90Y-oxine-ethiodol, a potential radiopharmaceutical for the treatment of liver cancer

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Abstract

Ethiodol (or lipiodol) is selectively retained in hepatocellular carcinoma and is used as a vehicle to deliver radioactive agents following intraarterial hepatic infusion. We prepared the lipophilic complex 90Y-oxine with a radiolabeling efficiency of 97.6±1.1%. After extraction into ethiodol, a stability test in serum at 37°C showed that 87.8% of the 90Y remained ethiodol-bound for 7 days. Bremsstrahlung imaging of a rabbit for 48 h confirmed that the homogeneous mixture of radiolabeled 90Y-oxine and ethiodol stayed in the targeted liver lobe. This radiopharmaceutical is thus a potential candidate for the treatment of non-resectable liver cancer.

Keywords: 90Y; Oxine; Lipiodol; Oxyquinoline; Radiopharmaceutical; Ethiodol; Radiolabeling; 111In; Liver cancer

1. Introduction

Ethiodol, or lipiodol, is a sterile injectable radio-opaque diagnostic agent used in lymphography and hysterosalpingography. It consists of 37% iodine (475 mg/ml) organically combined with ethyl esters of the fatty acids of poppyseed oil and is stabilized with 1% poppyseed oil (Stanbury et al., 2002). Because it is selectively retained in hepatocellular carcinoma (HCC), ethiodol has been used as a vehicle to deliver localized doses of chemotherapeutic and radioactive agents to HCC following intraarterial hepatic infusion, thereby reducing problems such as systemic toxicity of chemotherapy or low tolerance of the liver to external beam irradiation (Stansby et al., 1994; Park et al., 1986, 1990; Aguayo and Patt, 2001).

131I is the most popular radioisotope for labeling lipiodol to treat HCC, particularly in cases of unresectable liver tumors. 131I-labeled lipiodol has been investigated for clinical applications (Tilak et al., 1966; Partensky et al., 2000; Yumoto et al., 1992). A drawback of 131I labeled compounds is the radioisotope’s high γ-ray energy (364 keV, 81%). The whole-body dose can be significantly reduced by using a β-emitter instead with lower amounts of mostly low-energy γ-rays, such as 188Re, or a pure β-emitter such as yttrium-90 (90Y).

Wang et al. reported a method to label lipiodol with 188Re and test it in rats for liver cancer treatment (Wang et al., 1996a,b). The ratio of tumor concentration to the normal liver tissue concentration (T/N ratio) was 5.15 at 1 h and rose to 7.7 at 24 h and 10.84 at 48 h. Even more recently, Lee et al. published an elegant method of covalently coupling a diaminedithiol to a long chain alkyl group that could be labeled in a kit formulation with 188Re (Lee et al., 2002). This lipophilic agent could then be mixed with lipiodol and retained it by lipophilic interaction. Preliminary clinical results on the use of 188Re-lipiodol in patients with HCC are hopeful (Keng et al., 2002; Sundram et al., 2001). In the newest multicenter clinical phase I trial, Sundram et al. delivered up to 200 mCi of 188Re-Lipiodol without impairing liver...
function or bone marrow toxicity, and the disease could be stabilized in 13 of the 16 patients (Sundram et al., 2002). A phase II trial is now ongoing.

The $^{188}$ Re radioisotope is not ideal for radiotherapy as it is currently not available in clinical quality in terms of sufficiently high purity, high concentration, sterility and non-pyrogenicity. The pure $\beta$-emitter $^{90}$Y does not have this disadvantage, since it is available from several commercial sources in clinical quality. $^{90}$Y has a half-life of 64 h and emits $\beta$-electrons with a maximum energy $E_{\text{max}}$ of 2.27 MeV, giving it a maximum range in tissue of 12 mm. Bremsstrahlung imaging of $^{90}$Y with a $\gamma$-camera is possible (Shen et al., 1994), permits a determination of ethiodol deposition in both the tumor and liver, and therefore a semi-quantitative estimate of the dose given to the tumor by the pure $\beta$-emitter (Madsen et al., 1988; Park et al., 1986).

