Lipiodol solution of a lipophilic agent, $^{188}$Re-TDD, for the treatment of liver cancer

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Abstract

Radiolabeled lipiodol has been used for targeting liver cancer. We developed a lipiodol solution of $^{188}$Re-TDD (2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol) and investigated its feasibility for the treatment of liver cancer. The lipiodol solution of $^{188}$Re-TDD was well-retained in the lipiodol phase in vitro. After injection through the tail veins of mice, high lung-uptake was investigated which is evidence of embolizing activity. We also found high accumulation in hepatoma after injection through the hepatic arteries of hepatoma-bearing rats. In conclusion, the lipiodol solution of $^{188}$Re-TDD is a promising agent for liver cancer therapy. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Lipiodol; $^{188}$Re; Diaminedithiol; $N_2S_2$; Liver cancer; TDD

1. Introduction

Liver cancer, particularly hepatocellular carcinoma, is one of the most common malignant diseases in many developed and developing countries (especially East Asia, Southeast Asia, Africa, and Southern Europe). Although surgery is very effective in early-stage patients, other methods are considered for patients in whom surgery may not work well. Systemic administration of chemotherapeutic agents is not often considered in liver cancer patients due to discouraging results and adverse side effects [10,12]. Alternatively, local administration of embolizing agents, such as lipiodol or gel foam, through the hepatic artery or direct injection of alcohol into the tumor guided by sonography have been reported to be effective [5,16].

Liver cancer blood supply is primarily obtained from the hepatic artery, while that of normal hepatic cells is mainly obtained from the portal vein. Consequently, many embolic agents accumulate in liver cancer by embolization if administered through the hepatic artery. Lipiodol is an iodinated and esterified lipid of poppy seed oil and has been used as a contrast agent. Because of its high lipophilicity and viscosity, it has been used as an embolic agent for the detection of liver cancer, and has also been used for liver cancer therapy after being mixed with chemotherapeutic agents such as adriamycin.

Radiolabeled lipiodol has been tested for the targeting of liver cancer in both human and animal models. $^{131}$I-labeled lipiodol has been used for the treatment of liver cancer since 1986 [8,10,11,14,15,22]. Methods for the preparation of $^{90}$Y- and $^{188}$Re-labeled lipiodol have been reported by Wang et al. in 1995 and 1996 [19–21]. In 1998, a method for the preparation of a $^{188}$Re-sulfur colloid-suspended in lipiodol was reported [2]. This method was unique in that it did not involve the direct labeling of lipiodol. $^{188}$Re-sulfur colloid prepared by the reported methods [1,17,18] was washed by ethanol and diethyl ether, and then suspended in lipiodol.

Among the radioisotopes used for the treatment of liver cancer, we believe that $^{188}$Re is potentially the most useful radioisotope. Because it has 17-hr half-life, 2.1-MeV beta ray with average-path of 3.8 mm and maximum-path of 11

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2. Materials and methods

2.1. General

Reagents used in the syntheses were purchased from Sigma-Aldrich Company or Guerbet Company and used without further purification unless otherwise indicated. Analytical thin layer chromatography was performed on Kieselgel 60F254 (0.25 mm, Merck), and the spots were detected with ethanolic p-anisaldehyde solution and/or UV light. Nuclear magnetic resonance spectra (1H-NMR spectra) were obtained on a Varian 300-MHz Gemini 300 FT-NMR spectrometer. The samples prepared for NMR analysis were dissolved in CDCl3 with TMS as an internal reference or D2O purchased from Sigma-Aldrich Company. Melting point was determined on a Mel-temp II apparatus (Holliston Company) and are uncorrected. 188W/188Re-generator was purchased from Oak Ridge National Laboratory. ITLC-SG was purchased from Gelman Company. BAS-2500 was purchased from Fuji Company.

