Tumor Visualization with a Radioiodinated Phospholipid Ether

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The known ability of phospholipid ethers to accumulate in certain tumors prompted the synthesis and evaluation of a radioiodinated phospholipid ether analog as a potential tumor imaging agent. Tissue distribution studies with [^{125}I] -*rac*-1-0-[12-(*m*-iodophenyl)dodecyl-2-0-methylglycero-3-phosphocholine in rats bearing the Walker 256 carcinosarcoma showed the tumor to contain the highest concentration of radioactivity at 24 hr (15% of the dose) and a tumor-to-blood ratio of 13. Scintigraphic images taken at 24 hr compared favorably with those obtained with (^{67}Ga)-citrate. In contrast with the latter, however, the phospholipid ether showed little propensity to accumulate in an inflammatory lesion in the rat. Tumor visualization was also accomplished in a rabbit bearing the Vx2 adenocarcinoma. We conclude that phospholipid ethers may represent a new class of carrier molecules for the transport of radionuclides to tumors.

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Various strategies have been employed to achieve selective accumulation of a radiopharmaceutical within tumor cells. Many of these approaches have focused on either real or perceived differences between normal and tumor cell biochemistry. A difference that attracted our attention was the reported higher levels of alkyl and alk-1-enyl phospholipids in neoplastic cells as opposed to normal cells (*1,2*). An apparent explanation for this finding was the difference in the level of alkyl glycero-monooxygenase (alkyl cleavage enzyme, AGMO, E.C. 1.14.16.5), wherein low levels of this enzyme in tumor cells caused the accumulation of alkyl lipids (*3,4*).

This difference in phospholipid ether metabolism in tumor cells led Munder and coworkers (for review see ref. *5*) to synthesize a series of phospholipid ether analogs which, upon subsequent testing, were shown to have selective cytoxicity toward a variety of human murine tumors (*6*). The most widely studied of these analogs has been *rac*-1-0-octadecyl-2-0-methyglycero-3-phosphocholine (ET-18-OCH_3, I in Fig. 1), which is now in clinical trials as an anticancer agent (*6*).

Although the mechanism of action for ET-18-OCH_3 is not presently known, several groups have found it to accumulate in tumor cells in vivo (*7*) and in vitro (*8, 9*). Of particular interest was the report by Arnold and coworkers (*7*), which analyzed the biodistribution of [3H]-ET-18-OCH_3 in mice bearing 3-methyl cholanthrene-induced fibrosarcoma. They found the tumor and liver to contain the highest levels of radioactivity, which amounted to 40% and 7% of the total radioactivity, respectively.

These studies encouraged us to synthesize a radioiodinated analog of ET-18-OCH_3 (III in Fig. 1) as a potential tumor-imaging agent (*10–11*). We now wish to report our preliminary results with this agent in rats bearing the Walker 256 carcinosarcoma and rabbits bearing the Vx2 adenocarcinoma.

MATERIALS AND METHODS

Preparation of [^{125}I]-rac-1-0-[12-(*m*-iodophenyl)dodecyl]-2-0-methylglycero-3-phosphocholine (NM-294, III)

The radioiodinated phospholipid ether (III) was synthesized by slight modification of the method described previously (*11*). The unlabeled lipid (1.1 mg) was placed in a 1-ml serum vial, which was then sealed with a teflon-lined rubber septum and aluminum cap. Aqueous Na^{125}I (4.5 μl, 1.7 mCi, dilute NaOH solution, pH 6–11, Amersham Co., Arlington Heights, IL) and freshly distilled tetrahydrofuran (THF) (15 μl) were added to the vial and a gentle stream of nitrogen applied for 1 hr to remove the solvents. Pivalic acid (15 μl, dried by azeotropic distillation with toluene under nitrogen) was added via a preheated syringe, and the mixture was heated in an oil bath at 160°C for 45 min. The reaction vial was allowed to cool to room temperature and $CHCl_3$:MeOH (1:1) (30 μl) was added to dissolve the residue. A sample (1 μl) was added to a TLC plate (Merck silica gel-60) and developed with $CHCl_3$:MeOH (1:1) to estimate radiochemical yield. To the remaining solution was added H_2O (50 μl), and the mixture was extracted twice with $CHCl_3$ (30 μl, 50 μl). The combined extracts were applied to a silica gel-60 column (1 × 5 cm, 230–400 mesh). The column was initially eluted with $CHCl_3$:MeOH (1:1) to remove the Na^{125}I and then with

