

^{99m}Tc -HYNIC-Folate: A Novel Receptor-Based Targeted Radiopharmaceutical for Tumor Imaging

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The folate receptor is overexpressed in a wide variety of human tumors. Conjugates of folate have been shown to be selectively taken up by tumor cells via the folate receptor. In this study, a novel radiopharmaceutical, ^{99m}Tc -6-hydrazinonicotinamido-hydrazido (HYNIC)-folate, was synthesized and evaluated for its efficacy as a targeted agent for the imaging of tumors that overexpress the folate receptor. **Methods:** HYNIC-folate was synthesized and radiolabeled with ^{99m}Tc using tricine and trisodium triphenylphosphine-3,3',3''-trisulfonate as coligands. The receptor binding properties of ^{99m}Tc -HYNIC-folate were studied in cultured tumor cells that overexpress the folate receptor. The tumor-localizing properties of ^{99m}Tc -HYNIC-folate were then evaluated in C57BL/6 mice bearing subcutaneously implanted folate receptor-positive syngeneic tumors. Tissue distribution was determined at two different time points, and gamma camera images were collected on two animals. **Results:** The folate receptor-mediated uptake of ^{99m}Tc -HYNIC-folate by cultured tumor cells was approximately 300 times higher than the nonspecific binding determined in the presence of 1 mmol/L free folic acid. Excellent tumor selectivity was also shown in the animal model; tumor-to-blood ratios reached 55 ± 19 and 81 ± 6 at 4 and 24 h after injection, respectively. Tumor uptake of the radiotracer was blocked by the co-injection of 100 μg free folate. Tumors were clearly identifiable on the gamma camera images, with the kidneys and the bladder as the only normal organs showing high levels of the radiotracer. **Conclusion:** ^{99m}Tc -HYNIC-folate is a promising, novel receptor-specific radiopharmaceutical with potential applications in the imaging of human tumors.

Key Words: ^{99m}Tc ; folate receptor; tumor imaging

J Nucl Med 1999; 40:1563–1569

Receptor-targeted radiopharmaceuticals show promise to greatly improve the specificity and sensitivity of nuclear imaging procedures. The folate receptor is a glycosylphosphatidylinositol-anchored membrane protein overexpressed in approximately 100% of serous ovarian adenocarcinomas and a wide variety of other human cancers (1–13). Meanwhile, the normal tissue distribution of this receptor is highly restricted. Two folate receptor isoforms have been identified in humans, α and β . The type α receptor is frequently

overexpressed in epithelial-lined tumors and the type β in nonepithelial-derived tumors (4). Both isoforms show a high affinity for folic acid.

There has been much interest in exploiting the folate receptor for tumor-specific targeted delivery of imaging or therapeutic agents. Earlier efforts in targeting the folate receptor were focused on the use of conjugates of antibodies against the folate receptor. For example, radiolabeled MOv18, a monoclonal antibody against the folate receptor α , has been evaluated for radioimaging and radiotherapy treatment of ovarian carcinomas in clinical studies (3,14–17). An alternative strategy has been developed for folate receptor targeting in which the ligand, folic acid, is covalently linked to the molecule to be delivered (18–32). Folic acid (molecular weight 441.4) is a high-affinity ligand for the folate receptor ($K_d \sim 10^{-10}$ mol/L). The receptor binding properties of folic acid are retained when it is derivatized by its γ -carboxyl. Folate conjugates are taken into cultured tumor cell by binding to the folate receptor on the cellular surface followed by receptor-mediated endocytosis. This targeting strategy has been exploited in the receptor-mediated delivery of proteins, liposomes, gene transfer vectors, chemotherapeutic agents and radioimaging agents into tumor cells (18–32).

Two radiolabeled folate-chelator conjugates have been synthesized and evaluated for tumor imaging: ^{67}Ga -deferoxamine-folate and ^{111}In -diethylenetriamine pentaacetic acid (DTPA)-folate (26–29,31,32). Similar to other low-molecular-weight radiopharmaceuticals, such as radiolabeled peptide analogs of somatostatin (e.g., ^{111}In -DTPA-octreotide) (33,34), these radiolabeled folate conjugates showed high degrees of tumor specificity and rapid systemic clearance and are potentially nonimmunogenic (26–29,31,32). In a folate receptor-positive xenograft tumor model, a tumor-to-blood ratio of 409:1 was achieved 4 h after intravenous injection of ^{67}Ga -deferoxamine-folate (26). Although results of these earlier studies are promising, we believe that ^{99m}Tc , rather than ^{67}Ga or ^{111}In , represents the ideal radionuclide for radioimaging applications. The 140-keV γ -radiation of ^{99m}Tc is optimal for standard imaging equipment. Although the 6-h half-life of this radionuclide may prove too short for labeling monoclonal antibodies, which have a relatively slow rate of systemic clearance, it takes full advantage of the

Received Sep. 16, 1998; revision accepted Mar. 8, 1999.

