Applied Radiation and Isotopes 94 (2014) 241–246

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Mixed-ligand complexes of yttrium-90 dialkyldithiocarbamates with 1,10-phenanthroline as a possible agent for therapy of hepatocellular carcinoma

A. Lopez [a,b], N. Noiret [b,c], E. Garin [a,c], N. Lepareur [a,c,*]

[a] Centre Eugène Marquis, INSERM UMR-S 991, Avenue de la Bataille Flandres-Dunkerque, CS 44229, F-35042 Rennes, France
[b] ENSCR, CNRS UMR 6226, 11, Allée de Beaulieu, CS 50837, F-35708 Rennes, France
[c] Université Européenne de Bretagne, F-35000 Rennes, France

HIGHLIGHTS

- New radiolabelling of Lipiodol with yttrium-90.
- Fast and efficient synthesis.
- Radiolabelled Lipiodol is a promising treatment modality for HCC.

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form
16 August 2014
Accepted 26 August 2014
Available online 6 September 2014

Keywords:
Dithiocarbamate
1,10-phenanthroline
Hepatocellular carcinoma
Lipiodol
Radionuclide therapy
Yttrium-90

ABSTRACT

Yttrium-90 is a radioelement which has found wide use in targeted radionuclide therapy because of its attractive physical and chemical properties. Radioembolisation of hepatocellular carcinoma with radiolabelled Lipiodol is a method of choice. We have synthesised a series of alkyldithiocarbamate yttrium complexes, easily extracted into Lipiodol due to their high lipophilicity. Among the prepared series, a new radioconjugate, which is stable over an extended period of time, has been prepared, and could represent a potential treatment procedure for hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC), the most common form of primary liver cancers, is the fifth most common tumour worldwide, and even ranks third in terms of mortality (Ferlay et al., 2010; Jemal et al., 2011). For the vast majority of patients not eligible to curative treatments, such as resection or transplantation, there is a wide range of palliative treatments which can be proposed, among which are chemoembolisation and radioembolisation with Lipiodol or microspheres (Venook, 1994; Thomas and Zhu, 2005; Liapi and Geschwind, 2010; Lencioni, 2010; Raoul et al., 2010). Lipiodol is an oily medium which has shown to be selectively retained in tumour when administered intra-arterially (Chou et al., 1995). Lipiodol has been labelled with iodine-131 (Liebster and Kocandrle, 1964; Raoul et al., 1986), rhenium-188 (Lepareur et al., 2008), yttrium-90 (Wang et al., 1996b; Yu et al., 2003), and radiolanthanides (Das et al., 2009; Subramanian et al., 2010). Yttrium-90 (pure beta-emitter, $E_{\beta_{max}}$ = 2.27 MeV, $t_{1/2}$ = 64 h, max tissue penetration = 12 mm) has ideal properties for targeted radiotherapy, and has found wide use in peptide receptor radionuclide therapy (Goffredo et al., 2011), radioimmunotherapy (Sharkey et al., 2010) and radioembolisation (Salem and Hunter, 2006). It has been suggested as a suitable isotope to label Lipiodol as it should lead to a significantly reduced whole-body dose compared to ^{131}I-Lipiodol, since more than 90% of this dose is due to the emitted gamma rays (respectively

Abbreviations: Bipy, 2,2′-bipyridyl; DEDC, diethyldithiocarbamate; dtc, dithiocarbamate; HCC, hepatocellular carcinoma; Phen, 1,10-phenanthroline

* Corresponding author at: Centre Eugène Marquis, INSERM UMR-S 991, Avenue de la Bataille Flandres-Dunkerque, CS 44229, F-35042 Rennes, France.
Tel.: +33 2 99 25 31 44; fax: +33 2 99 25 32 60.
E-mail address: n.lepareur@rennes.unicancer.fr (N. Lepareur).

http://dx.doi.org/10.1016/j.apradiso.2014.08.015
0969-8043/

0.02 rad/mCi compared to 1.9 rad/mCi, based on estimated dosimetry) (Madsen et al., 1988). After having successfully developed radiolabelled Lipiodol with rhenium-188 (Lepareur et al., 2004, 2012), we decided to develop a new labelling of Lipiodol with yttrium-90 for the treatment of HCC, as a complementary tool in the treatment armamentarium. A series of heteroligand yttrium dialkyldithiocarbamate complexes with 1,10-phenanthroline has been prepared (Fig. 1), and their suitability to label Lipiodol has been investigated.

2. Experimental

2.1. Materials and methods

Yttrium-90 was obtained from IBA as yttrium chloride in HCl 0.04 M (Ytracis, CIS bio International/IBA, Gif-sur-Yvette, France). Lipiodol was obtained from Guerbet (Villepinte, France). 1,10-phenanthroline was purchased from Acros (Illkirch, France). Sodium diethyldithiocarbamate was purchased from Aldrich (Saint Quentin Fallavier, France). Other dithiocarbamates were synthesised according to the literature from the corresponding secondary amines and carbon disulphide. Briefly, in a 100 mL round-bottom flask, under nitrogen, 14 mmol of *N*-methylalkylamine was diluted in 30 mL diethyl ether. The round-bottom flask was cooled down to $-15\,^{\circ}C$ in an ice/salt bath. 532 mg (13.3 mmol) of sodium hydroxide in methanol was added under stirring. 1.1 g (14.6 mmol) of carbon disulphide was slowly added under stirring. The mixture was stirred for 30 min in the ice/salt bath, then at room temperature for 90 min. The obtained precipitate was filtered then dried under vacuum to give a white powder.

