^{125}I-Radioiodinated tyrosine-folate and tyrosine-click-folate

[^{125}I]-2 and [^{125}I]-4

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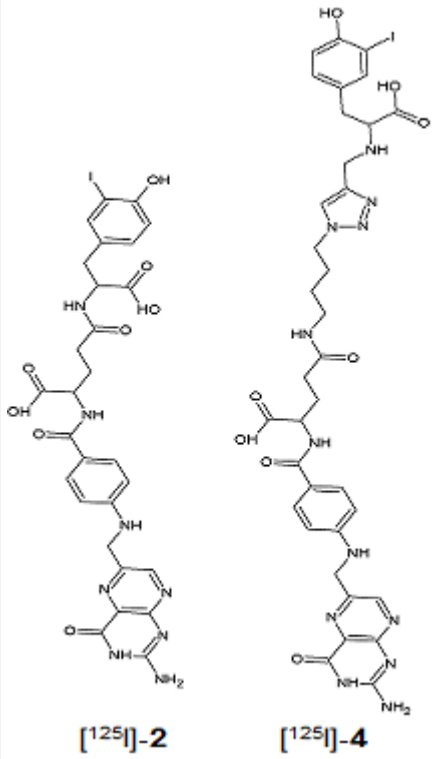
Chemical name:	^{125}I-Radioiodinated tyrosine-folate and tyrosine-click-folate
Abbreviated name:	[^{125}I]-**2** and [^{125}I]-**4**
Synonym:	
Agent Category:	Compounds
Target:	Folate receptor (FR)
Target Category:	Receptors
Method of detection:	Single-photon emission computed tomography (SPECT) and planar imaging
Source of signal / contrast:	^{125}I
Activation:	No
Studies:	• *In vitro* • Rodents



Structures of [^{125}I]-**2** and [^{125}I]-**4**

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Background

[PubMed]

^{125}I-Radioiodinated tyrosine-folate ([^{125}I]-**2**) and tyrosine-click-folate ([^{125}I]-**4**) are two folic acid conjugates synthesized by Reber et al. for imaging tumors by targeting folate receptor (FR) (1).

FR is a well-established target for molecular imaging and therapy. This receptor exhibits some favorable features as a target, such as high affinity for exogenous folate conjugates (K_d = ~100 pM), overexpression in a variety of human cancers, mediation of folate conjugates into cells, and self-recycling (2-4). In addition, conjugation of imaging labels *via* the γ-carboxyl group of folate has no apparent effect on its binding affinity to FR. However, FR expresses at a relatively high density on tumor cell surface (1–3 million FR/cell), and the folate-FR binding can be saturated rapidly. FR also expresses on the luminal side of proximal tubule cells in kidneys and is accessible to filtered radiofolates (5, 6). For most radiofolates, high renal accumulation (tumor/kidney ratio = ~0.1) and the resulting damage to kidneys are constant concerns in radiotherapy (1, 7).

Radioiodination of tyrosine residues is a common strategy for efficient radiolabeling of proteins and peptides (1, 4). The instability of radioiodinated tyrosine constructs as a consequence of *in vivo* deiodination is generally regarded as a disadvantage for radiopharmaceutical applications. However, studies have shown that the tumor/kidney ratios of radioiodinated conjugates are often higher than those of conjugates labeled with other radionuclides (8). The higher tumor/kidney ratios are considered to be due to partial deiodination of the radioiodinated conjugates.

Reber et al. designed two radioiodinated folic acid conjugates, [^{125}I]-**2** and [^{125}I]-**4**, based on the hypothesis that radioiodinated folate conjugates would exhibit favorable properties, similar to those found with other radioiodinated conjugates (1) and the partial deiodination of the radioiodinated folate conjugates might result in a quick clearance of the released iodide *via* kidneys and thus lead to improved tumor/kidney ratios. The findings obtained by Reber et al. confirm the hypothesis and indicate that the two radiofolates are potentially valuable for imaging applications (1). The radiofolate [^{131}I]-**4** was also tested in combination with Pemetrexed (PMX), a multitargeted antifolate drug (7), for therapeutic purposes. This chapter summarizes the data obtained with [^{125}I]-**2** and [^{125}I]-**4**.

