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Improved freeze-dried kit for the preparation of ^{188}ReN-DEDC/lipiodol for the therapy of unresectable hepatocellular carcinoma

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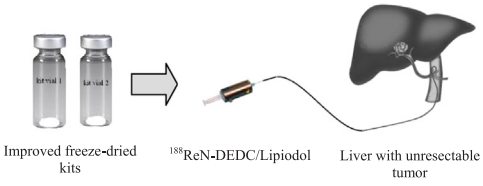
HIGHLIGHTS

- A glacial acetic acid free route for the preparation of $[^{188}ReN]^{2+}$ core.
- Use of oxalate buffer significantly improved rate of formation of $[^{188}ReN]^{2+}$ intermediate.
- > 95% RCP of $[^{188}ReN]^{2+}$ intermediate achieved within 5 min incubation at room temperature.
- Oxalate buffer kits provide an efficient and user-friendly route to ^{188}ReN-DEDC complex.

GRAPHICAL ABSTRACT



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ABSTRACT

Rhenium-188-N-$(DEDC)_2$/lipiodol (abbreviated as ^{188}ReN-DEDC, where DEDC = monoanionic diethyldithiocarbamate) is a clinically proven radiopharmaceutical for the therapy of unresectable hepatocellular carcinoma (HCC) through trans arterial radioembolization (TARE). A two-vial freeze-dried kit for the preparation of $[^{188}ReN(DEDC)_2]$ complex using sodium perrhenate ($Na^{188}ReO_4$) obtained from a commercial Tungsten-188/Rhenium-188 generator had been reported earlier. This method required addition of stipulated volume of glacial acetic acid into vial 1 by the user for efficient preparation of $[^{188}ReN]^{2+}$ intermediate. An error in this step can result in low radiochemical yield of $[^{188}ReN]^{2+}$ intermediate as well as sub-optimal pH of the reaction mixture for the second step, leading to poor radiochemical purity of ^{188}ReN-DEDC complex. In the present work, a solution to this problem was found by including an oxalate buffer of pH = 3 in vial 1, eliminating the need for the addition of glacial acetic acid by the user. This modification not only made the kits more user-friendly, it resulted in significant improvement in the kinetics of formation of $[^{188}ReN]^{2+}$ intermediate, wherein > 95% radiochemical purity could be achieved within 5 min incubation at ambient temperature. Moreover, the novel route for the preparation of $[^{188}ReN]^{2+}$ intermediate may be applied to any radiopharmaceutical based on ^{188}ReN-core.

1. Introduction

Liver cancer is one of the major causes of cancer deaths worldwide with the number of cases consistently increasing over the years (Ferlay et al., 2015). Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two most common primary cancer variants observed in patients (Swinburne et al., 2017; Wang et al., 2017). Asymptomatic nature of HCC and ICC often leads to patients presenting themselves with late stage disease when surgical intervention becomes difficult or impossible. The usual course of action under such conditions is systemic chemotherapy. However, other modes of loco-regional therapies such as thermal ablation of the affected tissue,

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teletherapy and trans arterial therapy are gaining popularity (National Comprehensive Cancer Network, 2015). Trans arterial radioembolization (TARE) is one of the minimally invasive, image-guided loco-regional liver therapies in clinical practice today (Gbolahan et al., 2017). This procedure embolizes the blood vessels feeding the tumor tissue to deny vital nutrients and oxygen. Additionally, the radiotherapy agent in the embolizing medium provides effective loco-regional therapy while sparing neighboring normal liver cells.