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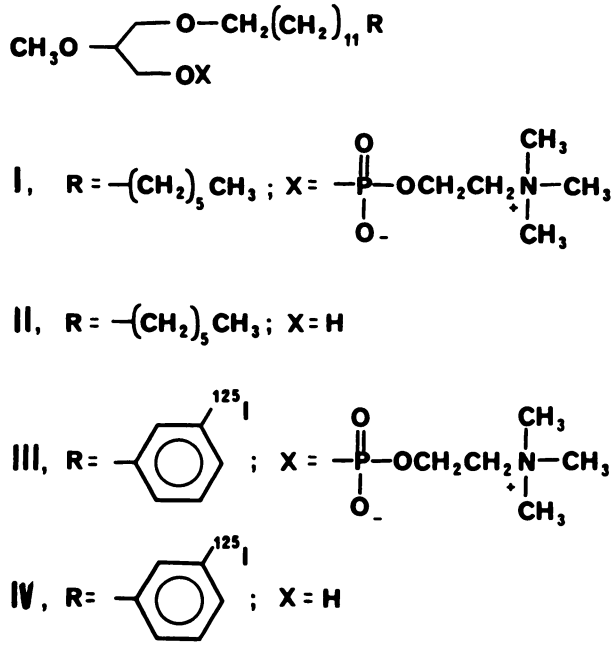

FIGURE 1
Structures of lipid ethers and their radioiodinated analogs.

$CHCl_3$:MeOH:H_2O (65:25:4) to obtain the product. The radiochemical yield for III based on TLC was 81%.

Preparation of [^{125}I]-rac-1-0-[12-(m-iodophenyl) dodecyl]-2-0-methylglycerol (NM-289, IV)

The diglyceride ether (IV) was synthesized as previously described (*11*) and 2 mg were placed in a 1-ml serum vial along with 10 mg of pivalic acid. The vial was flushed with nitrogen and sealed with a teflon-lined rubber septum and aluminum cap. Aqueous Na^{125}I (1.4 μl, 2 mCi) was added, and the mixture was heated in an oil bath at 150° for 1 hr. The reaction vial was allowed to cool to room temperature before THF (30 μl) was added. The solution was applied to a silica gel-60 column (1 × 10 cm) and eluted with hexane:ethyl acetate (1:1) to obtain the product. The radiochemical yield for IV based on TLC was 61%.

Animal Models

Female Sprague Dawley rats (200–275 g) were obtained from Charles River, Portage, MI. The Walker 256 carcinosarcoma was introduced to these animals by hindlimb injection of tumor cells. Continued passage of the tumor was accomplished by aseptic removal and excision of ~1–2 g of tumor tissue, which provided greater than 10^6 viable cells/ml once minced and suspended in physiologic saline. The cells (1 ml) were then inoculated in the left hind leg muscle of female rats. These animals were used for tissue distribution and scintigraphic studies at 7–10 days following implantation, at a time when the tumor mass averaged 10.7 g. In addition, an inflammatory lesion was developed in the rat by injecting 3 ml of a 2% carrageenan aqueous solution into an air pocket in the left hindlimb (*12*). The animal was used five days later.

Female New Zealand White rabbits were implanted intramuscularly in the right thigh with Vx2 adenocarcinoma maintained by serial animal passage (*13*). Scintigraphic studies were performed three weeks after implantation.

Tissue Distribution Studies

The radiolabeled compounds, NM-289 and NM-294, were dissolved in $CHCl_3$:MeOH:H_2O, 65:25:3, and Tween 20 (0.1 ml/mg compound) was added. The solvent was evaporated with a stream of nitrogen. Physiologic saline was added, and final traces of solvent were removed by passing nitrogen over the solution until it became clear and the final concentration of Tween was 2%–3%. The solubilized, radiolabeled compounds (5.0–16.4 μCi) were administered intravenously via the tail vein to rats bearing the Walker 256 tumor. At various times following injection, rats were killed by exsanguination while under ether anesthesia. A total of 12 tumored rats were employed, one each for the 0.5 and 3-hr times, three each for the 6- and 24-hr times, and four for the 48-hr timepoint. Selected tissues were removed, trimmed, blotted of excess blood, and weighed. These organs were then minced with scissors, and the samples were weighed in gelatin capsules and counted in a Searle 1185 well scintillation counter (85% counting efficiency). The results are expressed as the percent of administered dose per whole organ.