Related Resource Links:

Folate receptor–targeted imaging agents in MICAD

Nucleotide and protein sequences of folate receptor

Bioassays of folate receptor in PubChem

Folate receptor–related compounds in Pubchem

Folate receptor–related clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

Reber et al. described the synthesis of tyrosine-folate (compound **1**), iodo-tyrosine-folate (compound **2**), tyrosine-click-folate (compound **3**), and iodo-tyrosine-click-folate (compound **4**) in detail (1). The overall yield was ~10% for compound **1** and ~5% for compound **2**. Conjugation of the tyrosine moiety of compounds **1** and **2** with "click chemistry" resulted in compounds **3** and **4**, respectively, with an overall yield of ~25% for compound **3** and ~14% for compound **4**. All compounds were obtained with >95% purity as confirmed with high-performance liquid chromatography (HPLC). Compounds **1** and **3** were radiolabeled with [^{125}I]-iodine and [^{131}I]-iodine to obtain [^{125}I]-**2** and [$^{125/131}$I]-**4**. The specific activities for [^{125}I]-iodine and [^{131}I]-iodine were 78.6 MBq/nmol (2.12 mCi/nmol) and >24.2 MBq/nmol (0.65 mCi/nmol), respectively. [^{125}I]-**2** (retention time (R_t) ≈ 14.4 min) and [$^{125/131}$I]-**4** (R_t ≈ 15.7 min) were separated from traces of free iodide (R_t ≈ 2.9 min) and cold precursor **1** (R_t ≈ 12.4 min) or **3** (R_t ≈ 13.9 min) with HPLC. Compounds **2** and **4** were used as reference compounds in the experiments.

The radiochemical yields were >97% for both [^{125}I]-**2** and [$^{125/131}$I]-**4**. The actual experimental specific activities of [^{125}I]-**2** and [$^{125/131}$I]-**4** were not reported. For *in vitro* and *in vivo* experiments, the radiofolates were diluted in phosphate-buffered saline (PBS) at pH 7.4. The octanol/PBS distribution coefficient (log D value) for [^{125}I]-**2** was slightly higher (−2.87 ± 0.02) than that for [^{125}I]-**4** (−3.13 ± 0.07), indicating that [^{125}I]-**4** is more hydrophilic than [^{125}I]-**2**.

The *in vitro* stabilities of [^{125}I]-**2** and [^{125}I]-**4** were determined in mouse and human plasma and in mouse liver microsomes. During the incubation period of 7 days in both mouse and human plasma at 37°C, HPLC analysis always showed only one single peak, with an integrated area of 100% and a retention time that corresponded to the retention times for [^{125}I]-**2** and [^{125}I]-**4**, indicating no deiodination or decomposition of [^{125}I]-**2** and [^{125}I]-**4**. In murine liver microsomes, [^{125}I]-**2** and [^{125}I]-**4** exhibited no deiodination over a time period of 24 h in contrast to [^{125}I]iodo-l-tyrosine, which was almost completely deiodinated within 30 min.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Reber et al. analyzed the cell uptake and binding affinities of [^{125}I]-**2** and [^{125}I]-**4** with cultured KB cells (human cervix carcinoma), IGROV-1 and SKOV-3 cells (both are human ovarian adenocarcinoma cells), and PC-3 cells (human prostate carcinoma) (1). KB cells are known to express high levels of FR, whereas IGROV-1 and SKOV-3 cells

express lower levels of FR. PC-3 cells do not express FR and were used as a negative control.

Cell uptake studies were performed with KB cells. The results showed that [^{125}I]-**2** and [^{125}I]-**4** had similar characteristics. The maximal amount of uptake was reached after 90–120 min incubation at 37°C. The internalized fraction for both radiofolates accounted for 25%–30% of total cell uptake (sum of FR-bound radiofolates on the cell surface and internalized fraction). Co-incubation of [^{125}I]-**2** or [^{125}I]-**4** with excess folic acid inhibited uptake for [^{125}I]-**2** and [^{125}I]-**4** to <0.1% of the total added radioactivity.

Relative binding affinities were determined with a competition assay with ^{3}H-folic acid and the nonradioactive reference compounds **2** and **4**. The binding affinity of folic acid was set to 1.0, and a relative affinity value of 1.0 implies that the test compound has an affinity for FR equal to that of folic acid. Likewise, values <1.0 reflect a weaker affinity, and values >1.0 reflect a stronger affinity. Compounds **2** and **4** had a relative binding affinity of 0.78 and 0.67, respectively.

Reber et al. also analyzed cell viability after incubation of the cells with [^{131}I]-**4** (5 MBq/mL (0.14 mCi/mL)) (1). In the absence of folic acid, [^{131}I]-**4** reduced the viabilities of FR-positive KB, IGROV-1, and SKOV-3 cells to 4.5%, 15.3%, and 27.4% of the viabilities of the untreated control cells, respectively. However, in the presence of excess folic acid to block FR, the viabilities of these cells were not affected by [^{131}I]-**4** at the same radioactivity concentration. In addition, no reduction of the cell viability was observed with the same amount of [^{131}I]-**4** in the control experiments performed with FR-negative PC-3 cells. The half-maximal inhibitory concentrations of [^{131}I]-**4** were 0.39 ± 0.03 (10.54 ± 0.81), 0.95 ± 0.05 (25.68 ± 1.35), and 1.22 ± 0.09 (32.97 ± 2.43) MBq/mL (μCi/mL) for KB, IGROV-1, and SKOV-3 cells, respectively. The incubation of KB cells with PMX (1 μM) for 4 h and 24 h reduced tumor cell viability to 80% and 50% of the cell viability of the untreated controls, respectively. Application of [^{131}I]-**4** at a concentration of 0.01 MBq/mL (0.27 μCi/mL) did not affect tumor cell viability if applied as a single agent for 4 h. However, if this radioactivity concentration was applied in combination with PMX (1 μM), it resulted in an enhanced inhibitory effect, decreasing cell viability to 72% (after 4 h) and 23% (after 24 h) compared with untreated controls.

