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Radiosynthesis and Biodistribution of an ^{125}I-labeled Resveratrol Derivative

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An ^{125}I-labeled resveratrol derivative **1** was synthesized. It was purified by reverse phase HPLC. Radiochemical purity of the product was more than 98%, and the yield was 35% (decay-corrected). Its biodistribution in tumor-bearing mice was studied. The results showed that the highest radioactivity was located in intestine and stomach. The biodistribution profile suggests that compound **1** can be effectively used as a promising imaging probe for intestine and stomach.

Key Words : Radiosynthesis, Resveratrol, Iodoresveratrol, Biodistribution, Imaging agent

Introduction

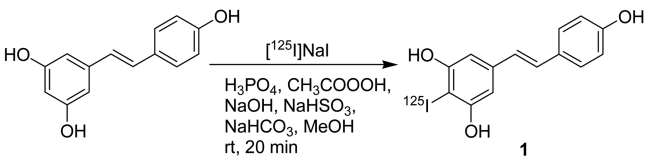
Resveratrol, 3,4',5-trihydroxy-*trans*-stilbene, is a naturally-occurring phytoalexin and polyphenol found in grapes and a variety of medicinal plants.[1-6] It is one of the best known "nutraceuticals". It possesses a plenty of biological properties including antifungal,[7] antibacterial,[8] anticancer,[9,10] antiviral,[11] estrogenic,[12,13] platelet-antiaggregating,[14] heart-protecting,[15] and atherosclerosis suppressing[16] activities.

The biodistributions of ^{14}C-labeled[17] and ^{18}F-labeled[18] resveratrol derivatives in mice tissues after oral administration were studied. The results showed that the highest accumulation of radioactivity was found in liver. In the present investigation, we report radiosynthesis and biodistribution profile of an ^{125}Iodine labeled resveratrol derivative in tumor-bearing mice.

Results and Discussion

Chemistry. The ^{125}Iodine-labeled resveratrol derivative **1** was synthesized as illustrated in Scheme 1. Resveratrol was iodinated in the presence of [^{125}I]sodium iodide, phosphoric acid, peracetic acid, sodium hydroxide, sodium bisulfite, and sodium bicarbonate. The optimum reaction time was 20 min.

5-(4-Hydroxystyryl)-2-iodobenzene-1,3-diol (iodoresveratrol) was synthesized by same method utilized for synthesis of **1**, but sodium iodide used was non-radiolabeled. 5-(4-Hydroxystyryl)-2-iodobenzene-1,3-diol was prepared as a reference for comparison of its R_f value on TLC and its retention time on HPLC column with the target ^{125}I-labeled resveratrol derivative **1**.



Scheme 1. Synthesis of ^{125}I-labeled resveratrol derivative **1**.

Purification by HPLC. A mixture of iodoresveratrol and resveratrol was analyzed by reverse phase HPLC (Fig. 1). In addition, the target radiolabeled product **1** was purified by reverse phase HPLC (Fig. 2). Radiochemical purity of the product was > 98% with decay-corrected yield of 35%. Its retention time (15.0-17.0 min) was identical with that of non-radiolabeled iodoresveratrol. From these data, we could confirm the identity of compound **1**. Moreover, compound **1**

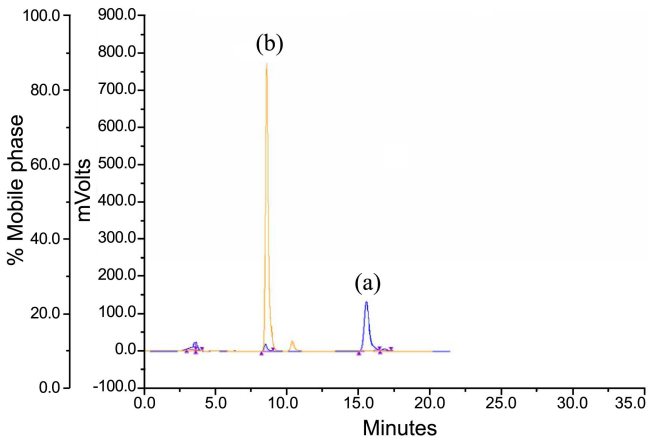


Figure 1. HPLC chromatogram of iodoresveratrol (a) and resveratrol (b) by γ-radioactivity detector (Waters, μBondapak RP-18, 10 μ, 3.9 × 300 mm; eluent 35:65 v/v solvents A/B, A: 0.1% trifluoroacetic acid in acetonitrile, B: 0.1% trifluoroacetic acid in water; flow rate: 1.0 mL/min, retention time of iodoresveratrol: 15.0-17.0 min).