Gamma Camera Scintigraphy

Animal imaging was performed using a low-energy mobile camera (Siemens Medical Systems, Hoffman Estates, IL) with a high sensitivity, low-energy collimator for ^{125}I and a low-energy all-purpose collimator for ^{99m}Tc. For ^{67}Ga, a wide-field camera (Ohio Imaging, Bedford, OH) fitted with a medium-energy collimator was used. Rats bearing either a Walker 256 tumor or a carrageenan-induced granuloma were injected with either 75 μCi NM-294 or 100 μCi [^{67}Ga]citrate intravenously via the tail vein. At 24 hr following administration, each rat was sedated with 87 mg/kg ketamine and 13 mg/kg xylazine i.m. The animals were placed supine on the appropriate camera, and posterior images of 100,000 counts were obtained. Rabbits bearing Vx2 tumor were administered 200 μCi of either NM-294 or ^{99m}Tc-human serum albumin (HSA) intravenously via the ear vein. At the appropriate time post-injection, the rabbits were sedated with 59 mg/kg ketamine and 9 mg/kg xylazine i.m., placed on a table, and posterior images containing 250,000 counts were obtained.

RESULTS

Tissue distribution studies with NM-294 in rats bearing the Walker 256 carcinosarcoma clearly demonstrated its ability to accumulate in the tumor (Fig. 2). By 24 hr, the amount of radioactivity in the tumor approximated 15% of the administered dose. At earlier times, the majority of radioactivity was present in the liver (~30% of the dose, Fig. 3), but this was found to decline to <10% of the administered dose by 24 hr (Fig. 3). Other tissues (Table 1) also showed an inability to retain radioactivity, and the thyroid levels were found to be particularly low for a radioiodinated compound given without prior administration of Lugol's solution. The diglyceride ether NM-289, an expected metabolite of NM-294, showed little capacity to accumulate in the tumor (Fig. 2).

Three animal models were employed for the scintigraphic studies. Administration of NM-294 to a rat

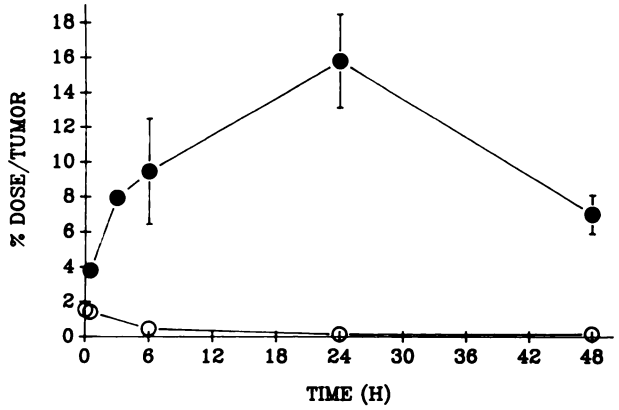

FIGURE 2
Uptake of radioactivity by Walker 256 carcinosarcoma in the rat at various times following i.v. administration of NM-294 (●) and NM-289 (○).

TABLE 1
Tissue Distribution of Radioactivity in Rats Bearing Walker 256 Adenosarcoma Following Intravenous Administration of NM-294

	6 hr (n = 3)	24 hr (n = 3)	48 hr (n = 4)
Adrenal	0.03 ± 0.00*	0.03 ± 0.00	0.02 ± 0.00
Blood	2.69 ± 0.07	1.14 ± 0.28	0.57 ± 0.13
Fat	1.20 ± 0.19	1.39 ± 0.54	1.42 ± 0.29
Heart	0.11 ± 0.00	0.06 ± 0.01	0.04 ± 0.00
Kidney	2.34 ± 0.16	0.84 ± 0.20	0.54 ± 0.14
Liver	31.51 ± 0.85	7.12 ± 0.93	2.71 ± 0.17
Lung	1.14 ± 0.07	0.40 ± 0.02	0.17 ± 0.01
Muscle	4.85 ± 0.30	3.03 ± 0.55	2.05 ± 0.13
Ovary	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Spleen	1.02 ± 0.05	0.57 ± 0.13	0.18 ± 0.03
Thyroid	0.07 ± 0.00	0.67 ± 0.11	0.61 ± 0.11
Tumor	9.46 ± 3.03	15.86 ± 2.67	7.05 ± 1.11

