^{99m}Tc-Labeled peptide derivative of folic acid

^{99m}Tc-EC20

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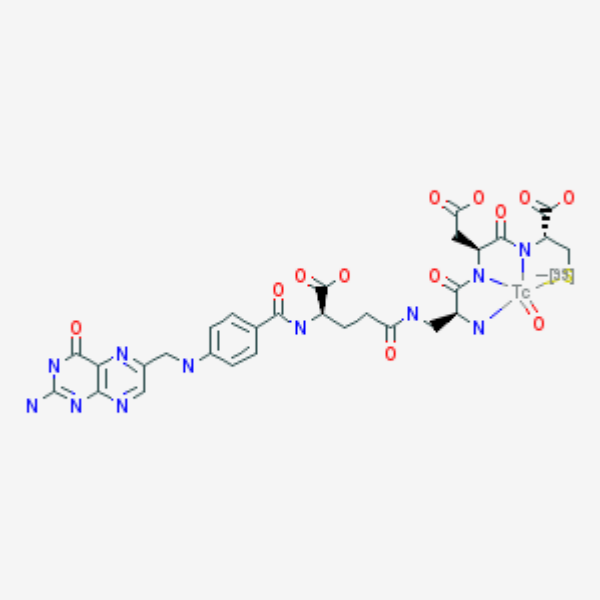
Created: September 11, 2009; Updated: November 12, 2009.

Chemical name:	^{99m}Tc-Labeled peptide derivative of folic acid
Abbreviated name:	^{99m}Tc-EC20
Synonym:	
Agent Category:	Compound
Target:	Folate receptor (Folate binding protein)
Target Category:	Receptor
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging
Source of signal / contrast:	^{99m}Tc
Activation:	No
Studies:	• *In vitro* • Rodents • Humans



Structure of ^{99m}Tc-EC20 according to Leamon et. al. (1)

Background

[PubMed

Folic acid (FA; folate) belongs to the B group of vitamins, and its activity is mediated through the membrane-bound folate receptor (FR; also known as the folate-binding protein), which has two isoforms (α and β). A third constitutively secreted form of the

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NLM Citation: Chopra A. ^{99m}Tc-Labeled peptide derivative of folic acid. 2009 Sep 11 [Updated 2009 Nov 12]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

receptor (γ) has also been identified in some hematopoietic cells (2). The α and β isoforms of FR show a high affinity for FA and exhibited restricted expression in certain tissues. FR-α is found in the lungs, glandular tissue, and choroid plexus of the brain. A functional β form is present only in activated monocytes and macrophages, although it is also borne by mature neutrophils and $CD34^+$ cells (as an inactive form) (2). Low amounts of FR-α are expressed in normal tissue, but it was shown to be overexpressed in several cancerous tumors such as those of the breast, colon, head and neck, kidneys, and lungs (3). In addition, overexpression of the FR in tumors indicates a poor prognosis for the patient (4, 5). Therefore, the FR has been targeted in clinical trials for the detection, diagnosis, and treatment of various cancers. Several folate-based imaging agents that use techniques such as magnetic resonance imaging, optical imaging, computed tomography, positron emission tomography, and single-photon emission tomography (SPECT) have been developed and evaluated for the noninvasive detection of malignant tumors that had excessive expression of FR (3). However, except for the SPECT-based agents, all imaging agents had limited use and were determined to be unsuitable for detection of tumors overexpressing the FR as detailed by Sega et al. (3).

An indium (^{111}In)-labeled folate-based SPECT agent with a good tumor specificity was developed earlier and evaluated in clinical trials, but the nuclide is expensive to produce, has a high γ energy emission (171 and 245 keV), and has a long half-life (~68 h) (3), and the detection of recurrent cancer with this agent is difficult (6). To overcome the problems associated with the use of ^{111}In, some investigators developed and evaluated the use of technetium (^{99m}Tc)-labeled FR imaging agents because this nuclide has a low energy emission (140 keV), has a short half-life (~6 h), is inexpensive to produce, and, compared with ^{111}In, produces high-quality images (1, 7-11). Among the different ^{99m}Tc-labeled FR imaging agents, only the peptide-based, folate-derived compound ^{99m}Tc-EC20 (folic acid-pteroic acid (Pte)-D-Glu-β-L-diaminopropionic acid(βDpr)-Asp-Cys) has been evaluated in a mouse model (1, 12, 13), and more recently it was used for an exploratory study in humans to detect the presence of FR-positive tumors (14).

