Nuclear Medicine & Biology, Vol. 26, pp. 23–25, 1999

ISSN 0969-8051/99/$–see front matter
PII S0969-8051(98)00076-6

Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts

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ABSTRACT. The radiochemical synthesis and stability of ^{67}Ga-deferoxamine-folate ([^{67}Ga]Ga-DF-Folate) were examined as a function of DF-Folate concentration. Optimal labeling occurred at DF-Folate concentrations ≥2.5 μg/mL. To define the possible biological significance of variations in product formulation, the biodistribution of [^{67}Ga]Ga-DF-Folate was examined as a function of administered deferoxamine-folate dose in an athymic mouse KB tumor model. The folate-receptor-positive KB tumors were found to concentrate the ^{67}Ga radiolabel in a dose-dependent fashion, consistent with saturable involvement of the folate receptor in mediating tumor accumulation of the radiopharmaceutical. NUCL MED BIOL 26;1:23–25, 1999. **© 1998 Elsevier Science Inc.**

KEY WORDS. ^{67}Ga-Deferoxamine-folate, Folate receptor, Tumor-targeting

INTRODUCTION

The tumor-cell-membrane-associated folate receptor is a potential molecular target for selective radiopharmaceutical delivery to ovarian, endometrial, and other human tumors known to overexpress folate binding protein (FBP) (1, 2). FBP is a glycosyl-phosphatidyl-inositol-linked cell membrane protein involved in cellular uptake of oxidized folates via endocytosis (4). Previous studies have shown that simple low-molecular-weight folate-chelate conjugates, such as ^{67}Ga-deferoxamine-folate ([^{67}Ga]Ga-DF-Folate; 6–8, 12) and ^{111}In-DTPA-Folate (9–11, 13), are able to target this tumor receptor system both *in vitro* and *in vivo*. The present study was undertaken to better define optimal conditions for radiochemical synthesis of [^{67}Ga]Ga-DF-Folate, and to define the effects of DF-Folate dose on [^{67}Ga]Ga-DF-Folate biodistribution in a mouse tumor model.

MATERIALS AND METHODS

General

The deferoxamine-folate(γ) conjugate (DF-Folate, Fig. 1) was prepared as described previously (12). No-carrier-added ^{67}Ga-gallium chloride was obtained as an aqueous HCl solution from Mallinckrodt Medical, Inc. (Maryland Heights, MO). Folate-deficient rodent chow was obtained commercially (ICN Biomedicals, Costa Mesa, CA) and ultraviolet (UV) irradiated prior to use. The KB cells, a human oral epidermoid carcinoma cell line, were cultured and prepared for implantation as described previously (6, 7).

Radiochemical Synthesis of [^{67}Ga]Ga-DF-Folate

The effects of DF-Folate conjugate concentration were evaluated with respect to radiolabeling yield and product radiochemical stability, following the general labeling procedure described previously (7, 12). Briefly, the dilute HCl solution of $^{67}Ga^{3+}$ (~1.7 mCi) was evaporated to dryness with heating under a stream of N_2. The ^{67}Ga was then reconstituted as $^{67}Ga(acac)_3$ by addition of 1,200 μL ethanol containing 0.001% acetylacetone (acacH). Aliquots (200 μL) of this solution were buffered by addition of 300 μL TRIS-buffered saline (pH 7.4), followed by addition of the aqueous DF-Folate conjugate and incubation at room temperature. The concentration of DF-Folate in the labeling solution varied from 25 ng/mL to 2.75 mg/mL (Table 1). The radiochemical purity of the product was evaluated by thin layer chromatography, as described previously (7, 12). Reported radiochemical purity results are the mean of two measurements.