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fast clearing kinetics of low-molecular-weight folate conjugates and the shortened waiting period between radiotracer administration and image collection.

In this study, a ^{99m}Tc -labeled folate conjugate, ^{99m}Tc -6-hydrazinonicotinamido-hydrazido (HYNIC)-folate, was synthesized and evaluated for receptor binding in tumor cell culture and for its tumor-localizing properties in a syngeneic mouse tumor model.

MATERIALS AND METHODS

Materials

Folic acid, 6-chloronicotinic acid, trisodium triphenylphosphine-3,3',3''-trisulfonate (TPPTS), tricine, dicyclohexylcarbodiimide, N-hydroxysuccinimide and hydrazine hydrate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Bicinchoninic acid (BCA) protein assay kit was obtained from Pierce Chemical (Rockford, IL). ^{99m}Tc pertechnetate was produced by an on-site generator.

Cell Culture

KB cells, a human oral carcinoma cell line that overexpresses the folate receptor, were obtained from Dr. Philip S. Low at Purdue University (West Lafayette, IN). 24JK-FBP cells, a methylcholanthrene-induced mouse sarcoma cell line transfected with the human folate receptor gene, were obtained from Dr. Patrick Hwu at the National Cancer Institute (Bethesda, MD). Both cell lines were cultured continuously as a monolayer at 37°C in a humidified atmosphere containing 5% CO_2 in folate-free RPMI1640 medium supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 $\mu\text{g}/\text{mL}$ streptomycin. The final folate concentration (with the fetal bovine serum as the only source of folate) falls in the range of the physiological concentration in human serum. Because of their high folate content (2 $\mu\text{mol}/\text{L}$), which leads to the downregulation and presaturation of the cellular folate receptor, regular culture media were not used.

Synthesis of Folate- γ -Hydrazide

First, N-hydroxysuccinimide (NHS) ester of folic acid was synthesized (Fig. 1). Five grams of folic acid were dissolved in 100