Synthesis

[PubMed]

The synthesis of EC20 and its labeling with ^{99m}Tc was described by Leamon et al. (1). The synthesized EC20 was purified with high-performance liquid chromatography (HPLC) and analyzed with nuclear magnetic resonance imaging and electrospray-mass spectrometry. Kits containing a sterile, nonpyrogenic mixture of purified EC20, sodium α-D glucoheptonate, tin chloride, and sodium hydroxide or hydrochloric acid (to maintain a pH of 6.8 ± 0.2) were subsequently prepared in reagent vials for lyophilization. The lyophilized preparation was sealed under argon and stored at -20°C until use. The shelf-life of the lyophilized kit under these storage conditions was reported to be >2 years (1).

The labeling of EC20 was done by injecting ^{99m}Tc-pertechnetate into the EC20 kit vial and by heating it in a boiling water bath for ~18 min (1). The labeled product was allowed

to cool for at least 15 min at room temperature and used within 6 h of preparation. From an HPLC analysis of the labeled product, the investigators concluded that it had a purity of >95% but probably contained a diastereomeric mixture of the labeled product (1). Radiochemical purity of the reconstituted labeled compound was reported to be >90% for up to 24 h when stored at room temperature. The specific activity of ^{99m}TC-EC20 was not reported. Chelation of ^{99m}Tc to EC20 is shown in the chemical structure of the compound as suggested by Leamon et. al. (1). Other ^{99m}Tc-labeled analogs of EC20 (^{99m}TC-EC14 and ^{99m}TC-EC28; for details regarding their respective structures, see Leamon et al. (1)) and ^{111}In-diethylenetriamine pentaacetic acid (DTPA)-folate, a previously used radiopharmaceutical (15), were also prepared for some studies by the investigators (1), but their radiochemical purity, specific activity, and stability were not reported.

In another study, an all-D isomer of EC20, designated EC53, was synthesized, purified, lyophilized, and labeled with ^{99m}Tc as detailed above (12). The radiochemical purity of ^{99m}Tc-EC53 was >90%. The stability of reconstituted EC53 was reported to be the same as that of EC20 for at least 24 h at room temperature. Specific activity of the labeled compound was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

An analysis of ^{99m}Tc-EC20 exposed to rat or human serum showed that ~70% of the radiochemical was bound to a >30-kDa serum protein in both sera (1). However, the investigators concluded that this did not affect biodistribution of the radiocompound because it was detected in all the FR-positive tissue (see below for details).

The FR binding affinity of ^{99m}Tc-EC20 in KB cells was reported to be 1.1 nM (1), and the binding was completely blocked in the presence of excess (100 nM) folic acid. Compared with folic acid, the relative affinity (RA; defined as the inverse molar ratio of compound required to displace 50% of ^{3}H-folic acid bound to FR on KB cells; the RA of folic acid was taken to be 1.0) values of ^{99m}Tc-EC20 and DTPA-folate was determined to be 0.92 ± 0.23 and 0.87 ± 0.16, respectively. In the same study, the diastereoisomers of ^{99m}Tc-EC20 (with either a syn or an anti configuration of the ^{99m}Tc-oxygen bond in the DAP-Asp-Cys chelating ring of EC20) were reported to have an RA of 1.42 ± 0.36 and 1.37 ± 0.23, respectively. In another study, EC53, an all-D isomeric molecule of EC20, was shown to have an RA of 0.23 (12).

Reddy et al. investigated the effect of FR expression level on the uptake of ^{99m}Tc-EC20 in tumor cells (12). Tumors derived from 4T1 (expressing 1.5 pmol FR/mg protein), M109 (expressing 22.9 pmol FR/mg protein), and 4T1-pico (expressing 7.2 pmol FR/mg protein) cells were harvested from mice exposed to ^{99m}Tc-EC20. The amount of radioactivity associated with cell membranes of the respective tumors was determined and reported to be proportional to the expression of FR by the various cells (2.6 ± 0.7, 8.7 ± 2.3, and 15.53 ± 1.90 percent injected dose/gram tissue (% ID/g) for the 4T1, 4T1-pico,

and the M109 tumor cells, respectively). In addition, when the radiotracers were co-injected with 100-fold excess unlabeled folic acid, <1% ID/g accumulation of radioactivity was observed in the three tumor types, indicating that the uptake of label in these tumors was primarily due to presence of the FRs.