Some of the clinically available options for TARE include ^{90}Y-microparticles (Fidelman et al., 2016; Hoffmann et al., 2012), ^{131}I-lipiodol (Lintia-Gaultier et al., 2013; Ahmadzadehfar et al., 2014) and ^{188}Re-lipiodol (Jeong et al., 2001; Kumar et al., 2007; Boschi et al., 2004). Recent studies had proved the efficacy and safety of ^{90}Y-microparticles (Wang et al., 2017). However, high cost of ^{90}Y-microparticle therapy limits its application to a small fraction of liver cancer patient population. Though ^{131}I-lipiodol therapy provides an economically viable alternative, long half-life ($t_{1/2}$ = 8.04 days), low β-energy [$E_{\beta max}$ = 0.61 MeV (89.3%), 0.33 MeV (7.3%), 0.25 MeV (2.1%)], need for the isolation of patient post therapy and high non-specific lung uptake, which drastically limits the administered dose, makes it a less preferred clinical choice.

Rhenium-188 radiopharmaceuticals for liver cancer therapy combine the benefits of low cost of ^{131}I-lipiodol therapy and, the safety and efficacy of ^{90}Y-microparticle therapy. Rhenium-188 has beta emission with energy [$E_{\beta max}$ = 2.12 MeV, E_{γ} = 155 keV (15%), $t_{1/2}$ = 16.9 h] close to that of ^{90}Y [$E_{\beta max}$ = 2.28 MeV, $t_{1/2}$ = 64.1 h] and hence comparable tissue penetration could be expected. Presence of gamma emission, which permits monitoring the localization of radiopharmaceutical in the target tissue, is an added advantage of ^{188}Re-agents over ^{90}Y-agents. Moreover, commercial availability of ^{188}W/^{188}Re generator permits preparation of the ^{188}Re-radiopharmaceutical "on demand" in any hospital radiopharmacy housing the generator.

General strategy for the development of non-particulate ^{188}Re-radiopharmaceuticals for liver cancer therapy involved initial preparation of a stable, highly lipophilic complex of ^{188}Re followed by its extraction into hydrophobic lipiodol phase. The resulting radiopharmaceutical-loaded lipiodol was used for TARE. A number of lipophilic ^{188}Re(V)-oxo complexes have been reported for lipiodol ^{188}Re-labeling following above strategy. Wang et al. reported ^{188}Re-EDTB/lipiodol [EDTB = N,N,N",N"-tetrakis(2-benzymidazolylmethyl)-l,2-ethanediamine] for liver cancer therapy (Wang et al., 1996). However, significant accumulation of activity in lungs and kidneys limited its clinical applications. The ^{188}Re-HDD/lipiodol (HDD = 4-hexadecyl-2,2,9,9- tetramethyl-4,7-diaza-1,10-decanedithiol) reported by Paeng et al., showed good retention in liver and it was found to be a clinically useful therapeutic agent for the treatment of liver cancer (Paeng et al., 2003). The ^{188}Re-HDD/lipiodol is an improved version of ^{188}Re-TDD/lipiodol (TDD = 2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol) reported by the same group (Jeong et al., 2001). In an IAEA-sponsored study, Kumar et al. reported that ^{188}Re-HDD/lipiodol is a safe and effective TARE agent for therapy of liver cancer (Kumar et al., 2007).

The ^{188}ReN-DEDC/lipiodol is another TARE agent, which had proven its efficacy for the therapy of unresectable liver cancer (Boschi et al., 2004). The structure of ^{188}ReN-DEDC comprises two deprotonated diethyldithiocarbamato ligands bound to a $[ReN]^{2+}$core. A two-vial kit method for the preparation of ^{188}ReN-DEDC complex was reported by Boschi et al. (Boschi et al., 2004). Their method involved preparation of $[^{188}ReN]^{2+}$ intermediate in the first step, which was used to prepare the complex ^{188}ReN-DEDC in the subsequent step. The neutral and lipophilic ^{188}ReN-DEDC complex thus prepared was extracted into lipiodol phase and used for TARE. Clinical trials with ^{188}ReN-DEDC/lipiodol showed retention of activity in liver with no detectable levels of activity in lungs, kidneys or any other vital organs (Boschi et al., 2004).