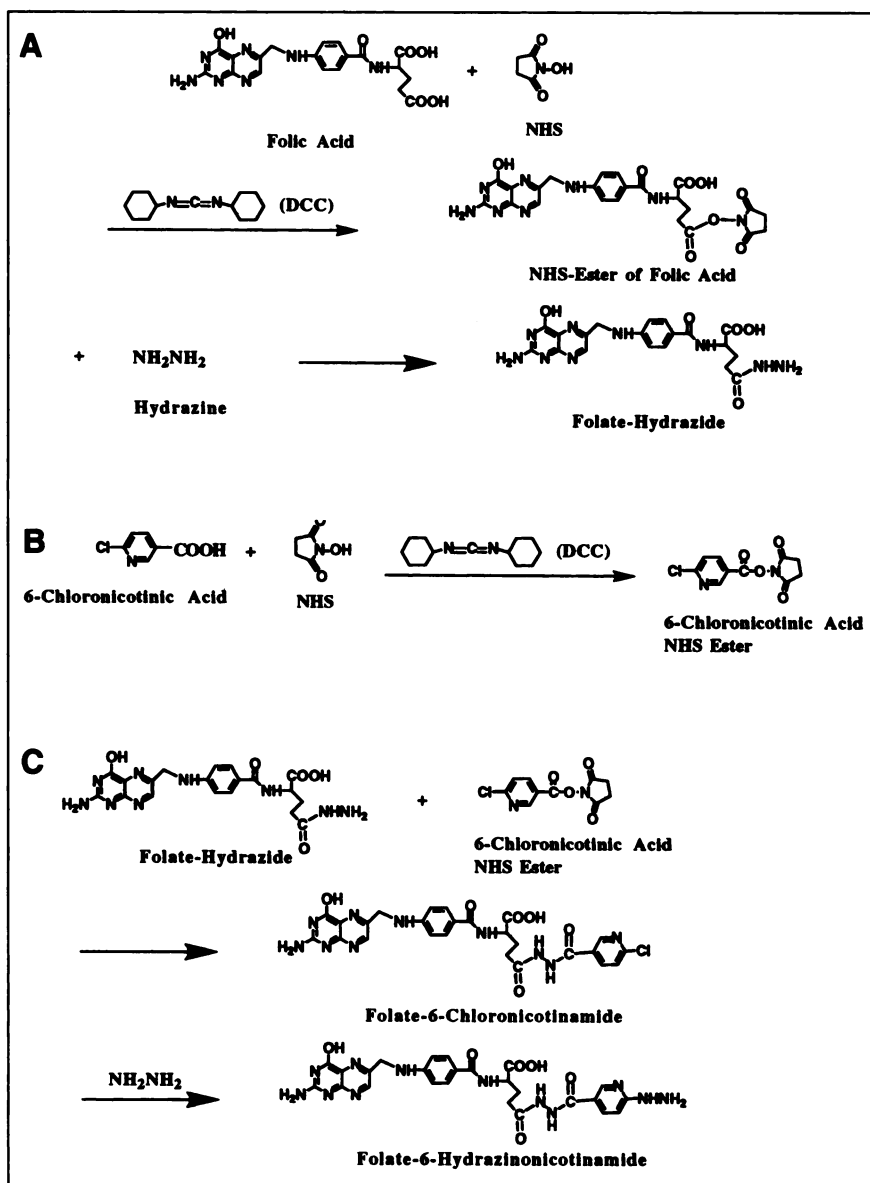


FIGURE 1. (A) Synthesis of folate-hydrazide, (B) 6-chloronicotinic acid NHS-ester and (C) HYNIC-folate.

mL dimethylsulfoxide (DMSO). A 1.1-molar excess of NHS and dicyclohexylcarbodiimide (DCC) were then added. The reaction was allowed to proceed for 4 h at room temperature under stirring and shielded from light. The by-product dicyclohexylurea was removed by filtration. The DMSO solution of the NHS-folate was stored at -20°C until use. Then, to synthesize folate-hydrazide (Fig. 1), 7 mL of the above NHS-folate solution were slowly added to 200 μL hydrazine hydrate under stirring at room temperature. The product folate-hydrazide was converted into a hydrochloride salt with the addition of 2 mL 0.5 N HCl and then precipitated with four volumes of acetonitrile/diethylether 1:1. The precipitate was pelleted by centrifugation, redissolved in a small volume of water and then reprecipitated with 10 volumes of ethanol. The pellet was then washed sequentially with ethanol and diethylether and then dried into a yellow powder under vacuum. The product mainly consisted of a mixture of α - and γ -carboxyl linked isomers of folate-hydrazide, which were further fractionated by anion-exchange chromatography, based on the different pK_a s of the underivatized carboxyl group on the folate conjugates ($\text{pK}_a \sim 2.5$ for a free α -carboxyl and ~ 4.5 for a free γ -carboxyl). Solution of the product mixture was loaded on to a 25 cm \times 10 mm diethylamine ethyl (DEAE)-trisacryl column and eluted with an ammonium bicarbonate gradient of 100–500 mmol/L (Fig. 2). Peaks corresponding to the two isomers of folate-hydrazide and folic acid were identified by their characteristic absorption spectrums and presence of hydrazide group assayed with ninhydrin. Fractions containing the folate- γ -hydrazide were pooled, lyophilized and stored at -20°C . The overall yield of the reaction was approximately 60%. Product purity as determined by ultraviolet absorbance at 360 nm was 94%.

Synthesis of HYNIC-Folate

First, an NHS-ester of 6-chloronicotinic acid was synthesized: 200 mg 6-chloronicotinic acid were dissolved in 2 mL DMSO, and 146 mg NHS and 261 mg DCC were then added. The reaction was allowed to proceed for 4 h at room temperature under stirring and shielded from light. The by-product dicyclohexylurea was pelleted by centrifugation. The pellet was rinsed with 2 mL DMSO, which

was combined with the supernatant to fully recover the product. Second, 1 mL of this DMSO solution was added to 50 mg folate- γ -hydrazide. The reaction mixture was incubated for 4 h at room temperature to yield folate-6-chloronicotinamide. Finally, 200 mg hydrazine hydrate were added to the reaction mixture. After overnight incubation at room temperature, the product HYNIC-folate was converted to a hydrochloride salt with the addition of 100 μL 0.5 N HCl and then precipitated with four volumes of acetonitrile/diethylether 1:1 and pelleted by centrifugation. The pellet was then washed three times by dissolution in a small volume of water followed by precipitation with four volumes of ethanol. Finally, the pellet was washed with ether and dried into a powder under vacuum and stored at -20°C . The molecular mass of HYNIC-folate was confirmed to be 591.14 by fast-atom bombardment mass spectroscopy (FAB-MS). The absorption spectrum of HYNIC-folate was analyzed on an ultraviolet-Vis spectrophotometer. Concentration of HYNIC-folate was determined using a molar extinction coefficient of 6500. The yield for HYNIC-folate synthesis was 73%.

