^{-1}$	$7.4\ 10^{-2}\pm 2.6\ 10^{-2}$	ND

% IA/g, Percentage injected activity per gram of tissue; % IA, percentage injected activity present in the organ; ND, not determined
[a] Intestine plus faecal matter

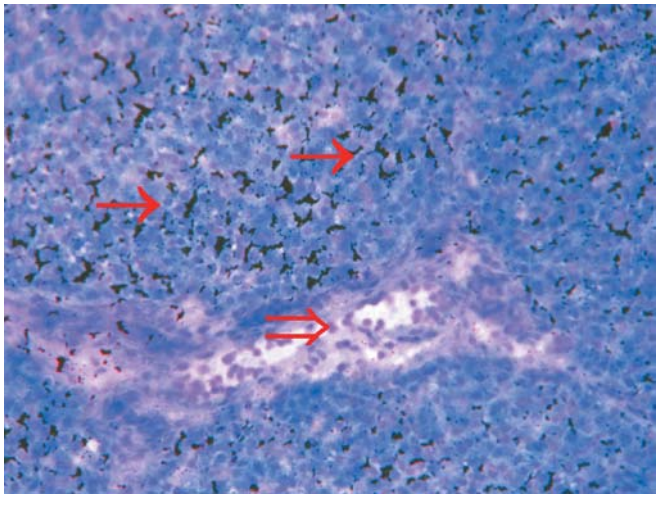

Fig. 2. Autoradiography of the liver, 48 h after intra-arterial injection of 130 MBq of ^{188}Re-SSS lipiodol via the left branch of the hepatic artery

On autoradiography carried out 48 h post injection (Fig. 2), the radioactivity was found exclusively in the sinusoids.

Discussion

Various attempts have been made to label lipiodol with therapeutic isotopes other than ^{131}I, in particular with ^{188}Re using the chelating agents TDD [7] and HDD [8, 9]. This approach currently seems to be the most promising, but the labelling yield is not optimal [8, 9]; in fact, it ranges between only 50% and 70% [8], which can pose problems for the synthesis of high therapeutic activities.

We have shown in this study that ^{188}Re-SSS lipiodol radiolabelling can be obtained with a very high yield (87%±9.1%) and with satisfactory radiochemical purity. This yield is much higher than that observed by Lee et al. [8], which is important for the production of therapeutic doses of high activity. This difference in radiolabelling yield is probably due to the fact that, once the ^{188}Re-SSS complex has been formed, the ^{188}Re is in oxi-

dation state III. This state is more difficult to obtain but is also more stable than oxidation state V, which is the speciation found in the ^{188}Re-HDD complex.

Using the reported procedure, our study also shows that the ^{188}Re-SSS lipiodol radiolabelling remains stable in vitro for at least 48 h, in accordance with the procedures already described for radiolabelling with ^{188}Re [7, 8].

The study of reproducibility carried out here gave satisfactory results in terms of radiochemical purity and in vitro stability at 48 h, and the yield was especially good, which is important given that the yield is the weak point in radiolabelling of lipiodol by ^{188}Re-HDD.

The ex vivo counting results obtained in the healthy pig after intra-arterial injection of ^{188}Re-SSS lipiodol via the hepatic artery are concordant with the published results for ^{188}Re-lipiodol in the rat, which also revealed predominant hepatic uptake associated with weak lung and intestinal uptake [7].

Scintigraphic acquisitions in this study demonstrated exclusive and homogeneous hepatic uptake whereas ex vivo counting additionally detected weak lung activity and the appearance of weak intestinal activity at 24 h. This clearly underlines the difficulties in detection that can be encountered with ^{188}Re [12]. Nevertheless, these scintigraphic acquisitions showed that the biodistribution of ^{188}Re-SSS lipiodol is similar to that observed for ^{131}I-lipiodol in humans. In the human, where only scintiscan data are available [13], ^{131}I-lipiodol is distributed preferentially in the liver (76%±12% of detectable activity is located in the liver), while the rest of the activity is detected only in the lungs [13].

On the other hand, the elimination mechanisms for ^{188}Re-SSS lipiodol and ^{131}I-lipiodol seem different: while weak intestinal elimination at 48 h (4.8%±1.9% of injected activity) and weak urinary elimination at 48 h (2.3%±0.5% of injected activity) were observed for ^{188}Re-SSS lipiodol, it has been reported that in the case of ^{131}I-lipiodol less than 3% of the injected activity is eliminated in faecal matter at day 5 and 30–50% of injected activity is eliminated in urine at day 8 [13]. Our observations conflict with the preliminary results published by Lambert et al. [14], who, in a series of seven patients treated with ^{188}Re-HDD lipiodol, found urinary elimination after 72 h corresponding to 43.5% (SD 13%) of the injected activity. The presence of very weak urinary and intestinal elimination of ^{188}Re-SSS lipiodol suggests better stability of this radiolabelling.

In addition, we performed an autoradiographic study to check whether the distribution of ^{188}Re-SSS lipiodol at the microscopic level is identical to the lipiodol distribution in the liver, i.e. in order to test the stability of the labelling in vivo. After injection via the hepatic artery, lipiodol is rapidly and unambiguously located in the sinusoids [15]; then, a variable quantity passes into the centrolobular veins and into the general bloodstream. In our study, the radioactivity was also located mainly in the sinusoids, and to a less degree in the vessels present in Kierman's space (arteries and veins) as well as the centrolobular veins. The present results show that, even in the absence of a chemical bond between the ^{188}Re-SSS complex and lipiodol, ^{188}Re-SSS lipiodol is stable in vivo for at least 48 h and has an intrahepatic distribution very similar to that observed for lipiodol alone.

In conclusion, this study shows that ^{188}Re-SSS lipiodol can be obtained with a high yield, a clear advantage over the previously described technique of radiolabelling using ^{188}Re. The radiochemical purity is satisfactory and the radiolabelling is stable for at least 48 h in vitro. The biodistribution of ^{188}Re-SSS lipiodol is close to that observed for ^{131}I-lipiodol in humans. The main difference is represented by very weak urinary elimination and more marked intestinal elimination.

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