^{68}Ga-1,4,7-Triazacyclononane,1-glutaric acid-4,7-acetic acid-1,2-diaminoethane-γ-folate (P3246)

^{68}Ga-P3246

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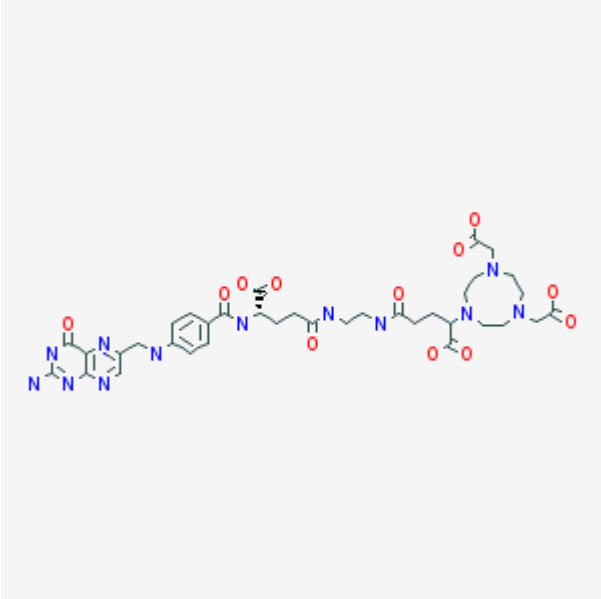
Chemical name:	^{68}Ga-1,4,7-Triazacyclononane, 1-glutaric acid-4,7-acetic acid-1,2-diaminoethane-γ-folate (P3246)
Abbreviated name:	^{68}Ga-P3246
Synonym:	^{68}Ga-NODAGA-folate
Agent category:	Compound
Target:	Folate receptor
Target category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal:	^{68}Ga
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

Folic acid (folate) is a water-soluble B vitamin (1) that is essential for methylation and DNA synthesis. The primary pathway for entry of folate into cells is through the facilitated transporter, which has a low affinity for folate (Michaelis constant (K_m) = 1–5 μM). Some cells in the choroid plexus, kidney, lung, thyroid, spleen, placenta, and thymus also possess a higher-affinity receptor (dissociation constant (K_d) = 0.5 nM) that allows folate uptake *via* receptor-mediated endocytosis. Some human epithelial tumor cells have been found to overexpress folate receptors (2). More than 90% of human ovarian and endometrial cancers express the high-affinity folate receptor, which is absent in the corresponding normal tissues. Breast, colorectal, renal, and lung carcinomas also overexpress the high-affinity folate receptor but at lower frequencies (20%–50%). Activated macrophages, but not resting macrophages, have also been found to have the high-affinity folate receptor (3).

Several folate-based conjugates (^{111}In-DTPA-folate, ^{99m}Tc-EC-folate, and [^{18}F]FBA-folate) have been studied in tumor imaging (4-8). Deferoxamine (DF), a chelating agent, was conjugated to folic acid to form a mixture of two isomers, DF-α-folate and DF-γ-folate. Only the DF-γ-folate isomer was able to displace [^{3}H]folic acid from its receptors, with a 50% inhibition concentration similar to that of folic acid (2.5 nM *versus* 2.4 nM) (9). Fani et al. (10) prepared a γ-folate conjugate with tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) and 1,2-diaminoethane as a spacer to form P3026, which was labeled with ^{68}Ga for positron emission tomography (PET) imaging of folate receptors in tumors. To further the quest for a ^{68}Ga-folate conjugate for clinical application, folic acid was conjugated to 1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid (NODAGA) *via* 1,2-diaminoethane as a linker between the NODAGA and folic acid (P3246) (11). ^{68}Ga-P3246 was evaluated as a PET agent for imaging folate receptor expression in a mouse tumor model. $^{67/68}$Ga-P3246 exhibited a higher tumor/blood ratio than $^{67/68}$Ga-P3026 in the same tumor model.