Radiochemical Synthesis of $^{99\text{m}}\text{Tc}$ -HYNIC-Folate

A ternary ligand system using tricine and TPPTS as coligands was developed for the radiolabeling of HYNIC-folate based on a method reported previously for peptide labeling (35). The following solutions were sequentially added to a 3-mL glass tube: 0.5 mL tricine solution (80 mg/mL in H_2O , pH 5), 17 μL HYNIC-folate solution (10 μg in H_2O), 0.2 mL TPPTS coligand solution (5 mg/mL in H_2O), 74 MBq (2 mCi) $^{99\text{m}}\text{Tc}$ -sodium pertechnetate (in 0.5 mL saline) and 25 μL $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ solution (1 mg/mL in 0.1 N HCl). The pH of the final mixture was approximately 4.5, and HYNIC-folate concentration was 13 $\mu\text{mol/L}$. The glass tube was heated to 80°C for 30 min, and the radiochemical product was then analyzed by instant thin-layer chromatography (ITLC) for purity. Briefly, a 5- μL sample was spotted at the origin of two ITLC-SG strips (2 \times 15 cm). After developing in one of two different solvent systems (0.9% sodium chloride solution or acetone), the strips were scanned on the multichannel analyzer radiochromatogram scanner system and calculations were made to determine the percentages of

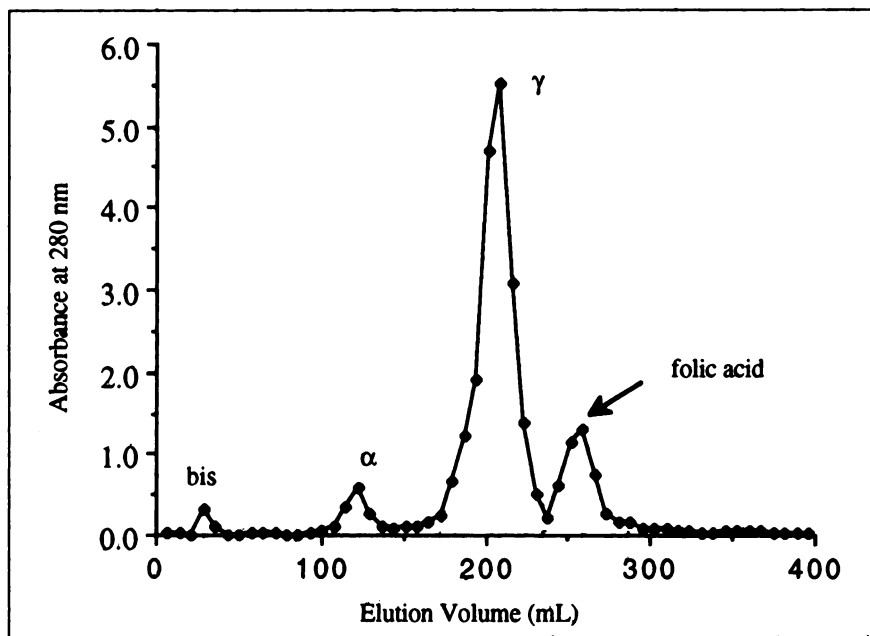


FIGURE 2. Ion-exchange chromatogram shows separation of folate-hydrazide isomers.

radioactivity at the origin and solvent front where peaks were located. The radiochemical purity was found to be greater than 95% using this analytical procedure. ^{99m}Tc -HYNIC-folate was then used in receptor binding or biodistribution studies.

Uptake of ^{99m}Tc -HYNIC-Folate by Cultured Cells

The folate receptor binding properties of ^{99m}Tc -HYNIC-folate were evaluated in two receptor-positive cell lines: KB, a human oral cancer cell line, and 24JK-FBP cells, a methylcholanthrene-induced mouse sarcoma cell line transfected with the human folate receptor- α gene. Eighty percent confluent monolayers of tumor cells in 24-well plates were incubated with various concentrations of ^{99m}Tc -HYNIC-folate diluted in 1 mL culture medium. After 20 min incubation at room temperature, the incubation medium was removed and the cells were gently washed three times with phosphate-buffered saline (136.9 mmol/L NaCl, 2.68 mmol/L KCl, 8.1 mmol/L Na_2HPO_4 , 1.47 mmol/L KH_2PO_4 , pH 7.4). The cells were then lysed in 0.1% Triton X-100 and the lysate counted for cell-associated radioactivity using a gamma counter. Cellular protein was determined using BCA protein assay reagent. The receptor specificity of ^{99m}Tc -HYNIC-folate was determined by addition of 1 mmol/L free folic acid to the incubation medium, which blocks binding by the folate receptor.