* Results expressed as % administered dose per organ ± s.e.m.

bearing a Walker 256 carcinosarcoma produced excellent images of the tumor at 24 hr (Fig. 4A). In order to assess the ability of NM-294 to distinguish between neoplastic lesions and inflammatory lesions, a comparative study was performed in rats bearing a carrageenan-induced granuloma. This granuloma was induced in the thigh of the rat in approximately the same location as tumor in the tumor-bearing rats. Histologic examination of the granuloma primarily revealed the presence of neutrophils, but some basophils and macrophages also were present. The identical dose of NM-294 used in the tumor-bearing animals failed to image the granuloma (Fig. 4C). Moreover, as a result of the decreased radioactivity in the region of the granuloma, the acquisition time for scan 4C was almost double that needed for 4A and resulted in an emphasis of the radioactivity present in the liver and other regions of the abdomen. Gallium-67 citrate, on the other hand, imaged both the tumor (Fig. 4B) and granuloma (Fig. 4D).

In a similar manner, NM-294 succeeded in localizing and visualizing a Vx2 adenocarcinoma in a rabbit (Fig. 5A). Scans taken at 5 min (Fig. 5B) and 24 hr (not shown) after administration of ^{99m}Tc-HSA to a similarly tumored rabbit showed that the uptake in the tumor was not a result of abnormal blood flow or tumor hypervascularity (Fig. 5B).

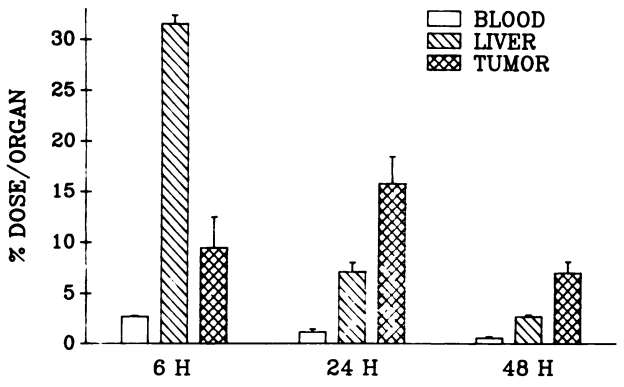

FIGURE 3
Distribution of radioactivity in blood, liver, and tumor in rats bearing Walker 256 adenocarcinoma at various times following i.v. administration of NM-294.

DISCUSSION

The ability of phospholipid ethers to accumulate in tumors following administration was confirmed in this study. In rats bearing the Walker 256 carcinosarcoma, the highest concentration of radioactivity (15% of dose) was found to be present in the tumor at 24 hr after the administration of NM-294. At this time, the tumor:blood ratio was ~13. These results are in accord with the earlier studies by Arnold and coworkers (*7*) with tritiated ET-18-OCH_3. In mice bearing a 3-methylcholanthrene-induced fibrosarcoma they found the tumor to contain the highest levels of radioactivity, although their protocol was quite different from the one followed in this study.

The Walker 256 tumor in rats is known to contain low levels of AGMO (*3*), the phospholipid ether cleavage enzyme, but it is uncertain at this time whether this property can account for the selective accumulation of NM-294 and similar phospholipid ethers in tumors. Recent studies with ET-18-OCH_3 have shown that it is not a substrate for AGMO (*14–16*). Thus, the accumulation of ET-18-OCH_3 in neoplastic tissue cannot be explained by the differences between the levels of AGMO in normal and malignant cells. In light of this finding, other investigators have examined alternative mechanisms of ET-18-OCH_3 metabolism. These studies have shown that 1-0-octadecyl-2-0-methylglycerol (II in Fig. 1) is the major metabolite arising from ET-18-OCH_3 in a variety of cells presumably by the action of