Lipiodol labeled with $^{90}$Y has already been studied for the treatment of liver cancer in rats (Wang et al., 1996a,b). For this purpose, $^{90}$Y was labeled with lipiodol conjugated with the lipophilic complex N,N,N‘,N‘-tetrakis (2-benzimidazolylmethyl)-1,2-ethanediamine (EDTB). The T/N ratio was 3.03 at 1 h and rose to 6.45 at 72 h. Drawbacks of the method were that the preparation of $^{90}$Y-EDTB-lipiodol was complex and long, and that some of the $^{90}$Y accumulated in the skeletal system, a fact which would be expected to limit clinical application (Wang et al., 1996a,b). For this reason, we investigated the labeling of lipiodol with $^{90}$Y using an alternative method. We prepared $^{90}$Y-labeled lipiodol based on the lipophilic oxine complex. Oxine has been used since the 1970s in indium-111 oxyquinoline ($^{111}$In-oxine) as a diagnostic radiopharmaceutical for the radiolabeling of autologous leukocytes (Thakur et al., 1977). $^{111}$In-oxine-labeled leukocytes constitute a valuable tool in the diagnosis and localization of both normal lymphoid structures and those with metastatic disease (Schell-Frederick et al., 1984; Schroeder et al., 1983; Santin et al., 2000). Because of similar chemical properties of yttrium and indium, and since oxine has been used to extract Y$^{3+}$ before (Czakis-Sulikowska et al., 1994), we optimized the preparation of the $^{90}$Y-oxine complex and studied the stability of $^{90}$Y-oxine-Ethiodol in human plasma for use as a new radiotherapeutic agent. The selective liver uptake and imaging properties were also investigated in a preliminary in vivo experiment.

2. Methods

2.1. Radiolabeling of $^{90}$Y-oxine

Two hundred microliters of 0.1 M 8-hydroxyquinoline (oxine; Sigma-Aldrich Co., St. Louis, MO) in ethanol was added to 200 $\mu$l of $^{90}$Y (yttrium chloride in 0.05 M HCl; Perkin Elmer, Boston, MA) in 0.02 M NH$_4$Ac buffer (pH 6–7) for 30 min of incubation at 50°C. The radiolabeling efficiency was analyzed by thin layer chromatography (TLC) (see 2.2). The lipophilic compound was extracted twice with 200 $\mu$l of methylene chloride (CH$_2$Cl$_2$) or chloroform. The extraction efficiency was determined by measuring the radioactivity in the buffer and the CH$_2$Cl$_2$ (or chloroform) phase and calculating the activity in the CH$_2$Cl$_2$ phase divided by the summed activity of both phases. Optimizations were performed for oxine concentration from 6.9 mM to 0.1 M, for the solvents chloroform and ethanol, for temperatures from 20°C to 99°C, and incubation times from 5 min to 20 h.

2.2. TLC system

The radiolabeling efficiency of $^{90}$Y-oxine was analyzed immediately after heating in the ethanol solution by Silica Gel TLC on glass fiber strips (Tec-strip, black, #151-005, Biodex Medical Systems) as the stationary phase and methanol as the mobile phase. In this system, free $^{90}$Y stays at the origin ($R_f = 0$) and $^{90}$Y-oxine develops to the top of the strip ($R_f = 0.9–1.0$).

2.3. Preparation of $^{90}$Y-oxine-ethiodol

The extracted $^{90}$Y-oxine in CH$_2$Cl$_2$ phase was evaporated to dryness at 60°C for about 1 h, followed by the addition of 500 $\mu$l of ethiodol (Savage Laboratories, Melville, New York, USA) to the vial and incubation for 1 h at 50°C on an Eppendorf Thermomixer shaking at 1400 rpm. $^{90}$Y-oxine-ethiodol was then washed twice with 1 ml of phosphate buffered saline (PBS). The binding efficiency was calculated by subtracting from 1 the radioactivity of the 2 washes divided by the total radioactivity before washing.

2.4. Stability study of $^{90}$Y-oxine-ethiodol

One milliliter of human plasma was added to 500 $\mu$l of $^{90}$Y-oxine-ethiodol. At four time points (1 h, 1, 3, 7 d), the vial with the $^{90}$Y-oxine-ethiodol was removed from the 37°C shaking water bath, the total radioactivity measured, and 1 ml of supernatant transferred to a clean vial. The activity of the supernatant vial was measured (supernatant radioactivity) and 1 ml of fresh plasma added to the original vial. The stability of the ethiodol-bound $^{90}$Y was calculated by subtracting from 1 the supernatant activity divided by the total radioactivity at each time point.

2.5. Imaging study in a rabbit

The initial imaging test to confirm the targeted localization and in vivo stability of radiolabeled ethiodol was performed in a white New Zealand rabbit weighing...
3.6 kg. The experiment was performed according to the guidelines for the Care and Use of Laboratory Animals published by NIH, using a protocol approved by the Cleveland Clinic’s animal review committee. The animal was anesthetized with a mixture of xylazine (5 mg/kg) and ketamine hydrochloride (35 mg/kg) administered intramuscularly. The right groin was shaved and prepared with povidone iodine, and a femoral cut down performed to allow access to the femoral artery through a 4 F sheath. Heparin at 50 units/kg was given through the sheath. A 3 F microcatheter was then manipulated into the celiac axis for angiography, and advanced into the common hepatic artery for the delivery of 90Y-oxine-ethiodol. A total of 0.6 ml of 90Y-oxine-ethiodol was slowly injected, the catheter withdrawn and femoral access ligated with surgical suture. Thirty minutes later, the animal was transferred to a Technicare 420 γ-camera and imaged using an energy centerline of 810 and a window setting of 222. These settings were chosen according to Shen et al., who described a procedure for 90Y Bremsstrahlung imaging using an energy window between 55 and 285 keV (Shen et al., 1994). Gamma camera images were taken for 5 min at the 1 h time point and 10 min at the 48 h time point.