Tumor Imaging and Biodistribution Studies

Ten 7- to 8-wk-old female C57BL/6 mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were placed on folate-free rodent diet (Dyets, Inc., Bethlehem, PA) on arrival (day 0). The animals were inoculated with 24JK-FBP cells on day 16. Approximately 2×10^6 trypsinized cells were injected subcutaneously using a 25-gauge needle at each of two sites on each animal in a volume of 100 μL : the first on the right shoulder and second on the left hip. Tumor imaging and biodistribution studies were performed on day 26, at which time all mice developed tumors in both locations. ^{99m}Tc -HYNIC-folate was injected through the tail vein in a volume of 100 μL (containing 1.3 nmol HYNIC-folate conjugate). Animals were killed and tissues harvested at 4 h (7 mice) and 24 h (3 mice) postinjection. Three mice of the 4 h time point were coinjected with 100 μg free folate to block receptor-mediated uptake of the radiotracer, and the rest of the animals were injected with only the radiotracer. Gamma camera images of two of the animals of the 4 h time point (injected with just the radiotracer) were taken immediately before kill. Harvested tissue samples were weighed and counted in an automatic well gamma counter. The biodistribution of the radiotracer in each sample was calculated as a percentage of the injected dose per gram of tissue weight (%ID/g) using counts from weighed and appropriately diluted samples of the original injectate for reference. Tumor-to-background tissue ratios were then calculated from the corresponding %ID/g values.

RESULTS

Synthesis of HYNIC-Folate

The synthetic scheme for HYNIC-folate is outlined in Figure 1. Folate-hydrazide was first synthesized by reacting an N-hydroxysuccinimide ester of folic acid with hydrazine hydrate. Because folic acid has two carboxyl groups with similar reactivity and because a free α -carboxyl is needed for binding to the folate receptor, it is necessary to separate the two regioisomers of the folate conjugate. This was accomplished by anion-exchange chromatography on a

DEAE-trisacryl column and a gradient of ammonium bicarbonate. Separation of the two isomers was based on the different pK_a s of the α - and γ -carboxyl of folic acid. The final product HYNIC-folate was identified by FAB-MS and had a molecular mass of 591.14.

Radiochemical Synthesis of ^{99m}Tc -HYNIC-Folate

A ternary ligand system using tricine and TPPTS was used in the radiolabeling of HYNIC-folate. Previous HYNIC-derivatized peptide ligand radiolabeling studies showed that such a ligand system produces technetium complexes in high yield and with high specific activity (>740 GBq/mmol) (35). TPPTS was chosen as a coligand because of its three negatively charged sulfonates, which confer the radiopharmaceutical a high degree of hydrophilicity. This is important because highly charged radiopharmaceuticals tend to exhibit fast clearance kinetics dominated by rapid excretion through the kidneys, which could be beneficial for applications in scintigraphic imaging. With our labeling method, a radiochemical purity $>95\%$ was readily achieved based on ITLC analysis.

Uptake of ^{99m}Tc -HYNIC-Folate by Cultured Cells

Two folate-receptor-positive cell lines were used in the in vitro characterization of this radiopharmaceutical. As shown in Figure 3, cellular uptake of ^{99m}Tc -HYNIC-folate was saturated at approximately 10 and 3.4 pmol/mg cellular protein for KB and 24JK-FBP cells, respectively. Cellular uptake was reduced by more than 100-fold by 1 mmol/L free folic acid, suggesting the uptake was exclusively through the folate receptor and that nonspecific cellular binding was minimal. These results are consistent with previous data obtained in similar assays performed with ^{67}Ga -deferoxamine-folate and ^{111}In -DTPA-folate, in which a high degree of receptor specificity was also observed (29,31,32).

