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Development of acetylated HDD kit for preparation of ^{188}Re-HDD/lipiodol

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Abstract

A lipiodol solution of ^{188}Re-4-hexadecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (^{188}Re-HDD/lipiodol) is in clinical study for liver cancer therapy. However, formulation of it is difficult due to highly active and unstable sulfhydryl groups. We produced new kits using diacetylated HDD (AHDD), in which sulfhydryl groups are protected. We found that AHDD kit can replace HDD kit due to an increased stability for formulation, the better radiolabeling efficiency (78%) and the equivalent biodistribution pattern in mice.

Keywords: Liver cancer therapy; HDD; ^{188}Re; Lipiodol

1. Introduction

Liver cancer is one of the most prevalent causes of death in East Asia, Southeast Asia and Africa. The incidence is also increasing in the United States and Europe.

An oily contrast agent—lipiodol has been used for targeting liver cancer because it accumulates in the cancer effectively if injected through the hepatic artery. ^{131}I-lipiodol has been developed for radionuclide therapy of liver cancer (Park et al., 1986, 1987; Yoo et al., 1986). ^{188}Re became of great importance (Jeong and Chung, 2003) due to excellent nuclear properties and development of ^{188}W/^{188}Re-generator (Knapp et al., 1997). To improve the therapeutic effect, ^{188}Re-lipiodol has been developed (Wang et al., 1996, 1996). However, it has not been practically used due to low radiolabeling efficiency, low stability and technical problems in preparation.

After development of a stable lipophilic diaminedithiol-based complex, ^{188}Re-4-hexadecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (HDD) (Jeong et al., 2001; Lee et al., 2002), ^{188}Re-labeled lipiodol became practical. Clinical application of ^{188}Re-HDD/lipiodol was initiated by the International Atomic Energy Agency (IAEA) (Sundram et al., 2002, 2004; Lambert et al., 2005a, b).

Kit formulation is essential for the clinical use of ^{188}Re-HDD/lipiodol in order to simplify the radiolabeling procedure and to reduce radiation exposure during preparation. A large-scale synthesis for kit formulation and the characterization of HDD have been delayed because of the instability of sulfhydryl groups and hygroscopic characters of various salts of HDD

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(Lever et al., 1985). To overcome this problem, we developed a diacetylated HDD (AHDD) kit formulated with AHDD in which the reactive sulfhydryl groups were protected with acetyl groups. We worked to establish the optimal condition for ^{188}Re radiolabeling using an AHDD kit and its lipiodol solution. We also compared the biodistribution of ^{188}Re-HDD/lipiodol in mice from an AHDD kit (^{188}Re-HDD-A) with ^{188}Re-HDD/lipiodol from an HDD kit (^{188}Re-HDD-H).

2. Materials and methods

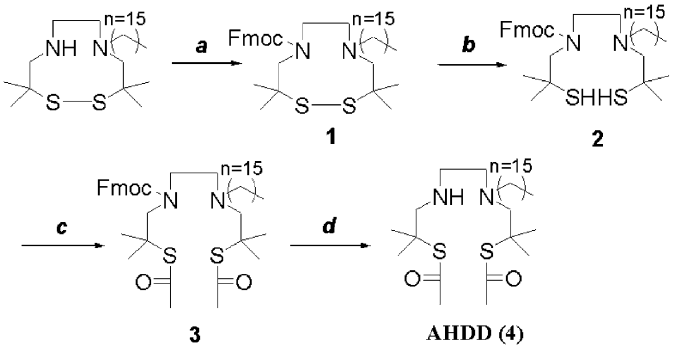
2.1. General

A NaI well counter (Packard, Canberra Co., USA) and a dose calibrator (Atomlab 100, Medical Systems Inc., USA) were used to measure the low and high levels of radioactivity, respectively. An AR-2000 TLC Imaging Scanner (Bioscan, Washington, DC, USA) was used to perform the Radio-TLC scan. For TLC, we purchased aluminum backed silica gel 60 F_{254} from the E. Merck Company (Darmstadt, Germany); ITLC-SG plates were obtained from the Pall Company (New York, NY, USA). A centrifuge (Union 32R Plus, Hanil Science Industrial Co., Ltd., Seoul, Korea) was used for lipiodol extraction of ^{188}Re-HDD. A freeze dryer (Heto FD 8.0, JOUAN Nordic A/S, Allerød, Denmark) was used for lyophilizing of the kit. The ^{188}W/^{188}Re generator was purchased from the Oak Ridge National Laboratory (Oak Ridge, TN, USA). AHDD was offered by Dong-A Pharmaceutical Co., Ltd., (Kyunggi-do, Korea). All reagents and solvents, if not specified, were purchased from Sigma-Aldrich or Fluka, and were used with no further purification. Mice were purchased from Orient Co., Ltd., (Seoul, Korea) and animal study was carried out in compliance with the local institutional regulations.