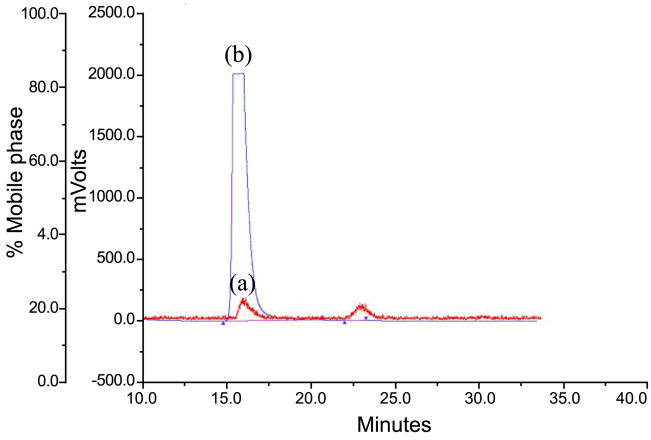


Figure 2. HPLC chromatogram of a mixture of ^{125}I-labeled iodoresveratrol derivative (a) and iodoresveratrol (b) by γ-radioactivity detector (Waters, μBondapak RP-18, 10 μ, 3.9 × 300 mm; eluent 35:65 v/v solvents A/B, A: 0.1% trifluoroacetic acid in acetonitrile, B: 0.1% trifluoroacetic acid in water; flow rate: 1.0 mL/min, retention time of the target compound: 15.0-17.0 min).

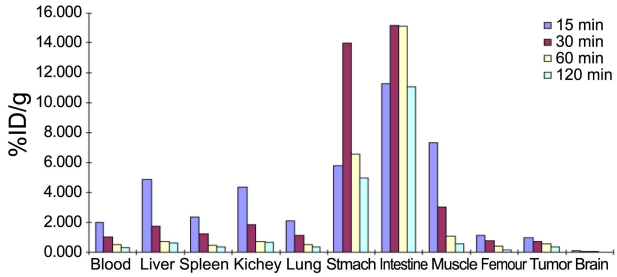


Figure 3. Tissue distribution of radioactivity after intraperitoneal injection of I-125 labeled resveratrol derivative **1** in mice. The radiolabeled resveratrol derivative (26 μCi) was intraperitoneally injected into nude mice bearing A549 lung cancer cells.

and iodoresveratrol had the same R_f value on TLC (R_f = 0.63 upon using ethyl acetate:hexane 1:1 v/v as an eluent).

Biology. Table 1 and Figure 3 summarize the results of the biodistribution of ^{125}I-labeled resveratrol derivative **1** to a range of tissues in nude mice bearing A549 lung cancer cells, which were used as a tumor model due to our interest in the uptake into tumors.

From the biodistribution profile of I-125 labeled resveratrol derivative **1** in mice (Fig. 3 and Table 1), we can observe the highest concentration of radioactivity in intestine with the maximum uptake [15.1536 ± 2.09 (%ID/g)] at 30 min. post-injection, which gradually decreased with time. Intestinal absorption of resveratrol in human intestinal Caco-2 cell model and in rat small intestine model *in vitro* has been demonstrated earlier.[19-22]

The second greatest uptake was in stomach. The uptakes in other organs, such as liver, spleen, kidney, lung, and bone were relatively low. As the brain uptake was minimal, we can conclude that compound **1** could not pass the blood-brain barrier (BBB). Tumor uptake was less than blood uptake. Due to minimal tumor uptake, compound **1** cannot be developed as cancer imaging agent.

Preclinical studies in mice, rats, and dogs suggest that resveratrol is rapidly glucuronidated and sulfated in liver and in intestinal epithelial cells.[19,23,24] This accounts for gradual decrease of radioactivity with time, and for minimal tumor uptake of the radiolabeled compound **1**. In addition, compound **1** can be readily metabolized and eliminated from the body in the forms of glucuronide and sulfate conjugates, and the radioactivity will not be retained in the body.

Conclusion

An ^{125}I-labeled resveratrol derivative was synthesized. It was purified by reverse phase HPLC. Radiochemical purity of the product was > 98% with decay-corrected yield of 35%. Its biodistribution profile was examined in tumor-bearing mice. The highest uptakes were found in intestine and stomach. But the uptakes were low in blood, liver, spleen, kidney, lung, muscle, bone, brain, and tumor tissues. Because of low tumor uptake of radioactivity, compound **1** cannot be used for tumor imaging. The high intestinal and gastric uptakes of the new radiolabeled resveratrol derivative **1** suggest that it can be developed as an imaging agent for intestine and stomach.