3. Results

3.1. Optimization of the radiolabeling efficiency of 90Y-oxine

Important factors for the optimal radiolabeling of 90Y-oxine are the solvent, the temperature, the incubation time and the concentration of the components. When chloroform was used to dissolve oxine, no 90Y-oxine was formed (Table 1). Replacing chloroform with ethanol then improved the radiolabeling efficiency considerably, reaching 35.7% even at unoptimized room temperature conditions (Table 1).

A key factor for higher radiolabeling efficiencies is the concentration of oxine. At a concentration of 6.9 mM (1 mg/ml), the radiolabeling efficiency was 65.2% after 30 min of incubation at 50°C. Increasing the oxine concentration to 0.1 M reached a radiolabeling efficiency of 96.1% under the same conditions (Table 1).

Temperature and incubation time are also important factors that affect the radiolabeling efficiency. Using ethanol as a solvent, we showed that incubation at 50°C for 1 h was optimal for both the 6.9 mM and 0.1 M oxine concentrations (Fig. 1). The higher oxine concentration of 0.1 M, though, reached much higher radiolabeling efficiencies. Optimizing the length of incubation at 50°C showed that the radiolabeling efficiency of the 6.9 mM oxine solution could be improved with longer incubation times (Fig. 2), however, there was no beneficial effect in incubation times exceeding 15 min for the 0.1 M oxine solution (Fig. 2).

Our final radiolabeling conditions combined a temperature of 50°C with a reaction time of 15 min. Under these conditions, we reached a radiolabeling efficiency of 97.6% ± 1.1% (n = 10).

3.2. TLC system for the analysis of 90Y-oxine

Fig. 3 shows that most of the radiolabeled product after CH2Cl2 extraction is in the form of 90Y-oxine. The TLC system with the TecStrips and methanol as a solvent separates free and chelated 90Y very well.

3.3. Preparation of 90Y-oxine-ethiodol and its in vitro stability

The freshly prepared 90Y-oxine is bound to ethiodol by lipophilic interaction. Simply adding ethiodol to the

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<thead>
<tr>
<th>Conditions tested for the radiolabeling of 90Y-oxine</th>
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<td>90Y activity, volume</td>
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<tr>
<td>116.7 μCi, 300 μl</td>
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<td>79.7 μCi, 300 μl</td>
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<td>172.2 μCi, 300 μl</td>
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<td>10 μCi, 200 μl</td>
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<td>167.7 μCi, 200 μl</td>
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<td>281.2 μCi, 200 μl</td>
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<td>160.9 μCi, 200 μl</td>
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<sup>a</sup>Room temperature.
90Y-oxine-ethanol mixture resulted in an extraction efficiency of only 25.6%. The extraction efficiency could be considerably improved by extracting 90Y-oxine into the chlorinated solvent CH2Cl2, followed by depositing (drying) it onto the glass wall of a 5ml vial and then taking it up into ethiodol. In this way, we reached an extraction efficiency of 87.2%.

CH2Cl2, therefore, proved to be the most effective solvent for the extraction of 90Y-oxine.

The binding efficiency of 90Y-oxine-ethiodol after two PBS washes was 98.4±0.7% (n = 3). The stability tests showed that 97.9%, 93.2%, 90.1% and 87.8% of the total radioactivity were still ethiodol-bound after 1h, 1, 3 and 7 days.

In order to make sure that all of the radioactive 90Y-oxine was dissolved in ethiodol and not bound to the wall of the vial at the end of the 1h incubation, the radiolabeled ethiodol was pipetted to a new vial. About 86.2%±5.5% (n = 3) of radioactivity was found in the new vial. The missing activity was detected in a small amount of ethiodol that could not be removed from the original vial and in the pipette tip. The high viscosity of the liquid makes such transfers difficult and such losses in the transfer materials (pipettes, catheters, vials, dead volumes of connectors) should thus always be taken into account during the planning stage of a clinical trial.

3.4. Preparation of 111In-oxine-ethiodol

Sometimes it is required to quantitatively analyze the in vivo distribution of a radiopharmaceutical. Since 90Y-oxine-ethiodol as a pure β-emitter can only be used qualitatively, we applied the optimized 90Y labeling conditions to 111In and prepared 111In-oxine-ethiodol with the same method. The radiolabeling efficiency of
111In-oxine was 99.0%, and the extraction efficiency 92.2%. After incubation of ethiodol with the dried 111In-oxine for 1 hr at 50°C, 83.7% of the total radioactivity was bound to the ethiodol. The radiolabeled 111In-oxine-ethiodol could thus be used for γ-camera imaging, if necessary, before using 90Y-oxine-ethiodol for the treatment of liver cancer.