Related Resource Links:

- Chapters in MICAD (folate receptor)
- Gene information in NCBI (folate receptor)
- Articles in OMIM (folate receptor)
- Clinical trials (folate receptors)
- Drug information in FDA (folate receptor)

Synthesis

[PubMed]

Fani et al. (11) coupled P3246 (12 nmol) with ^{68}Ga in sodium acetate buffer (pH 4.0) for 10 min at 25°C to yield ^{68}Ga-P3246 with >92% radiochemical purity. Radiochemical

yields exceeded 95% with a specific activity of ~30 MBq/nmol (0.81 mCi/nmol). ^{67}Ga-P3246 was similarly radiolabeled with a specific activity of ~3 MBq/nmol (0.081 mCi/nmol). ^{68}Ga-P3238 was prepared with similar specific activity as ^{68}Ga-P3246.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The human nasopharyngeal carcinoma KB cell line folate receptors were studied with ^{67}Ga-P3246 saturation binding studies at 4°C (11). ^{67}Ga-P3246 showed a K_d (affinity constant) of 5.61 ± 0.96 nM, which was similar to the K_d value (4.65 ± 0.82 nM) for ^{67}Ga-P3026 (the DOTA-folate conjugate). ^{67}Ga-P3246 (2.5 nM) was rapidly associated (bound to the cell surface and internalized) with KB cells at 37°C, with 60% of incubation dose (ID) at 30 min and 72% ID at 4 h. Approximately 15% ID ^{67}Ga-P3246 was internalized at 4 h. Excess folate blocked the cell-associated radioactivity to <1% ID. Approximately 76% of radioactivity was retained in the cells after 4 h incubation in fresh medium.

Animal Studies

Rodents

[PubMed]

Fani et al. (11) performed *ex vivo* biodistribution studies of 0.4 nmol $^{67/68}$Ga-P3246 in nude mice (n = 3–5/group) bearing KB tumor xenografts. Accumulation of $^{67/68}$Ga-P3246 in the KB tumors was 15.56 ± 3.67, 18.42 ± 0.74, 16.29 ± 4.46, and 14.32 ± 5.80% injected dose/gram (ID/g) at 1, 2, 4, and 24 h after injection, respectively. The organ with the highest accumulation at 4 h after injection was the kidney (130% ID/g), followed by the salivary gland (8.6% ID/g), pancreas (2.9% ID/g), adrenal (2.8% ID/g), heart (2.0% ID/g), muscle (1.8% ID/g), stomach (1.5% ID/g), and liver (1.1% ID/g). The accumulation in the blood was low (0.06% ID/g) at 4 h. The tumor/blood ratios were 81, 207, 254, and 391 at 1, 2, 4, and 24 h, respectively. Pretreatment with excess folate (40 nmol, 5 min before $^{67/68}$Ga-P3246 injection) reduced the radioactivity accumulation by >85% in the folate receptor-positive tumor, salivary glands, and kidneys at 4 h after injection. Pretreatment with pemetrexed (60 min before ^{67}Ga-P3246 injection), a folate analog metabolic inhibitor, significantly reduced the kidney accumulation of $^{67/68}$Ga-P3246 by >70% at 1 h and 4 h after injection. On the other hand, little inhibition by pemetrexed was observed in the tumor and other organs. $^{67/68}$Ga-P3246 exhibited higher tumor/blood ratios than its $^{67/68}$Ga-DOTA counterpart ($^{67/68}$Ga-P3026 with tumor/blood ratios of 36 at 1 h, 50 at 2 h, and 70 at 4 h).

Whole-body PET imaging scans were performed for 60 min after injection of 10 MBq (0.27 mCi) ^{68}Ga-P3246 (0.4 nmol) in nude mice bearing KB tumor xenografts (the number of mice used was not reported) (11). The highest radioactivity levels were visualized in the KB tumor, salivary glands, and kidneys, with standard uptake values of 2.2, 1.8, and 10 at 60 min, respectively. The accumulation of ^{68}Ga-P3246 in the kidneys

was blocked by pre-injection of pemetrexed, whereas the accumulation in the tumor and salivary glands remained at the same levels. No blocking studies using excess folate were reported for the PET studies.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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