The two-vial method reported by Boschi et al., however, requires addition of stipulated volume of glacial acetic acid into vial 1 for efficient preparation of $[^{188}ReN]^{2+}$ intermediate. An error by the radiopharmacist in this step can negatively influence the formation of $[^{188}ReN]^{2+}$ intermediate as well as the optimum pH required for the reaction mixture for the crucial second step, leading to low radiochemical purity of ^{188}ReN-DEDC complex. In routine conventional radiopharmacy operations, an "acetic acid free" procedure for the preparation of ^{188}ReN-DEDC complex could be more reliable and reproducible, thus helping to avoid any inappropriate usage of the radiopharmaceutical. It should be noted that an alternative procedure for the preparation of ^{188}ReN-DEDC/lipiodol using an automated synthesis module has been described (Uccelli et al., 2011). As with most of PET agents, an automated procedure allows overcoming a number of human errors otherwise possible in conventional radiopharmaceutical preparation. However, this procedure is usually very expensive and much dependent on the commercial availability of a dedicated synthesis module.

In the present work, we report on the preparation of a two-vial lyophilized kit for the in-house preparation of ^{188}ReN-DEDC complex via a "glacial-acetic-acid-free" route. This improvement is expected not only to simplify the procedure for preparing the therapeutic agent ^{188}ReN-DEDC/lipiodol, but also to have a beneficial impact in promoting the search of new stable ^{188}Re-radiopharmaceuticals based on the ^{188}ReN core.

2. Materials and methods

Oxalic acid, sodium bicarbonate and stannous chloride dihydrate were purchased from Sigma Aldrich. Disodium oxalate was purchased from Alfa Aesar. N-methyl-S-methyl dithiocarbazate (DTCz) was synthesized as reported previously (Patent No. B.P.WO 90/06137; Ali et al., 1972). Rhenium-188 as Na^{188}ReO4 was obtained from a ^{188}W/^{188}Re generator purchased from ITG, Germany. Glass vials and other materials used for the preparation of lyophilized kits were thoroughly cleaned and autoclaved before use. All lyophilizations were carried out using the Alpha 1–2 LD plus lyophilizer (Martin Christ, GmBH). The HPLC analyses were performed on a JASCO PU 2080 Plus dual pump HPLC system, Japan, with a C18 reversed phase HiQ Sil (5 μm, 4 × 250 mm) column attached to a JASCO 2075 Plus tunable UV detector and a Gina Star radiometric detector system.

2.1. Preparation of ReN kit (Vial 1) for glacial acetic acid method (ReNGAA kit)

A twenty-vial batch of lyophilized kits, each kit containing DTCz (2 mg), $SnCl_2{\cdot}2H_2O$ (0.8 mg) and sodium oxalate (28 mg), as described earlier by Uccelli et al. (Uccelli et al., 2011), was prepared as follows. Sodium oxalate (588 mg) and DTCz (42 mg) was dissolved in 51.5 mL of nitrogen purged autoclaved water. To this, stannous chloride dihydrate solution (1 mL, 16.8 mg) was added. The solution was mixed thoroughly, filtered through a 0.22 μm syringe filter and 2.5 mL each was dispensed into 10 mL glass vials. The contents of the glass vials were lyophilized and sealed under vacuum.

2.2. Preparation of ReN kit (Vial 1) for oxalate buffer method (ReNOxb kit)

ReNOxb kits were prepared with three different concentration of oxalate buffer viz. 0.5 M, 0.25 M and 0.1 M to study the influence of buffer concentration on the formation of $[^{188}ReN]^{2+}$ intermediate. Procedure followed for the preparation of ReNOxb kits with different oxalate buffer concentration is briefly mentioned below. All procedures were carried out in clean room under sterile conditions.

2.2.1. 0.5 M oxalate buffer kit (20 vials per batch)

Disodium oxalate (630 mg), oxalic acid (592 mg) and DTCz (42 mg)