3.5. Imaging study in rabbits

The targeted, selective delivery of ethiodol to the left liver lobe of a rabbit worked well, as seen by the fluoroscopic pictures taken (Figs. 4A). No lung uptake was seen at 1 hr post-injection, as confirmed by Bremsstrahlung imaging with a γ-camera (Fig. 4B). In addition, there was no redistribution of the radioactive 90Y-oxine-ethiodol detected 48 hr after injection. All of the radiolabeled ethiodol was still in the liver, as seen in Fig. 5.

4. Discussion

Liver cancer, and specifically HCC, is a very common malignant disease seen in many developed and developing countries, especially of Eastern Asia (Jeong et al., 2001). In recent years, its prevalence has been increasing with the mounting incidence of viral hepatitis. In terms of patient survival and tumor response, radioactive 131I-labeled lipiodol and chemoembolization are equally effective in the treatment of HCC. However, tolerance to treatment with 131I-labeled lipiodol—or (in the United States) ethiodol—was significantly better compared with the large number of side effects observed with chemoembolization (Raoul et al., 1997; Raoul et al., 1999). Al-Mufti described an interesting phenomenon (Al-Mufti et al., 1999), a marked cytotoxic effect from 131I-ethiodol on all tested cancer cell lines in vitro. When he used the radioisotope 131I without ethiodol, none of the cell lines tested showed a cytotoxic effect, even at much higher doses and despite the cells being in direct contact with radioiodine. In addition, Towu et al. reported that a ‘cocktail’ containing 131I- and 125I-labeled ethiodol was more cytotoxic to the cells than either 131I- or 125I-labeled ethiodol alone, as measured in vitro by clonogenic assays of hepatoblastoma cells and human hepatocyte lines. Furthermore, the same activities of 125I or 131I, alone or in combination, showed no demonstrable effect on the liver tumor cell lines (Towu et al., 2001).

We thus believe that ethiodol has a therapeutic effect on cancer treatment, especially in combination with a therapeutic β-emitter. This reasoning encouraged us to develop an ethiodol-based radiopharmaceutical that does not use 131I, but employs the readily available, more energetic, β-emitting radioisotope 90Y.

Most procedures described in the literature for the radiolabeling of ethiodol involve attaching the radioisotopes directly to the oily ethiodol or to an ethiodol-bichelating agent conjugate. The procedures are thus necessarily complex, involved and time consuming (Wang et al., 1996a, b). Jeong developed a simple
method in which ethiodol binds $^{188}$Re while passing through an aqueous phase which contains a $^{188}$Re-labeled lipophilic agent (Jeong et al., 2001). Jeong suggested that this method could be applied to other lipophilic $\beta$-emitting radiopharmaceuticals that were soluble in ethiodol, as was confirmed here in our work for the lipophilic $^{90}$Y-oxine. Using our final preparation conditions, we were able to prepare the lipophilic $^{90}$Y-complex in 15 min, extract it into CH$_2$Cl$_2$, and then bind it to ethiodol with another incubation of 1 h. Using our optimized method, a radiolabeling efficiency of more than 97% and extraction efficiency into the lipophilic phase of more than 85% is consistently possible. The radiolabeled $^{90}$Y-oxine-ethiodol was routinely prepared in our lab in 2 h, including the quality control by TLC and a final sterile filtration of the radiopharmaceutical.

Drawbacks currently include the use and evaporation of the chlorinated solvent CH$_2$Cl$_2$, and the time it takes to extract the dried $^{90}$Y-oxine into ethiodol.

The stability of the radiopharmaceutical is a very important parameter, especially since much of the $^{90}$Y released in non-chelated, free form will be taken up by the skeletal system (bone seeker) and only be released with a very long metabolic half-life (Graul and Hundeshagen, 1959; Stevenson, 2000). Our stability results are promising. In vitro, mixed with plasma, about 90% of the radioactivity was still ethiodol bound after two half-lives. Measured in vivo in a rabbit using $\gamma$-camera imaging, no difference could be seen between the images taken 30 min after injection and 48 h later (Figs. 4 and 5). All radioactivity was confined to the (targeted) left liver lobe, with no activity in the lungs and the bladder. More involved biodistribution studies with full organ analyses and animals containing HCC are planned.

In conclusion, we prepared a lipophilic complex, $^{90}$Y-oxine. High radiolabeling and ethiodol-binding efficiencies, good stability in plasma and selective retention in the targeted liver lobe suggest that $^{90}$Y-oxine in ethiodol is a potential candidate for non-resectable HCC treatment.

References