Experimental

General. All reagents and solvents were purchased from

Table 1. Biodistribution of [^{125}I]iodoresveratrol (**1**) in tumor-bearing mice (%ID/g)

	15 min	30 min	60 min	120 min
Blood	1.9612 ± 0.19	1.0190 ± 0.17	0.4970 ± 0.17	0.3315 ± 0.11
Liver	4.8287 ± 1.39	1.7523 ± 0.62	0.6999 ± 0.48	0.6133 ± 0.21
Spleen	2.3300 ± 0.59	1.2502 ± 0.42	0.4614 ± 0.37	0.3509 ± 0.15
Kidney	4.3471 ± 0.41	1.8530 ± 0.34	0.7133 ± 0.30	0.6414 ± 0.27
Lung	2.0561 ± 0.19	1.1122 ± 0.20	0.5210 ± 0.24	0.3584 ± 0.13
Stomach	5.7610 ± 3.59	13.9628 ± 5.78	6.5545 ± 3.27	4.9601 ± 3.31
Intestine	11.2509 ± 2.06	15.1536 ± 2.09	15.1170 ± 4.07	11.0462 ± 5.14
Muscle	7.3249 ± 2.14	3.0110 ± 0.77	1.0396 ± 0.62	0.5691 ± 0.14
Femour	1.1088 ± 0.25	0.7348 ± 0.41	0.4163 ± 0.20	0.1604 ± 0.05
Tumor	0.9472 ± 0.20	0.7019 ± 0.07	0.5445 ± 0.19	0.3785 ± 0.10
Brain	0.0898 ± 0.02	0.0470 ± 0.01	0.0267 ± 0.01	0.0197 ± 0.01

Aldrich Chemicals Co. and used without further purification. Radio-TLC was monitored on a Bioscan AC-3000 scanner (Washington D.C., USA) and HPLC was performed on a Waters system using a 515 pump, 2487 UV detector (254 nm), and Raytest GABI γ-detector using a semi-preparative C18 reverse phase column (Waters, ìBondapak RP-18, 10 μ, 3.9 × 300 mm). ^{1}H NMR spectrum of iodoresveratrol was obtained using a Bruker 300 MHz FT-NMR spectrometer. Mass spectrum (MS) of iodoresveratrol was taken in ESI mode on a Waters 3100 Mass Detector (Waters, Milford, MA, USA). All animal experiments were approved by the pertinent committees of our institutions, and performed in compliance with institutional guidelines for the conduct of animal experimentation.

Synthesis of 5-(4-Hydroxystyryl)-2-iodobenzene-1,3-diol (iodoresveratrol). To a solution of resveratrol (100 mg, 0.44 mmol) in methanol (20 mL), phosphoric acid aqueous solution (0.5 M, 6 mL) and peracetic acid (0.4 M, 6 mL) were added. Then a solution of sodium iodide (85 mg, 0.57 mmol) in aqueous sodium hydroxide solution (0.01 M, 2 mL) was added thereto. The reaction mixture was stirred at room temperature for 20 min. After that, sodium bisulfite (0.048 M aqueous solution, 6 mL) and sodium bicarbonate (0.06 M aqueous solution, 12 mL) were added. The reaction mixture was stirred at room temperature for 20 min. The mixture was concentrated under reduced pressure. Water (15 mL) was added to the residue, then extracted with ethyl acetate (3 × 10 mL). The combined organic layer extracts were washed with brine then dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate:hexane 1:6 v/v then switching to ethyl acetate:hexane 1:3 v/v) to yield the pure product (93 mg, 60%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.07 (brs, 2H), 9.58 (brs, 1H), 7.42 (d, 2H, J = 8.6 Hz), 6.94-6.74 (m, 4H), 6.51 (brs, 2H); ESI-MS: 354.93 $[M + 1]^+$.

Radiosynthesis of 5-(4-Hydroxystyryl)-2-[^{125}I]iodobenzene-1,3-diol ([^{125}I]iodoresveratrol 1). It was prepared by the same procedure as described for synthesis of iodoresveratrol, except for using [^{125}I]NaI instead of non-radiolabeled NaI. The crude product was purified by reverse phase HPLC; C-18 semi-preparative column (Waters, μBondapak RP-18, 10 μ, 3.9 × 300 mm; eluent 35:65 v/v solvents A/B, A: 0.1% trifluoroacetic acid in acetonitrile, B: 0.1% trifluoroacetic acid in water; flow rate: 1.0 mL/min) The product **1** was collected at 15.0-17.0 min (Fig. 2). The purity was > 98% with decay-corrected yield of 35%.

Biodistribution Study. A suspension of A549 lung cancer cells (10^6 cells) in 100 μL of phosphate buffer saline (PBS) was subcutaneously injected into the right thigh of cann,cg-foxn1 nu/crljorl nude mice (8 week age, male, weighing 15-20 g). After 21 days, the presence of tumor mass was confirmed. Tumor-bearing mice were injected intraperitoneally with I-125 labeled resveratrol derivative **1** (26 μCi). At 15 min, 30 min, 1 h, and 2 h, the tumor-bearing mice were sacrificed and each organ was excised and examined. The radioactivity of each organ was measured with a gamma counter and expressed as a percentage of the injected dose per gram of tissue (%ID/g).

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