2.2. Synthesis of TDD

TDD was synthesized using minor modifications of the previously reported methods [6,7,9]. 2,2'-Dithio-bis(2-methylpropanal) was synthesized by reacting isobutyaldehyde (10 ml, 100 mmol) and sulfur monochloride (3.58 ml, 43.8 mmol) in chloroform (30 ml) for 4 hr at 50°C and overnight at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in ethylacetate (30 ml), washed with 1-M sodium hydroxide and subsequently with saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was dissolved in benzene (20 ml). To this solution, 1,2-diaminoethane (4.08 ml, 61 mmol) was added dropwise with stirring. After refluxing for 2 hr, the solvent was evaporated under reduced pressure, the residue was dissolved in chloroform (30 ml) and filtered with a sintered glass filter. After removal of the solvent from the filtrate by rotary evaporation, the residue was recrystallized in acetonitrile (30 ml) and washed with cold petroleum ether to produce 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene as a pale yellow powder. mp 167~169; TLC (silica gel/ethylacetate:n-hexane = 1:2): Rf 0.2; 1H NMR (CDCl3) δ 1.36 (s, 6H), 2.34 (bs, 2H), 2.55 (d, 2H, J = 12.0 Hz), 2.80 (s, 4H), 2.98 (s, 4H), 3.24 (d, 2H, J = 6.0 Hz), 4.14~4.16 (d, 2H, J = 6.0 Hz), 6.85 (s, 2H).

To a solution of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene (2.0 g, 8.7 mmol) in methanol (30 ml), sodium cyanoborohydride (3.45 g, 52.1 mmol) was slowly added. Glacial acetic acid was added to adjust pH 5.0. After 2-hr stirring at room temperature, the solution was heated to 60°C for 6 hours. The reaction was quenched with saturated ammonium chloride solution (10 ml) and the solvent was evaporated under reduced pressure. The residue was dissolved in 1-M sodium hydroxide (5 ml) and extracted with chloroform (20 ml × 3). The chloroform layer was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated in vacuo to give 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (2.02 g, 99%) as a yellowish oil. TLC (silica gel/acetonitrile:water = 5:1): Rf = 0.3; 1H NMR (CDCl3) δ 1.24 (s, 6H), 1.36 (s, 6H), 2.34 (bs, 2H), 2.55~2.59 (d, 2H, J = 12.0 Hz), 2.80 (s, 4H), 2.98~3.02 (d, 2H, J = 12.0 Hz).

To a solution of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (1.0 g, 4.27 mmol) dissolved in anhydrous tetrahydrofuran (20 ml), 1-M lithium aluminum hydride (8.98 ml, 8.97 mmol) in tetrahydrofuran was added dropwise. After stirring for 10 hours, the reaction was quenched with saturated ammonium chloride solution (5 ml) and the solvent was evaporated under reduced pressure. The residue was dissolved into water and the pH was adjusted to 5 with 2-M hydrochloric acid. After washing with diethyl ether (10 ml × 2), the pH was adjusted to 8.0 with 2-M sodium hydroxide and extracted with diethyl ether (10 ml × 3). The organic layer was washed with saturated sodium chloride solution (10 ml) and dried over anhydrous sodium sulfate.
To this solution, 4-M hydrogen chloride solution in 1,4-dioxane (2 ml) was added. The resulting precipitate was collected by filtration and recrystallized in water and isopropyl alcohol mixture to give 2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (0.21 g, 0.68 mmol, 16.0%) as a white hygroscopic powder. TLC (silica gel/acetonitrile:water 5:5:1): Rf 0.2; 1H NMR (D2O) δ 1.51 (bs, 12H), 3.32 (bs, 4H), 3.65 (bs, 4H).

2.3. Radiochemistry

The TDD-labeling kits were prepared by lyophilizing vials containing 1-mg TDD, 10-mg SnCl2.2H2O and 200-mg tartaric acid. 188Re-perrhenate was obtained as a solution in normal saline by using published method [3,4]. To label TDD, 3-ml 188W/188Re-generator-eluted 188Re-perrhenate was added to the kit vial and boiled for 30 min. The labeling efficiency was checked using ITLC-SG as a stationary phase and ethylacetate as a mobile phase. The Rf values of 188Re-TDD, 188Re-perrhenate, 188Re-tartaric acid, and 188Re-colloid were 1.0, 0.8, 0.0, and 0.0, respectively.

Lipiodol (1~3 ml) was added to the vial which was then inverted several times to extract the labeled 188Re-TDD into lipiodol phase. Vigorous shaking was not helpful because of emulsification. The mixture was transferred to a centrifuge tube and centrifuged for 10 min at 3,000 rpm to separate the water and the lipiodol phase. The upper phase that contained hydrophilic 188Re, in the form of 188Re-perrhenate, 188Re-tartaric acid and 188Re-tin colloid was removed using syringe. The lower lipiodol phase was washed with 3-ml of normal saline by centrifuge for 10 min, 3,000 rpm. The lower lipiodol phase, which contained lipophilic 188Re-TDD, was carefully collected using a syringe fitted with a long needle (Fig. 2).