Tumor Imaging and Biodistribution Studies

Ten 7- to 8-wk-old female C57BL/6 mice were placed on a special folate-free rodent diet on arrival. This is necessary

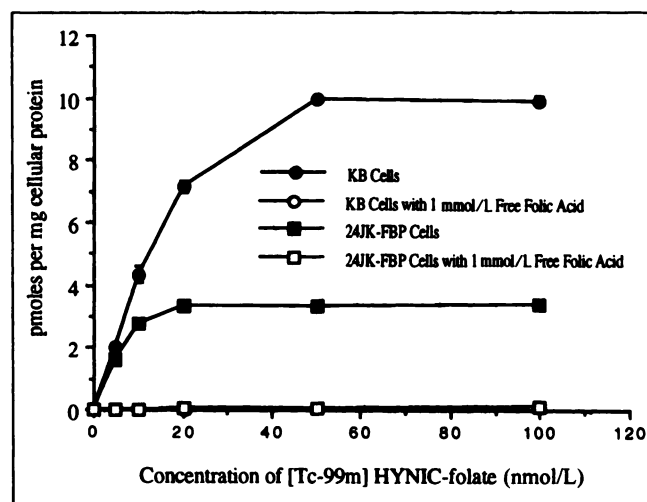


FIGURE 3. Uptake of ^{99m}Tc -HYNIC-folate by culture tumor cells.

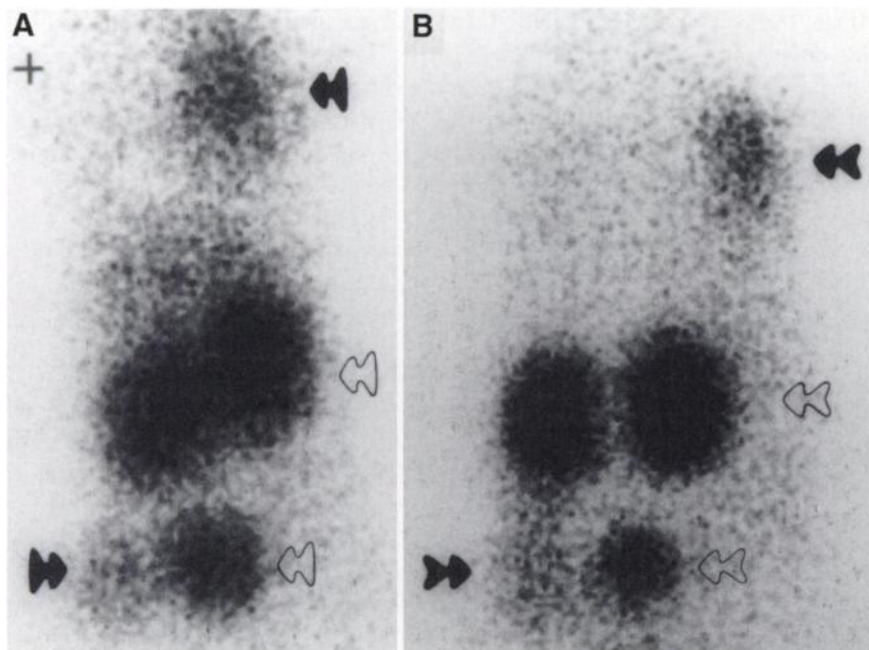


FIGURE 4. (A and B) Gamma camera images of mice treated with ^{99m}Tc -HYNIC-folate. Posterior images of two mice implanted with tumor cells on right shoulder and left hip obtained 4 h after intravenous administration of ^{99m}Tc -HYNIC-folate. Solid arrows point to tumor implants; open arrows indicate kidneys and urinary bladder of each animal.

because the high folic acid content of regular rodent diet (6 mg/kg) leads to high serum folate levels in mice. Previous studies show that animals placed on folate-free diet for 3 wk maintained a serum folate level of 25 ± 7 nmol/L (i.e., slightly higher than levels found in human serum [9–14 nmol/L]) and experienced no adverse effects (26). To create a suitable animal tumor model for evaluation of folate receptor targeting, the mice were subcutaneously implanted with 24JK-FBP cells. These were syngeneic chemically induced mouse sarcoma cells retrovirally transfected with the human folate receptor type α gene. Compared with the athymic mouse-KB cell xenograft model used in previous folate-receptor targeting studies, this syngeneic tumor model is more convenient to use and the folate receptor expression level of 24JK-FBP cells more closely resembles that of human tumors. Approximately 2×10^6 trypsinized 24JK-FBP cells were subcutaneously implanted on the right shoulder and the left hip of each test animal. Measurable tumors developed consistently 10 d after tumor cell implantation. At this point, each mouse was given an intravenous injection of approximately 3.7 MBq (100 μCi) ^{99m}Tc -HYNIC-folate, and 3 mice were also coinjected with 100 μg free folate. Animals were killed at 4 or 24 h after injection and tissues harvested for further analysis. Gamma camera images were collected for 2 mice at the 4 h time point immediately before kill. Tissue samples were weighed and counted for radioactivity. As shown in Figure 4, the highest levels of the radiotracer were found in the kidneys and the urinary bladder, whereas some liver activity was also detected in 1 mouse. The two tumor implants on the right shoulder and the hip regions on both animals were readily detectable in the gamma camera image. Meanwhile, other organs showed only background levels of radioactivity. The high kidney levels were likely a result of the presence of

folate receptor in the proximal tubules. The biodistribution of the radiotracer was calculated, and the results summarized in Table 1. The data showed tumor-to-blood ratios of 55 ± 19 and 81 ± 6 for the animals killed at 4 and 24 h