Athymic Mouse Model

All animal studies were performed in accordance with procedures approved by the Purdue Animal Care and Use Committee. Male athymic mice (Nu/Nu strain, 4–5 weeks old) were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN) and housed as described elsewhere (7). After a 7-day acclimation period on folate-free diet, the mice were inoculated subcutaneously with suspended KB tumor cells (2.8×10^{-6}/0.1 mL) in the interscapular region. Radiotracer biodistribution was evaluated 2 weeks after KB cell implantation. [^{67}Ga]Ga-DF-Folate was administered via the femoral vein to animals temporarily anesthetized by inhalation of diethyl ether. The total DF-Folate conjugate administered with the [^{67}Ga]Ga-DF-Folate was 133, 27, 2.8, 0.29, or 0.030 mg/kg body weight. All mice were sacrificed 4 h after radiotracer injection and tissues of interest removed, weighed, and counted to quantitate the biodistribution of the ^{67}Ga.

RESULTS AND DISCUSSION

Radiochemical purity of the [^{67}Ga]Ga-DF-Folate product after 24 h incubation was found to exceed 95%, except at the two lowest DF-Folate conjugate concentrations (≤0.25 μg/mL), in which labeling yields remained below 60% at 24 h (Table 1 and Fig. 2). At

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Received 4 February 1998.
Accepted 7 August 1998.

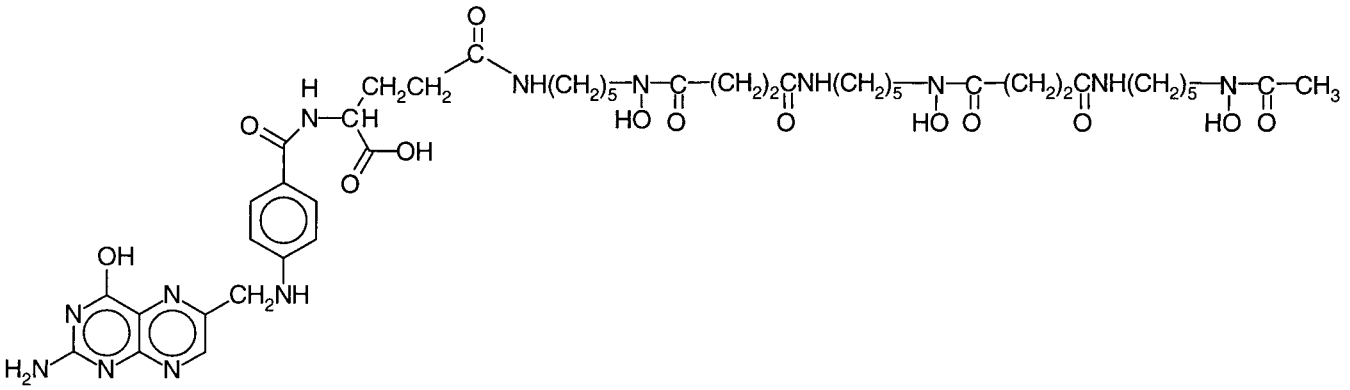

FIG. 1. Structural formula of the amide-linked deferoxamine-folate conjugate.

DF-Folate concentrations of 0.0025–2.75 mg/mL, radiolabeling yields always exceeded 80% by 10 min following DF-Folate addition to the [^{67}Ga]Ga-acetylacetonate solution (Table 1).

The biodistribution of [^{67}Ga]Ga-DF-Folate was determined in athymic mice with subcutaneous folate-receptor-positive KB tumor xenografts (Table 2, Fig. 3). All animals were sacrificed 4 h following intravenous co-administration of the [^{67}Ga]Ga-DF-Folate radiopharmaceutical with varying doses of DF-Folate. The tumor uptake of the ^{67}Ga was found to progressively decrease at DF-Folate doses above 0.29 mg/kg, presumably due to the competitive folate receptor binding by the excess unlabeled DF-Folate conjugate (Table 2 and Fig. 3). This result is consistent with earlier findings that tumor delivery of [^{67}Ga]Ga-DF-Folate can be competitively blocked by free folic acid (7).