Animal Studies

Rodents

[PubMed]

Biodistribution of [^{125}I]-**2** and [^{125}I]-**4** was investigated in CD-1 *Foxn-1/nu* mice (n = 6–8) (1). Animals were fed a folate-deficient rodent diet starting 5 days before KB cell inoculation. [^{125}I]-**2** and [^{125}I]-**4** were given to mice *via* tail vein injection (0.2 MBq (5.41 μCi)). Potassium iodide dissolved in PBS was injected intraperitoneally (4 mg) 1 h before the radioactive agents. Animals were euthanized at 1, 4, or 24 h after administration of the radiofolate. The radioactivity in organs was recorded as the percentage of the injected

dose per gram of tissue weight (% ID/g). Blocking experiments were performed 4 h after coinjection with excess folic acid (100 μg). The results showed that both [^{125}I]-**2** and [^{125}I]-**4** accumulated in FR-positive KB tumor xenografts and in kidneys (Tables 1 and 2).

Table 1: Biodistribution data of [^{125}I]-**2** at different time points after injection

Tissues	1 h	4 h	24 h	4 h (FA)[1]	4 h (KI)[2]
Blood	3.00 ± 0.56	2.15 ± 0.42	0.09 ± 0.02	2.25 ± 1.00	2.71 ± 0.52
Kidneys	18.33 ± 2.47	9.80 ± 1.67	0.85 ± 0.06	1.79 ± 0.58	8.66 ± 1.86
Liver	16.53 ± 1.20	2.74 ± 0.45	0.09 ± 0.01	0.83 ± 0.32	2.90 ± 0.63
Thyroid	261 ± 67	1059 ± 184	2830 ± 1272	868 ± 367	13.74 ± 5.01
Tumor	4.42 ± 0.66	3.43 ± 0.28	1.25 ± 0.18	1.65 ± 0.61	4.03 ± 0.22
Tumor/blood	1.48 ± 0.18	1.65 ± 0.41	14.38 ± 3.66	0.74 ± 0.04	1.53 ± 0.30
Tumor/liver	0.27 ± 0.04	1.28 ± 0.23	14.45 ± 1.88	1.98 ± 0.18	1.43 ± 0.24
Tumor/kidney	0.24 ± 0.03	0.36 ± 0.06	1.47 ± 0.14	0.90 ± 0.09	0.48 ± 0.09

[1]FR blockade with folic acid (FA, 100 μg).

[2]Thyroid blockade with potassium iodide (KI, 4 mg).

Table 2: Biodistribution data of [^{125}I]-**4** at different time points after injection

Tissues	1 h	4 h	24 h	4 h (FA)[1]	4 h (KI)[2]
Blood	0.10 ± 0.01	0.18 ± 0.15	0.01 ± 0.00	0.03 ± 0.00	0.08 ± 0.01
Kidneys	19.12 ± 0.58	16.44 ± 2.64	7.10 ± 1.44	0.08 ± 0.00	17.84 ± 2.60
Liver	0.28 ± 0.08	0.15 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.16 ± 0.03
Thyroid	8.36 ± 1.77	10.81 ± 4.99	47.59 ± 28.07	9.53 ± 8.10	2.47 ± 0.56
Tumor	2.36 ± 0.05	2.28 ± 0.46	1.92 ± 0.24	0.03 ± 0.01	2.53 ± 0.39
Tumor/blood	23.21 ± 2.17	17.41 ± 9.66	286.75 ± 53.44	1.08 ± 0.47	30.93 ± 6.87
Tumor/liver	8.73 ± 2.13	15.19 ± 3.99	84.82 ± 12.71	2.20 ± 0.96	15.78 ± 4.33
Tumor/kidney	0.12 ± 0.00	0.14 ± 0.02	0.27 ± 0.05	0.41 ± 0.15	0.14 ± 0.02

[1]FR blockade with folic acid (FA, 100 μg).

[2]Thyroid blockade with potassium iodide (KI, 4 mg).