Non-radioactive compounds were characterised by ^{1}H and ^{13}C NMR recorded with a BRUKER ARX 400 (Billerica MA, USA) at 400.13 and 100.62 MHz, respectively, in $CDCl_3$, calibrated internally to the residual solvent. ES mass analysis was done on a Shimadzu LCMS 2020 (Kyoto, Japan). Activity measurements were done in a CRC-127R well-counter (Capintec Inc., Ramsey NJ, USA). TLC analyses were done on ITLC-SG plates (Pall Life Sciences, Ann Arbor, MI, USA) with methanol as mobile phase. The plates were analysed with a Perkin-Elmer Cyclone Storage Phosphor Imager, using the Packard Optiquant v04.00 software.

2.2. Preparation of [^{89}Y(DEDC)$_3$(Phen)] (C1)

MW = 713

In a 20 mL round-bottom flask, under nitrogen, 180 mg (1 mmol) of 1,10-phenanthroline, dissolved in 2 mL methanol, and 675 mg (3 mmol) of sodium *N,N*-diethyldithiocarbamate trihydrate, dissolved in 2 mL methanol, are placed. 200 mg (1 mmol) of yttrium chloride was dissolved in 2 mL methanol and was added dropwise to the ligands. The complex precipitated. The mixture was stirred for 20 min at room temperature then filtered, washed with methanol, and dried under vacuum to give **C1** as white crystals (619 mg, 87%). ^{1}H NMR ($CDCl_3$): 9.99 (dd, J=4.9, 1.5 Hz, 2H, H-Phen), 8.37 (dd, J=8.1, 1.6 Hz, 2H, H-Phen), 7.85 (s, 2H, H_4), 7.72 (dd, J=8.1, 4.9 Hz, 2H, H-Phen), 3.83 (q, J=7.1 Hz, 12H, NCH_2), 1.12 (t, J=7.1 Hz, 18H, CH_3). ^{13}C NMR ($CDCl_3$): 205.8 (CS_2), 151.8 (CH-Phen), 144,9 (C-Phen), 137.9 (CH-Phen), 129.4 (C-Phen), 126.8 (CH-Phen), 123.5 (CH-Phen), 46.1 (NCH_2), 12.4 (CH_3). m/z=713.40. R_f (MeOH) =0.76.

2.3. Preparation of [^{90}Y(dtc)$_3$(Phen)] and Lipiodol radiolabelling

100 μL of $^{90}YCl_3$ in acetate buffer (pH 4.75) was added to a solution of 100 μL of sodium dithiocarbamate 15×10^{-2} M in ethanol and 100 μL of phenanthroline 5×10^{-2} M in ethanol. Several parameters such as concentration of ligands, volume and pH of the reaction mixture, incubation time, and temperature were varied extensively to arrive at the optimised protocol. For the preparation of ^{90}Y-Lipiodol, [^{90}Y(dtc)$_3$(Phen)] complex prepared under optimised reaction conditions was extracted in 2 mL Lipiodol. After vigorous shaking for 2 min to ensure homogeneous dispersion of the complex in Lipiodol, the phases were separated by centrifugation. Chemical identity of the radiotracer was assessed for [^{90}Y(DEDC)$_3$(Phen)] by comparing its TLC profile in MeOH with that of the fully characterised analogous non-radioactive complex **C1**. Other dithiocarbamates tracers were assumed to have similar structures.

2.4. Determination of complexation yield of [^{90}Y(dtc)$_3$(Phen)]

The complexation yield of the [^{90}Y(dtc)$_3$(Phen)] radiotracer prepared was determined by solvent extraction technique. 300 μL of chloroform was added. The mixture was vigorously shaken, then centrifuged (3840 rpm, 15 min) to separate the phases. The two phases were carefully collected and counted in a well-counter. The complexation yield was determined as the organic layer activity on the total (aqueous+organic) activity.

2.5. Determination of labelling yield of Lipiodol

The labelling yield of Lipiodol was determined by an analogue technique. 900 μL of saline and 2 mL of Lipiodol were added. The mixture was vigorously shaken, and then centrifuged (3840 rpm, 15 min) to separate the phases. The two phases were carefully collected and counted in a well-counter. The complexation yield was determined as the Lipiodol layer activity on the total (aqueous+Lipiodol) activity.

2.6. In vitro stability of ^{90}Y-Lipiodol

To check the *in vitro* stability of ^{90}Y-labelled Lipiodol, 2 mL of saline was added to the preparation and mixed vigorously. The mixture was allowed to settle at room temperature. 100 μL of aliquots was withdrawn from both layers at different time intervals post-preparation and the associated activity was counted. The stability of the radiolabelled preparation at various time points was determined by calculating the percentage of activity associated with the lipid phase from these data.

3. Results and discussion

When injected through the hepatic artery, Lipiodol, an iodinated mixture of esterified poppyseed oil, is selectively trapped in tumour cells (HCC and some hepatic metastases) (Nakakuma et al., 1985). Besides, Lipiodol displays a prolonged retention within the tumour, while it is more quickly cleared from the healthy liver (Chou et al., 1995; Kan, 1996). It has thus been used for the detection of HCC and as a vector for chemotherapeutic drugs (Bhattacharya and Dusheiko, 1995; Dalla Palma,1998).

When radiolabelled with iodine-131, Lipiodol has shown a high tumour-to-liver ratio, with some activity in the lungs (depending on arterioveinous shunts) and no activity in the thyroid, thus excluding iodine-131 release (Madsen et al., 1988; Raoul et al., 1988; Yoo et al., 1994). Covalently labelled Lipiodol with rhenium-188 and yttrium-90 demonstrated a biodistribution similar to ^{131}I-Lipiodol (Wang et al., 1996a, 1996b). This approach was however disappointing, and it was demonstrated that solubilisation of a labelled lipophilic chelate into Lipiodol was a better approach (Jackson et al., 2000). To efficiently interact with Lipiodol, the radiocomplex has to be conveniently lipophilic. Other important