The biodistribution results in the animals from Group C (Table 2) are generally consistent with those previously reported at a similar DF-Folate dose (7), except for the results in the kidney. For reasons that are unclear, [^{67}Ga]Ga-DF-Folate renal uptake in Group C was four-fold higher than observed previously (7).

As seen with [^{111}In]In-DTPA-Folate (10), the renal uptake of the folate-receptor-targeted [^{67}Ga]Ga-DF-Folate varies as a function of conjugate dose, dropping significantly as the DF-Folate dose was increased from 0.03 to 2.8 mg/kg (Table 2). This finding is consistent with the known occurrence of folate receptors in the proximal tubule of the kidney (5). The rise in renal ^{67}Ga at the highest DF-Folate doses is believed to be an artifactual result from precipitation of the DF-Folate conjugate in the kidneys as the urine is concentrated and acidified, resulting in mechanical obstruction of the collecting system (analogous to the known behavior of similarly soluble antifolates, such as methotrexate, in the kidney [3]). Overall, tumor-to-background contrast appeared optimal at the 2.8 mg/kg DF-Folate dose. Whereas the primary excretion pathway appears to be via urine, at all doses >20% of the tracer was cleared into the intestines, where the conjugate might interfere with rapid imaging of abdominal tumors.

TABLE 1. Variation of Radiochemical Yield with Labeling Conditions in the Synthesis of [^{67}Ga]Ga-DF-Folate

DF-folate concentration (μg/mL)	Total volume (mL)	Incubation time	Radiochemical purity (%)
2,750	0.8	10 min	78.7
		1 h	92.3
		4 h	94.6
		8 h	96.1
		24 h	97.7
275	0.8	10 min	82.8
		24 h	96.8
27.5	0.8	10 min	84.6
		24 h	95.7
2.5	0.8	10 min	87.8
		24 h	97.6
0.25	0.8	10 min	52.3
		24 h	60.4
0.025	0.8	10 min	40.2
		24 h	43.6

CONCLUSIONS

These data support our previous findings (6, 7) of saturable, receptor-mediated, uptake of [^{67}Ga]Ga-DF-Folate in folate-receptor-positive KB tumor xenografts.

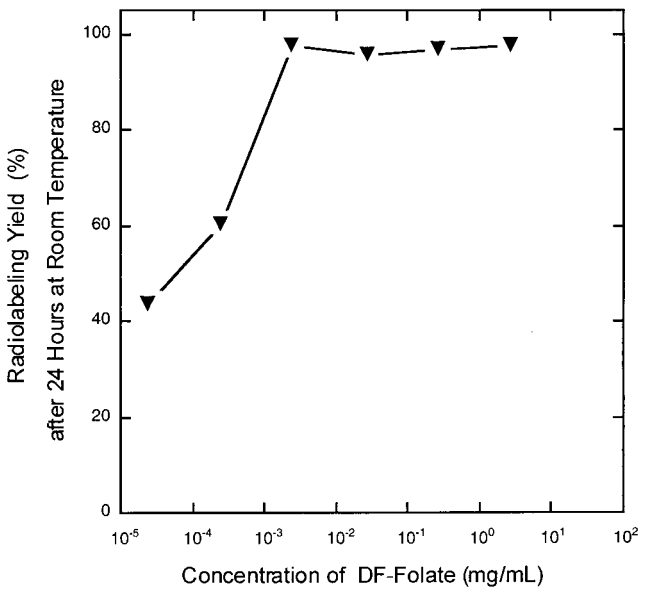

FIG. 2. Effect of DF-Folate concentration on radiolabeling yield in the synthesis of [^{67}Ga]Ga-DF-Folate. All radiolabeling yields were measured (in duplicate) after 24 hours incubation at room temperature.

