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Automated preparation of Re-188 lipiodol for the treatment of hepatocellular carcinoma

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Abstract

The iodinated oil lipiodol is commonly used as a carrier for in situ delivery of drugs or radioactivity to hepatic tumors. Recently, we reported a new kit formulation for high-activity labeling of lipiodol with the β-emitting radionuclide Re-188. Since the whole preparation involves different steps and complex manipulations of high-activity samples, we describe here an automated synthesis module that allows the easy preparation of sterile and pyrogen-free samples of Re-188 lipiodol ready to be administered to the patient. Important advantages include the possibility to incorporate high Re-188 activity into the lipiodol hydrophobic phase and a sharp reduction of radiation exposure of the operator assisting the labelling procedure. Application of this modular reaction system could be also extended to the preparation of other Re-188 radiopharmaceuticals and to compound labelled with different β-emitting therapeutic radionuclides.

Keywords: Rhenium-188; Hepatocellular carcinoma; Lipiodol; Radionuclide therapy; Synthesis module; ^{188}ReN-DEDC

1. Introduction

Hepatocellular carcinoma (HCC) is the most prevalent primary liver cancer [1] and constitutes the third most frequent cause of cancer-related deaths annually worldwide [2]. The selection of an appropriate treatment strategy for patients with HCC depends on careful tumor staging and assessment of the underlying liver disease [3]. Unresectable hepatocellular carcinoma is extremely difficult to treat because of the limited efficacy of non-surgical approaches employing delivery to the tumor site of chemoembolization-inducing drugs or infrared radiation [4]. A different approach involves targeting tumour with a radionuclide by means of administration of a radiolabeled substance [5,6]. This strategy includes the use of glass or plastic spheres absorbed with radioactivity [7,8] or, alternatively, of radiolabeled hydrophobic substances [9]. Lipiodol is a mixture of iodinated ethyl ester derivatives of a poppy-seed fatty oil, and has been used as a X-ray contrast agent for detection of HCC [10]. Administration of lipiodol by a catheter introduced through the hepatic artery of HCC patients results in its selective and prolonged retention within the tumor [11,12]. Therefore, lipiodol has been soon considered as a potential carrier of radioactivity when labeled with a suitable therapeutic radionuclide. Actually, radiolabeled lipiodol has proven to be an effective treatment for hepatoma in humans, and encouraging results have been reported with ^{131}I-labeled lipiodol [13–16]. However, the observed in vivo release of a significant amount of ^{131}I, which subsequently accumulates in the lungs, has hampered its routine clinical implementation. In recent years, ^{90}Y has been proposed as a suitable candidate for radionuclide therapy. Lipiodol was first labeled with ^{90}Y through its conjugation to the radiometal mediated by the lipophilic ^{90}Y-complex with *N*,*N*,*N*′,*N*′-tetrakis(2-benzymidazolmethyl)-1,2-ethanediamine [17,18]. However, after administration, a moderate concentration of free ^{90}Y was always noted in the skeletal system. As free ^{90}Y has a very long metabolic half-life in the bone, the clinical potential of this radiopharmaceuticals appears limited.

Recently, the radionuclide ^{188}Re has attracted much interest for therapeutic application due to its favorable nuclear properties [19]. This nuclide decays through the emission of a β-particle (E_β=2.1 MeV, $t_{1/2}$=16.9 h) with the

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concomitant emission of a γ radiation (E_γ=155 keV) that can be conveniently utilized for imaging the course of therapy. Another important advantage is that ^{188}Re is obtained from a transportable ^{188}W/^{188}Re generator system. Various attempts at labeling lipiodol with ^{188}Re have been proposed. The most simple and elegant approach involves dissolution of a lipophilic ^{188}Re-compound into the strong hydrophobic lipiodol phase. Following this strategy, ^{188}Re remains tightly retained as a consequence of the strong hydrophobic interaction between the lipophilic metal complex and the fatty oil. Obviously, a key requirement for the successful application of this approach is that a ^{188}Re complex, possessing sufficient stability and high lipophilicity, has to be first produced in high yield and then mixed with lipiodol.

An elegant example of the application of this labelling method has been reported [20–25]. A series of hydrophobic oxocomplexes of ^{188}Re was prepared by reacting [^{188}Re][ReO_4]$^-$ with derivatives of the tetradentate ligand 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane and, then, tested for they solubility in lipiodol. Despite good retention into the hydrophobic phase, the final radiochemical yield of the ^{188}Re complex was low and uncomplexed activity, probably in the form of colloidal [^{188}Re]ReO_2, was not stably retained into hepatoma. This result reflects the difficulty in obtaining ^{188}Re complexes in satisfactory yield using similar reaction conditions usually employed for the preparation of analogous ^{99m}Tc complexes. Higher labelling yields has been achieved, recently, through the preparation of a new class of lypophilic Re(III) complexes with dithiocarboxylate ligands [26,27].