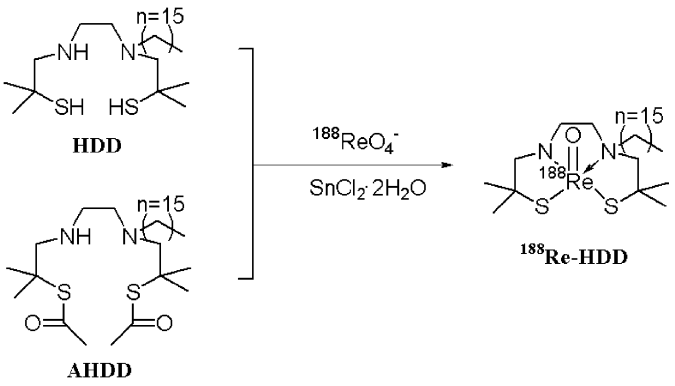
2.2. Preparation of kits

2.2.1. Chemical syntheses

AHDD was prepared from dithiol form of HDD by the acetylation reaction in the presence of base (Schemes 1 and 2).



Scheme 1. Chemical synthesis of AHDD. Reagents and conditions: Reagents and reaction conditions: (a) Fmoc-Cl, Et_3N, THF, rt; (b) 35% HCl, Zn powder, ethanol, 35 °C; (c) acetyl chloride, pyridine, CH_2Cl_2, rt; (d) DBU, CH_2Cl_2, rt, 20 min.



Scheme 2. ^{188}Re radiolabeling of HDD and AHDD.

The chemical structure of AHDD was confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS.

*2.2.1.1. 3,3,10,10-tetramethyl-5-hexadecyl-8-(9-fluorenyl-methyloxycarbonyl)-1,2-dithia-5,8-diazacyclodecane (**1**).* To a stirred solution of secondary amine 3,3,10,10-tetramethyl-5-hexadecyl-1,2-dithia-5,8-diazacyclodecane (5.0 g, 10.9 mmol) and triethyl amine (2.2 g, 21.8 mmol) in THF (50 mL) was added Fmoc-Cl (3.4 g, 13.1 mmol) in THF (25 mL) at 0 °C and the resulting solution was stirred at room temperature overnight. After concentration, the residue was partitioned between ethyl acetate (50 mL) and water (40 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by column chromatography using ethyl acetate and ethanol (10/1) as an eluent to afford **1** as an oil (5.2 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, $J = 7.59$ Hz, 2H), 7.57 (d, $J = 7.19$ Hz, 2H), 7.39 (t, $J = 7.19$ Hz, 2H), 7.31 (t, $J = 7.19$ Hz, 2H), 4.47 (d, $J = 5.60$ Hz, 2H), 4.21 (brs, 1H), 3.21 (brs, 2H), 3.02 (m, 2H), 2.82 (brs, 2H), 2.63 (d, $J = 13.19$ Hz, 2H), 1.44–1.13 (m, 42H), 0.88 (t, $J = 6.39$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 143.97, 141.28, 127.54, 126.92, 124.65, 119.86, 70.48, 66.73, 58.88, 58.25, 51.76, 48.90, 47.43, 46.69, 31.97, 29.73, 29.42, 26.93, 25.48, 22.75, 14.20; HRMS (FAB^+) calcd. for $C_{41}H_{64}N_2O_2S_2$ 680.4409, found 680.4525.

*2.2.1.2. 2,2,9,9-tetramethyl-4-hexadecyl-7-(9-fluorenyl-methyloxycarbonyl)-4,7-diaza-1,10-decanedithioacetate (**3**).* To a stirred solution of diazacyclodecane **1** (5.2 g, 7.63 mmol) in a mixture of methanol (100 mL) and ethanol (30 mL) was added 35% HCl (12 g, 114.45 mmol) and zinc powder (3.5 g, 53.44 mmol) successively and the resulting mixture was stirred at 35 °C overnight. After filtration and concentration, the residue was partitioned between ethyl dichloromethane (50 mL) and water (40 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness to give crude **2** (5.0 g, 96%), which was used in the next step without further purification. To a stirred solution of dithiol **2** (5.0 g, 7.32 mmol) and pyridine (1.39 g, 17.57 mmol) in dichloromethane (100 mL) was added