The stability of 188Re-TDD in lipiodol at room temperature was checked for 48 hr. Its stability in human serum was also checked at 37°C for 48 hr. The same chromatography conditions as mentioned above were used to analyze the radioactive components.

To check the possibility of back-extraction of 188Re-TDD into the aqueous phase, a 1-ml lipiodol solution of 188Re-TDD was extracted with 1-ml of phosphate-buffered saline (PBS, pH 7.4) three times. The radioactivity in each phase was measured by a dose-calibrator.

2.4. Animal experiment

188Re-TDD in normal saline (0.1 ml, 0.56 MBq) was injected to normal mice (ICR male) through the tail vein. The mice were killed after 10 min, 1 hr and 24 hr; the blood, muscle, fat, heart, lung, liver, spleen, stomach, intestine, kidney, brain, and bone were removed, weights and radioactivities were then measured. The percentage of injected dose per gram of each organ was calculated.

The biodistribution of 188Re-TDD in lipiodol (0.03 ml, 0.74 MBq) in normal mice after injection through the tail vein was investigated using the same method as used above.

Sprague-Dawley rats were anesthetized with intraperitoneal injection of pentobarbital (3 mg/100 g) and opened the abdomen. The 4 × 10⁶/0.1 ml of N1-S1 cells (ATCC CRL-1604, rat hepatoma) were injected into the liver. After stitching the incisions, the recovered rats were fed for 10 days. The lipiodol solution of 188Re-TDD was then injected through the hepatic artery of the tumor-inoculated rats (Fig. 3). The rats were killed after 5 min, 1 hr and 24 hr. The radioactivities and weights of the blood, muscle, fat, heart, lung, liver, spleen, stomach, intestine, kidney, bone, and
hepatoma were measured, and the percentage of injected dose per gram of each organ was calculated.

The tumor-bearing livers of rats were removed after the hepatic arterial injection of lipiodol solution of $^{188}$Re-TDD and frozen in dry ice. The frozen livers were sectioned and exposed to phosphoimaging plates. Phosphoimages were analyzed using BAS-2500. Hematoxylin and eosin staining was performed to identify tumor and normal tissue in the sections.

3. Results

3.1. Synthesis of TDD

TDD was synthesized using a previously reported method with minor modifications (13.5% overall yield). The final step including reduction with lithium aluminum hydride showed the lowest yield (16.0%) in the overall synthesis. TDD is slightly different from the compound that was reported in literature, in that the reported one has a dimethyl group on the ethylene bridge between the nitrogen atoms [7]. This difference did not affect the synthesis. The final product was purified by recrystallization in isopropyl alcohol and water as a hydrogen chloride salt.

3.2. Radiochemistry

The labeling efficiency of $^{188}$Re-TDD reached 88% after boiling for 15 min (Fig. 4). Boiling for a longer period did not improve the labeling efficiency.

The lipiodol solution of $^{188}$Re-TDD was stable in atmospheric condition and in human serum at 37°C for at least 2 days (Fig. 5).

The $^{188}$Re activities remaining in the lipiodol phase after the first, second and third extractions with PBS were 95.8%, 97.1% and 98.3%, respectively (Table 1).

3.3. Animal experiment

Following the intravenous injection of an aqueous solution of $^{188}$Re-TDD through the tail vein of normal mice, radioactivity was found to distribute mainly in the liver at 10 min (17.0 ± 2.5% ID/g) and in the intestine at 1 hr (21.6 ± 3.2% ID/g) (Fig. 6A). However, the lipiodol solution of $^{188}$Re-TDD showed a high lung-uptake (62.4 ± 20.6% ID/g) at 10 min after intravenous injection. Radioactivity in the lung decreased to 4.7 ± 1.3% ID/g at 1 hr (Fig. 6B). High uptake in the lung is evidence of the capillary-blocking activity of the agent.

High initial tumor-uptake (33.1 ± 24.1% ID/g, 5 min) was found after injection through the hepatic artery in hep-
atoma-bearing rats. Liver-uptake was 4.3 ± 2.8% ID/g at 5 min. The tumor to liver ratio increased from 7.9 ± 4.9% ID/g (5 min) to 11.7 ± 10.3% ID/g (24 hr). Uptakes to other organs were negligible (<1% ID/g) comparing with the tumor and the liver, except for a temporary increase in the kidney at 1 hr (1.6% ID/g) (Table 2).