Following the same labelling strategy, a few years ago, we developed an efficient procedure for labelling lipiodol with ^{188}Re, at tracer level and under sterile and pyrogen-free conditions, and the resulting radiolabelled product has been successfully employed in the treatment of a number of HCC patients [28,29]. This labelling procedure was based on the preliminary preparation of the highly lipophilic complex *bis*-(diethyldithiocarbamato) nitrido [^{188}Re]rhenium ([^{188}Re]ReN-DEDC) carried out using a two-vial, freeze-dried kit formulation. This complex was, subsequently, mixed with lipiodol to yield the final radiopharmaceutical. The whole preparation involves different steps and complex manipulation of high-activity samples that dramatically increases radiation exposure of the operator, particularly in routine treatment of HCC patients. To overcome this problem, we attempted to design an automated system for the remote-controlled preparation of ^{188}Re-lipiodol using our labeling method. This work describes the essential features and advantages of this new synthesis module.

2. Materials and methods

All chemicals and reagents were of analytical grade unless otherwise specified. Tungsten-188/Rhenium-188 generator was purchased from the National Institute for Radioelements (IRE, Fleurus, Belgium). SepPak Cartridges were obtained from Waters Corporation (Milford, MA, USA). On Guard II Ag Cartridges (5.0–5.5 mEq per cartridge) were purchased by Dionix (Sunnyvale, CA, USA). 0.22-μm Millex-LG sterile filters were obtained from Millipore (Milan, Italy). The two-vials (A and B), sterile, freeze-dried kit formulation (Lipiokit) for the preparation of the complex [^{188}Re]ReN-DEDC was produced at IZOTOPE, Budapest, Hungary and kindly provided by Dr. Joseph Kornyei. Vial A contained 2.0 mg of DTCZ (S-metyl N-methyl-dithiocarbazate), 0.8 mg of $SnCl_2{\times}2H_2O$ and 28.0 mg of sodium oxalate under a nitrogen atmosphere. Vial B contained a carbonate buffer (pH, 9.0) and 20.75 mg of DEDC under a nitrogen atmosphere. In addition to vials A and B, a sterile empty vial (C) was also provided. Oxygen was excluded from all solutions and from the automated system by flushing a nitrogen gas. Lipiodol was purchased from Guerbet, Roissy, France. Thin-layer chromatography (TLC) was carried out on silica-gel plates (Merck, Darmstadt, Germany) using ethanol/chloroform/benzene (1.5:2:1.5) as mobile phase. High-performance liquid chromatography (HPLC) was performed on a System Gold instrument equipped with a programmable solvent Module 126, a scanning detector Module 166 and a radioisotope detector Module 170 (Beckman Instruments, Fullerton, CA, USA). Chromatographic analyses were carried out using a reversed-phase Hamilton PRP-1 pre-column (45×4.1 mm) and a reversed-phase Hamilton PRP-1 column (250×4.1 mm) at a flow rate of 2 ml min^{-1} (Reno, NV, USA). The following gradient was used as mobile phase: A=trifluoroacetic acid (TFA) (0.1% v/v in water), B=TFA (0.1% v/v in CH_3CN); 0–1 min, B=0% (isocratic); 1–8 min, B=100% (gradient); 8–12 min, B=100% (isocratic); 12–13 min, B=0% (gradient).

2.1. Kit formulation

A schematic drawing of the different steps required for the preparation of Re-188 labelled lipiodol using the two-vial kit formulation is illustrated in Fig. 1. The procedure can briefly described as follows. (1) Glacial acetic acid (0.1 ml) and generator-eluted [$^{188}ReO_4$]$^-$ (3.0 ml) were added to vial A and the mixture was kept at room temperature for 15 min. (2) Water for injection (1.5 ml) was added to vial B, and the resulting solution (1.0 ml) was withdrawn with a syringe and introduced into vial A, which was, then, heated at 80°C for 30 min to afford the complex [^{188}Re]ReN-DEDC. (3) After cooling to room temperature, an equal volume of lipiodol was added to vial A and the mixture was vigorously vortexed for 15 min. Vial A was finally centrifuged for 10 min at 1600×*g* to separate the aqueous and hydrophobic layers. The aqueous layer was carefully removed with a syringe and the two phases were separately counted.

Radiochemical purity (RCP) of the complex [^{188}Re]ReN-DEDC was checked by HPLC (Fig. 2) at the end of step (2), and results gave a value of 94±1.28% for this parameter.