TABLE 2. Biodistribution of [^{67}Ga]Ga-DF-Folate (% Injected Dose per Gram Tissue Wet Mass) in Athymic Mice with Folate-Receptor-Positive KB Tumors at Varying DF-Folate Doses

	Group A	Group B	Group C	Group D	Group E[a]
DF-folate dose (mg/kg)	133 ± 24	27 ± 2	2.8 ± 0.3	0.29 ± 0.05	0.030 ± 0.001
Animal mass (g)	29.2 ± 3.1	28.4 ± 2.2	28.1 ± 1.5	28.3 ± 3.6	28.2 ± 1.7
Tumor mass (g)	0.22 ± 0.13	0.26 ± 0.05	0.33 ± 0.07	0.35 ± 0.07	0.32 ± 0.08
Blood	0.20 ± 0.07	0.058 ± 0.019	0.024 ± 0.010	0.055 ± 0.002	0.098 ± 0.012
Heart	0.14 ± 0.08	0.062 ± 0.051[a]	0.10 ± 0.02	0.33 ± 0.03	0.67 ± 0.09
Lungs	0.25 ± 0.10	0.38 ± 0.41	0.073 ± 0.016	0.19 ± 0.02	0.39 ± 0.01
Liver	6.2 ± 3.3	0.67 ± 0.63	0.30 ± 0.11	0.86 ± 0.55	1.1 ± 0.09
Kidney	67.7 ± 19.8	11.0 ± 5.9	8.4 ± 0.4	35.8 ± 2.8	60.9 ± 7.3
Intestines and contents	13.7 ± 2.2	30.3 ± 6.0	18.1 ± 4.0	15.0 ± 1.7	12.1 ± 1.2
Tumor	0.96 ± 0.17	3.7 ± 1.4	6.9 ± 1.5	8.5 ± 0.4	8.7 ± 1.4
Tumor/blood	5.3 ± 2.4	72 ± 40	289 ± 61	154 ± 3	89 ± 3
Tumor/liver	0.16 ± 0.07	8.1 ± 5.4	23.8 ± 6.9	11.4 ± 4.1	8.0 ± 2.0
Tumor/kidney	0.014 ± 0.005	0.54 ± 0.55	0.82 ± 0.16	0.24 ± 0.02	0.14 ± 0.02

Values shown represent mean ± SD of data from four animals, except for Group E.
[a] n = 3.

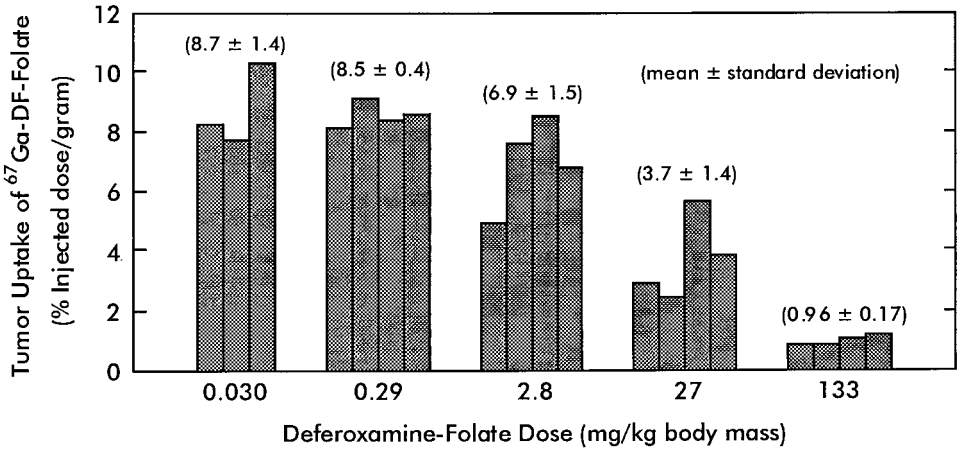

FIG. 3. Tumor uptake of [^{67}Ga]Ga-DF-Folate in mice with KB tumor xenografts. Each bar represents the data from one tumor-bearing mouse.

This work was supported in part by The Walther Cancer Institute, a "Jump-Start" Grant awarded by the Purdue Cancer Center, and a grant from the National Cancer Institute (RO1-CA70845).

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