Animal Studies

Rodents

[PubMed]

To investigate the biodistribution of ^{99m}Tc-EC14 (compared to EC20 an additional D-Glu residue is present in the EC14 peptide sequence), ^{99m}Tc-EC20, ^{99m}Tc-EC28 (negative control; compared to EC20 the sequence of the peptide in EC28 is Pte-D-Glu-D-Glu-βDpr-Asp-Cys), and ^{111}In-DTPA-folate (positive control), BALB/c mice (n = 3 animals per cohort) bearing M109 cell tumors were injected with the radiotracers through the lateral tail vein and euthanized 4 h later (1). The major tissues, including tumors, were removed from the animals, weighed, and counted for accumulated radioactivity. Compared with ^{99m}Tc-EC14, ^{99m}Tc-EC20, and ^{111}In-DTPA-folate, only a small amount of ^{99m}Tc-EC28 accumulated in the tumors and kidney (this organ contains high levels of FR). The other three folate-containing radiochemicals accumulated mainly in the tumors and the kidneys of the animals. The kidneys showed a higher uptake of the label compared to the tumors. The accumulation of ^{99m}Tc-EC20 and ^{111}In-DTPA-folate in the tumors was similar (17.2 ± 1.02 and 19.3 ± 5.86% ID/g, respectively), and for ^{99m}Tc-EC14 it was 9.83 ± 2.77% ID/g. When the radiolabeled folate compounds were injected along with a 100-fold excess of unlabeled folic acid, a significantly reduced uptake (P value not reported) of the respective labeled compounds in the tumors and kidneys was reported. This indicated that cell binding of the radiolabeled folate compounds was specific for FR.

Mice bearing M109 cell tumors underwent whole-body gamma planar imaging 4 h after treatment with ^{99m}Tc-EC20. The imaging showed that the radioactivity had accumulated primarily in the tumors and kidneys of the animals, which confirmed observations made during the biodistribution studies (1). No blocking studies were reported.

Reddy et al. studied the biodistribution of ^{99m}Tc-EC20 and ^{99m}Tc-EC53 in BALB/c mice (n = 3 animals/labeled compound) bearing M109 cell tumors (12). The animals were injected with the respective radiochemicals and euthanized 4 h later, and the radioactivity accumulated in the various organs was measured as described elsewhere (1). Although both tracers accumulated mainly in the tumors and the kidneys, the level of radioactivity in these tissues for animals treated with ^{99m}Tc-EC53 was ~33.0% less than that observed with ^{99m}Tc-EC20. In addition, tumor size, tumor location (subcutaneous or intraperitoneal), and route of radiotracer administration were reported to have no effect on uptake of the radiochemicals.

Other Non-Primate Mammals

[PubMed]

No reference is currently available.

Non-Human Primates

[PubMed]

No reference is currently available.

Human Studies

[PubMed]

The accumulation of ^{99m}Tc-EC20 in solid tumors and its correlation with FR expression, as determined with immunohistochemical (IHC) analysis, was investigated in a preliminary study of 154 cancer patients (14). Among these patients, 77% had a diagnosis of renal cell carcinoma and the remaining patients had a variety of other solid tumors. Gamma planar imaging was performed 1–2 h after treatment with ^{99m}Tc-EC20, followed by single-photon emission computed tomography of the index lesion regions. Data obtained with ^{99m}Tc-EC20 correlated with the IHC analysis of tissue available after surgery or from biopsies to detect the expression of FR-α. The accumulation of radioactivity in tumors was reported in 68% of the patients, and 67% of the tumors expressed FR-α. The two methods had an overall agreement of 61% (k = 0.096; 95% confidence interval = -0.085–0.277). There was 72% and 38% agreement of the positive and negative results, respectively, between the two methods.

From these results the investigators concluded that ^{99m}Tc-EC20 was safe and well tolerated by humans. The radiochemical could probably be used for a noninvasive procedure to select patients who could potentially benefit from FR-targeted therapy (14).

Supplemental Information

[Disclaimers]

No supplemental information is currently available.

References

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