TABLE 1
Biodistribution of ^{99m}Tc -HYNIC-folate in C57BL/6 Mice Carrying Subcutaneous Grafts of 24JK-FBP Cell-Derived Syngeneic Tumors that Express the Folate Receptor

Organs	4 h postinjection		24 h postinjection
	^{99m}Tc -HYNIC-folate only (n = 4)	^{99m}Tc -HYNIC-free folate + 100 μg (n = 3)	^{99m}Tc -HYNIC-folate only (n = 3)
Blood	0.29 ± 0.10	0.29 ± 0.05	0.07 ± 0.00
Heart	0.97 ± 0.36	0.17 ± 0.02	0.72 ± 0.28
Kidney	61.38 ± 34.32	13.99 ± 2.09	30.30 ± 10.15
Liver	3.40 ± 1.85	0.96 ± 0.91	2.12 ± 0.43
Lung	1.02 ± 0.40	0.39 ± 0.06	0.88 ± 0.08
Muscle	0.57 ± 0.08	0.12 ± 0.03	0.55 ± 0.27
Spleen	2.84 ± 2.96	0.73 ± 0.81	2.68 ± 1.93
Intestine	1.98 ± 2.22	0.41 ± 0.22	0.36 ± 0.15
Tumor 1 (shoulder)	17.84 ± 11.43	1.93 ± 0.33	6.75 ± 0.89
Tumor 2 (hip)	15.69 ± 9.91	1.87 ± 0.39	5.14 ± 1.12
Ratios			
Tumor-to-blood	55.0 ± 19.2	6.80 ± 2.19	80.9 ± 6.4
Tumor-to-muscle	28.2 ± 14.8	17.3 ± 6.2	12.1 ± 4.4
Tumor-to-liver	5.78 ± 2.92	3.02 ± 1.70	2.94 ± 1.05
Tumor-to-kidney	0.26 ± 0.03	0.13 ± 0.03	0.20 ± 0.04

HYNIC = 6-hydrazinonicotinamido-hydrazido.

Values shown are mean \pm SD of % injected dose per gram of tissue. Tumor-to-tissue ratios calculated based on average of two tumors.

postinjection, respectively. Coinjection of 100 µg free folate greatly reduced the tumor and kidney levels of the radiotracer, suggesting radiotracer localization in these tissues was mediated by the folate receptor. These results are similar to those obtained previously with ⁶⁷Ga-deferoxamine-folate in a KB cell xenograft tumor model (26).

DISCUSSION

The folate receptor is frequently overexpressed in human tumors, including approximately 100% of serous ovarian adenocarcinomas. Folic acid conjugates have been shown to bind the folate receptor with high affinity and are taken up by tumor cells by folate receptor-mediated endocytosis. This provides a useful approach for targeted delivery of imaging or therapeutic agents into tumor cells. A radiolabeled monoclonal antibody against the folate receptor, MOv18, has been evaluated in clinical studies for imaging and therapy against ovarian cancer (3,14–17). Although somewhat promising results were obtained in these studies, the sensitivity of this procedure was limited by the relatively slow systemic clearing kinetics of monoclonal antibodies. Moreover, these antibodies consistently elicit a human antimouse antibody immune response that may prevent the repeated use of the imaging procedure (15,17). By contrast, folate conjugates are low-molecular-weight agents that are rapidly cleared from the blood stream and are presumably nonimmunogenic. This should allow for much higher tumor-to-background tissue ratios to be achieved in a much shorter time span, thereby greatly accelerating the entire imaging procedure. Such advantages have been demonstrated for low-molecular-weight imaging agents such as the radiolabeled somatostatin analog ¹¹¹In-DTPA-octreotide and in two-step (or three-step) imaging procedures in which the radiolabeled agent is administered in the final step as a low-molecular-weight conjugate (33,36).