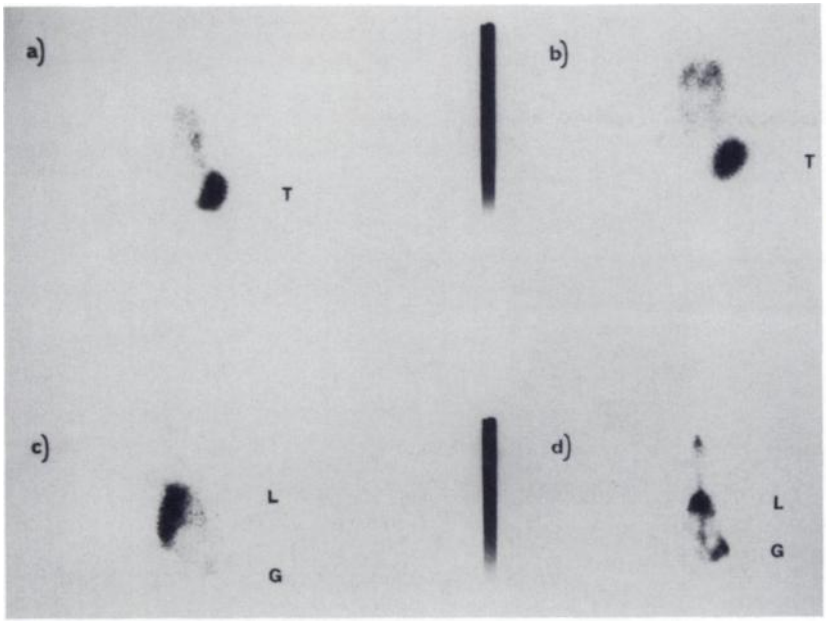

FIGURE 4
Scintiscans at 24 h following i.v. administration of (A) NM-294 in Walker 256 carcinosarcoma-bearing rat; (B) [^{67}Ga] citrate in Walker 256 carcinosarcoma-bearing rat; (C) NM-294 in granuloma-bearing rat; and (D) [^{67}Ga]citrate in granuloma-bearing rat. (G = granuloma, L = liver, and T = tumor).

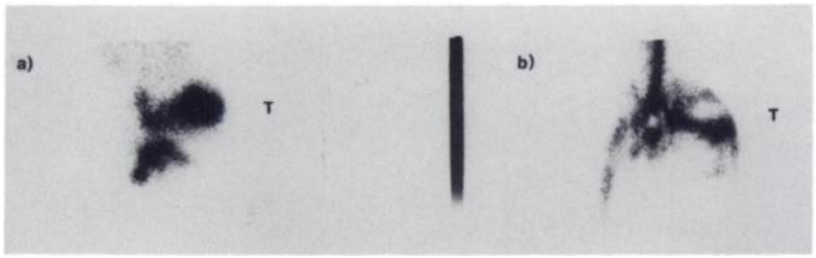

FIGURE 5
Scintiscans of rabbit-bearing Vx2 adenocarcinoma (A) at 24 hr following administration of NM-294 and (B) at 5 min following administration of ^{99m}Tc-human serum albumin.

phospholipase C (*17–18*). It has even been proposed that this metabolite may contribute to the anti-tumor activity of the parent compound (*19*).

These reports led us to examine the diglyceride ether NM-289 (IV in Fig. 1), the product expected to arise from the action of phospholipase C on NM-294. Unlike NM-294, compound IV showed little capacity to accumulate in the tumor (Fig. 2). Thus, if the diglyceride ethers have antitumor activity, our preliminary results indicate that it would be important to administer them as a phospholipid in order to achieve sufficient intracellular concentrations.

Gallium-67 citrate is widely used today in nuclear medicine for tumor imaging. This radiopharmaceutical has several limitations, however, which include: (1) multiple photons ranging from 91 keV to 294 keV; and (2) a lack of specificity to distinguish neoplasms from inflammatory lesions (*20*). Our results with NM-294 show that this or a similar agent may offer hope towards overcoming both of these limitations. First of all, NM-294 is well-suited for labeling with iodine-123, a single photon emitter. Moreover, these preliminary studies show its potential for distinguishing tumors and inflammatory lesions. Such findings justify further studies with NM-294 and related analogs as potential tumor-imaging agents.

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