The tumor uptake of [^{125}I]-**2** was higher than the uptake of [^{125}I]-**4** at 1 h after injection ($P = 0.02$). However, the tumor retention over time was superior for [^{125}I]-**4** compared to [^{125}I]-**2**. Coinjection of excess folic acid (4 h after injection) reduced tumor uptake to <2% of the uptake of the control values for [^{125}I]-**4** but only to 48% for [^{125}I]-**2**. The radioactivity in the kidneys for both [^{125}I]-**2** and [^{125}I]-**4** was high at 1 h after injection; however, radioactivity reduced significantly ($P < 0.05$) at 4 and 24 h after injection. Preinjection of PMX resulted in a significantly reduced uptake of [^{125}I]-**4** in the kidneys

($P < 0.05$), while uptake in the tumor xenografts was largely retained. The radioactivity in the liver was high for [^{125}I]-**2** (>16% and >2% ID/g at 1 h and 4 h after injection) but was almost negligible for [^{125}I]-**4** (<0.3% and <0.2% ID/g at 1 h and 4 h after injection). In the thyroid glands, large amounts of radioactivity (>1,000% ID/g at 4 h after injection) were found for [^{125}I]-**2**, whereas a >10-fold lower accumulation was observed for [^{125}I]-**4** at the same time point. Intraperitoneal administration of potassium iodide 1 h before administration of the radiotracer significantly ($P < 0.05$) reduced the uptake of iodide in the thyroid glands at 4 h after injection ([^{125}I]-**2**, 13.74 ± 5.01% ID/g; [^{125}I]-**4**, 2.53 ± 0.39% ID/g). In the gastrointestinal tract, the radioactivity was comparable for both [^{125}I]-**2** and [^{125}I]-**4** (4.5% ID/g at 1 h after injection).

Biodistribution studies were also performed with [^{125}I]-**4** in combination with preinjection of variable doses of PMX (1 h before injection of [^{125}I]-**4**). Nonradioactive potassium iodide was also injected intraperitoneally (4 mg) 1 h before injection of [^{125}I]-**4**. Biodistribution data obtained at 4 h after [^{125}I]-**4** injection showed that the tumor uptake was slightly reduced after administration of the highest dose (400 μg) of PMX (1.15 ± 0.17% ID/g, $P = 0.08$), whereas almost no tumor uptake reduction was observed after administration of 50% of the PMX dose ($P = 0.64$) and 25% of the PMX dose ($P = 0.19$). In contrast, the radioactivity in the kidneys was significantly reduced after administration of PMX in a dose-dependent manner ($P = 0.01$). Thus, by using the highest dose of PMX, the tumor/kidney ratio could be improved >10-fold without significant changes in the tumor/blood, tumor/liver, or tumor/muscle ratio.

Metabolite studies were performed in female *Balb/c* mice ($n = 2$/time point). Animals were fed a folate-deficient rodent diet starting 5 days before the experiments. All mice received an intraperitoneal injection of potassium iodide (4 mg) 1 h before injection of ~5 MBq (0.135 mCi (~64 pmol)) [^{125}I]-**2** or [^{125}I]-**4**. Urine and blood samples were taken 5 min and 30 min after injection of the radiotracer. The analysis of blood samples confirmed greater deiodination of [^{125}I]-**2** compared to that of [^{125}I]-**4**. As a consequence, a high concentration of free [^{125}I]-iodide was found in the blood samples from mice injected with [^{125}I]-**2** but not in blood samples from mice that received [^{125}I]-**4**. Intact [^{125}I]-**2** was not detectable in urine samples. In contrast, almost the whole radioactive fraction of [^{125}I]-**4** in the urine samples taken at 5 min after injection was intact, and only a small amount (~10%) was free [^{125}I]-iodide. At 30 min after injection, the intact [^{125}I]-**4** in the urine reduced to 5%.

SPECT/CT imaging was performed in nude mice bearing KB tumor xenografts after administration of [^{125}I]-**4** (~5.0 MBq (0.135 mCi)). PMX (400 μg) and/or potassium iodide (4 mg) was injected 1 h before [^{125}I]-**4** injection. SPECT scans (60 min) were performed ~24 h after injection of [^{125}I]-**4**. The administration of potassium iodide effectively blocked the uptake of radioactivity in the thyroid gland. With preinjection of PMX, renal radioactivity was reduced to background levels. SPECT/CT scans of a mouse that received both potassium iodide and PMX before injection of [^{125}I]-**4** resulted in a radioactivity uptake that was restricted to FR-positive tumor xenografts, and only traces of radioactivity were observed in non-targeted organs and tissues.

Ex vivo autoradiography of a mouse that received PMX showed that the radioactivity uptake of [^{125}I]-**4** in the tumor and kidneys decreased to 56% and 4% of the uptake in the control animals, respectively.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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