In this study, ^{99m}Tc was chosen as the radionuclide. Compared with other possible choices of radionuclides for gamma imaging, such as ¹¹¹In and ⁶⁷Ga, ^{99m}Tc is clearly the preferred choice. ^{99m}Tc is the most widely used radionuclide in nuclear medicine because its physical properties (6-h half-life, 140-keV gamma photon) are ideally suited for labeling fast-clearing radiopharmaceuticals for imaging. Moreover, the easy and inexpensive availability of this radionuclide accounts for its use in approximately 80% of nuclear medicine procedures. Therefore, development of a ^{99m}Tc-based agent is important for the potential clinical application of folate receptor-targeted radiopharmaceuticals.

HYNIC was linked to folic acid and used as the ligand for ^{99m}Tc radiolabeling. Recent studies show that a ternary system consisting of HYNIC, tricine and a phosphine (e.g., TPPTS) forms stable complexes with ^{99m}Tc with high yield and specific activity (35). TPPTS was chosen as a coligand because of its three negative charges, which should increase the overall hydrophilicity of the radiotracer complex, ensuring its rapid systemic clearance through the kidneys. When

^{99m}Tc-HYNIC-folate was prepared using only tricine as the coligand, highly specific binding to the folate receptor was also observed (data not shown). However, because previous studies showed that HYNIC-tricine labeling methods lead to the formation of multiple radiolabeled species, we did not further characterize this agent *in vivo* (35).

In the cellular uptake study, receptor binding was evidently saturable and was completely inhibited by 1 mmol/L free folic acid. This showed that ^{99m}Tc-HYNIC-folate was highly specific for the folate receptor and that nonspecific binding to the cellular membrane was negligible.

In this study, a syngeneic mouse tumor model was chosen based on subcutaneously implanted 24JK-FBP tumor cells grown in C57BL/6 mice. Compared with the KB cell xenograft athymic mouse model used in previous studies characterizing the ⁶⁷Ga-deferoxamine-folate, and ¹¹¹In-DTPA-folate conjugate (26,27), this tumor model has a lower level of folate receptor expression. This may explain why tumor-to-blood ratios (55:1 at 4 h postinjection) obtained in this study with ^{99m}Tc-HYNIC-folate were approximately one seventh of those obtained using the KB cell xenograft model with ⁶⁷Ga-deferoxamine-folate (409:1 at the same time point) (26). Because these mouse tumors have folate receptor levels more representative of tumors found in humans, we believe this animal model is a good alternative to the athymic mouse model. Moreover, an immune-competent animal model also allows for the future assessment of the immunogenicity of the folate conjugates.

The folate conjugate (^{99m}Tc-HYNIC-folate) used in this study was approximately one twentieth (1.3 nmol per animal) that of previous biodistribution studies on two other folate conjugates, ⁶⁷Ga-deferoxamine-folate and ¹¹¹In-DTPA-folate (26,27). As a result, the %ID/g values for the tumor were 3–54 times higher and the kidney values 20–30 times higher than reported previously, despite the use of a tumor model (carrying 24JK-FBP cell grafts) with fewer folate receptors per cell compared with the athymic mouse models (carrying KB cell xenografts).

Also, in the receptor blocking studies, we used 24 times less free folate (100 µg) than in previous studies on ⁶⁷Ga-deferoxamine-folate and ¹¹¹In-DTPA-folate (26,27). In those studies, 2.4 mg folate were administered intravenously 5 min before radiotracer injection, which apparently led to serious damage to the kidney functions and significant inhibition of blood clearance of the radiotracer, as suggested by a 30-fold increase in the blood radiotracer level and a more than 10-fold increase in the kidney levels over animals not receiving free folate (26,27). The 100 µg coinjected free folate used for receptor blocking in this study did not significantly increase the blood levels of the radiotracer and actually significantly decreased the radiotracer levels in the kidneys, presumably because of blocking of the kidney folate receptors. Meanwhile, free folate coinjection led to an eight-fold reduction in tumor-to-blood ratio, showing that tumor localization of ^{99m}Tc-HYNIC-folate was a result of the folate receptor.

CONCLUSION

Because of the frequent overexpression of folate receptor among human tumors, folate conjugate-based radiopharmaceuticals are likely to find widespread clinical applications as imaging agents. Analogous rhenium complexes may also be developed for receptor-targeted radiation therapy. This study shows that ^{99m}Tc -HYNIC-folate is capable of efficiently imaging tumors in an animal model. Further preclinical and clinical characterization of this promising radiopharmaceutical is warranted.

ACKNOWLEDGMENTS

The authors thank Dr. Sanjoy Saha and Bonnie Williams for technical assistance. This work was partially supported by a seed grant from The Ohio State University Comprehensive Cancer Center (Columbus, OH).

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