High tumor to liver ratio was observed in the phospho-image of the hepatoma-bearing liver. In the 1-hr image, radioactivities in blood vessels were seen in the normal liver (Fig. 7). In the 24-hr image, the contrast between tumor and liver tissue became stronger (Fig. 7). The tumor and normal tissue were identified by hematoxylin and eosin staining.

4. Discussion

There have been many attempts to label lipiodol with therapeutic radioisotopes [19,21,22]. However, the labeling procedures were cumbersome, because these efforts involved attaching the radioisotopes directly to the oily lipiodol or lipiodol-bichelating agent conjugate. One method has been reported which does not label the lipiodol itself, rather it labels lipiodol by suspending a 188Re-labeled sulfur-colloid in lipiodol [2]. This method showed a high tumor to non-tumor ratio when injected through the hepatic arteries of hepatoma-bearing rats.

In this experiment, we used TDD, a compound that forms a stable lipophilic chelate with 188Re. The TDD was synthesized using a previously published method [6,7,9]. Labeling of the TDD was done in a kit vial after injection of generator-eluted 188Re. This was easily performed compared to other published methods, because the labeling took place in aqueous solution. The labeled 188Re-TDD was readily dissolved in lipiodol by extraction in situ. After preparation, the stability of the 188Re-TDD-lipiodol was tested.

Our hypothesis was that the 188Re-TDD in lipiodol would behave exactly as lipiodol in vivo. Before proving the hypothesis, we tried to extract the 188Re-TDD from lipiodol with PBS and found that the majority of radioactivity remained in the lipiodol phase. The prepared lipiodol solution of 188Re-TDD was stable in atmospheric environment and in serum for at least 48 hours.

Following intravenous injection through the tail vein of mice, we found that the 188Re-TDD-lipiodol accumulates in the lung, which evidences that the agent works as an embolic agent like the lipiodol itself. When injected through the hepatic artery of hepatoma-bearing rats, the radioactivity accumulated primarily in the hepatoma, as expected. The radioactivity in the normal liver was only about 1/8 of that of the hepatoma at 5 min. Radioactivity uptakes in other tissues were very low when compared with the hepatoma and liver. The following hypotheses are generally accepted to explain the mechanism of lipiodol accumulation in liver cancer.

1. The blood flow of cancer tissue is higher than that of normal liver due to its hypervascularity. Consequently, the absolute amount of lipiodol flowing into the cancer is higher than the normal liver if injected through the hepatic artery.

2. The flow rate inside the capillaries of the cancer tissue
is lower than that of the normal liver due to their increased curvature, lower elasticity and decreased regulation. Thus, the congestion of lipiodol occurs inside the capillaries of the cancer.

3. Sinusoids tend to expand in liver cancer, and the lipiodol floods and blocks the expanded sinusoids.

4. The permeability of the capillaries in cancer tissue increases due to a secreted permeability-enhancing factor, and as a consequence lipiodol infiltrates into the interstitial tissue of cancer.

These hypotheses explain both the high lipiodol-uptake in cancer tissue and its rapid clearance from normal tissue. Actually, the lipiodol taken up by normal lungs in mice was rapidly cleared (Fig. 6B), and radioactivity in the normal liver decreased faster than in the cancer tissue, resulting in an increased tumor to liver ratio (Table 2). Moreover, radioactivity observed in the blood vessels of the liver at 1 hr disappeared at 24 hr (Fig. 7).

One important advantage of this method is that it can
be applied to other lipophilic beta ray-emitting radiopharmaceuticals that are soluble to lipiodol; for example, $^{131}$I-fatty acid derivatives, $^{90}$Y-oxine and $^{188}$Re-fatty acid derivatives.

5. Conclusion

The lipophilic chelate $^{188}$Re-TDD was easily prepared in aqueous solution. The lipiodol solution of $^{188}$Re-TDD was obtained by extracting with lipiodol from aqueous solution. It was stable and showed embolizing activity in normal mice. A high accumulation in the hepatoma was observed in hepatoma-bearing rats after injection through the hepatic artery. Thus lipiodol solution of $^{188}$Re-TDD is a potentially excellent therapeutic agent for the treatment of liver cancer by injection through the hepatic artery. Moreover, this method would also be applicable to label lipiodol with other lipophilic therapeutic radiopharmaceuticals.

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References


