^{111}In conjugated to benzyl-diethylenetriaminepentaacetic acid-human epidermal growth factor

[^{111}In]Bz-DTPA-hEGF

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Created: February 4, 2009; Updated: February 25, 2009.

Chemical name:	^{111}In conjugated to benzyl-diethylenetriaminepentaacetic acid-human epidermal growth factor	
Abbreviated name:	[^{111}In]Bz-DTPA-hEGF	
Synonym:		
Agent Category:	Receptor ligand	
Target:	Epidermal growth factor receptor (EGFR)	
Target Category:	Receptor	
Method of detection:	Single-photon computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click here for the protein and nucleotide sequence of human EGF.

Background

[PubMed]

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NLM Citation: Chopra A. ^{111}In conjugated to benzyl-diethylenetriaminepentaacetic acid-human epidermal growth factor . 2009 Feb 4 [Updated 2009 Feb 25]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Gliomas are the most common form of malignant brain tumors in humans. Although surgery, chemotherapy, or radiotherapy alone, or a combination of these therapies, comprise the most common treatment methods for this ailment, the prognosis for the patient is usually very poor (1). As an alternative, investigators have been exploring the use of receptor-based therapy for this cancer because with this technique the anti-cancer drugs can be targeted specifically to the tumor. Because the epidermal growth factor (EGF) receptor (EGFR) is known to be overexpressed in many glioma tumor types, it has been targeted for the treatment of this cancer with small-molecule drugs (SMDs) or antibodies (Abs) directed against the EGFR (click here for references: PubMed). The EGFR is a membrane-bound receptor with an extracellular region that can bind several different ligands, and it mediates its activity through an intracellular tyrosine kinase (TK) signaling pathway. This receptor has been shown to have a major role in the pathogenesis and progression of different cancers, as detailed elsewhere (2, 3). When a ligand, including an anti-EGFR Ab, is bound to the EGFR, the TK signaling pathway is activated and the receptor-ligand complex is rapidly internalized by the cell (4). The phenomenon of receptor internalization has been used by some investigators in an effort to improve the detection or treatment for a disease, including cancers, by linking a toxin, a SMD, or a radionuclide to Abs directed to a specific receptor (5-7).

Dadparvar et al. used anti-EGFR Abs labeled with radioactive indium (^{111}In, imaging gamma rays and Auger electrons emitter) to detect gliomas with scintigraphy in 23 human patients, and the investigators observed that uptake of the radiolabeled Ab by the tumors was variable (8). They concluded that uptake of the labeled Ab by the gliomas had almost no prognostic value even if the tumors were overexpressing the EGFR. This could be attributed to the large size of the Abs (~150 kDa), which may interfere with diffusion into deeper parts of the tumors. Because of its small size (~6 kDa), the use of radioiodinated (^{131}I) EGF for the radiotherapy of malignant gliomas has also been investigated, but this radiolabeled ligand was not very effective because ^{131}I dissociated quickly from the peptide and was excreted out of the cells (9). Subsequently, radioiodinated EGF-dextran (RED) conjugates were evaluated using human cultured glioma cell spheroids as a tumor model (10). Tumor penetration of the RED was reported to be slow, probably because of the large size of the radiolabeled peptide-dextran conjugate, and it took several hours for the radioactivity to penetrate into deeper parts of the spheroids. Also, synthesis of the RED is a complicated and laborious process. As an alternative to radioiodination, investigators labeled peptides with radiometals using chelating agents such as mercaptoacetyl-triglycerine, 2-iminothiolane, or a bifunctional metal-chelating peptide, but all of these chelating agents require complicated synthesis and are not readily available (11-13). Investigators have shown that EGF could be easily coupled to ^{111}In *via* diethylenetriaminepentaacetic acid (DTPA), a readily available metal chelating agent, and used for *in vitro* studies with cultured cells (14, 15). The labeled EGF was internalized by the cells and translocated to the nucleus, and the radioactivity was shown to be bound to the chromatin. Orlova et al. reported that ^{111}In conjugated to EGF, when internalized by the cells, had a superior cellular retention compared with ^{125}I derived from directly radioiodinated EGF (16). In addition, ^{111}In conjugated to EGF was

reported to have a selective toxicity toward MDA-MB-468 cells, a human cancer cell line with high EGFR expression (15).

The use of radioactive yttrium (^{90}Y, a high energy beta and Auger electrons emitter) conjugated to monoclonal Abs (MAbs) *via* DTPA was used to target human clonic tumors in nude mice, but the ^{90}Y-DTPA complex was determined to be unstable, to accumulate in the liver, and to have a rapid blood clearance compared with the ^{90}Y conjugate with the four isothiocyanatobenzyl (ITC-Bz) derivatives of DTPA (17). From this study the investigators concluded that the ITC-Bz derivatives of ^{90}Y were superior to the DTPA conjugates. Because different Auger-emitting radiometals can be conjugated to peptides with DTPA and the DTPA-radiometal conjugates are not satisfactorily stable, Sundberg et al. decided to investigate the biological characteristics of ^{111}In conjugated to benzyl-DTPA-human EGF(Bz-DTPA-hEGF) using the EGFR-rich U343MgaCl12:6 (U343) human glioma cell line under *in vitro* conditions (18). In another study, the biodistribution of ^{111}In-DTPA-hEGF and ^{111}In-Bz-DTPA-hEGF was compared in normal mice (19).

Synthesis

[PubMed]

The synthesis of ^{111}In-Bz-DTPA-hEGF was described by Sundberg et al. (18). Briefly, hEGF was obtained from commercial sources, and isothiocyanatebenzyl-DTPA (ITC-Bz-DTPA) was synthesized as described elsewhere (20). A hEGF solution in borate buffer (pH 9.1) was mixed with a solution of ITC-Bz-DTPA and left at room temperature overnight, and the Bz-DTPA-EGF conjugate was purified with reverse-phase high-performance liquid chromatography (RP-HPLC) with a SPEC C_{18} column using acetonitrile:water as the eluent. The buffer was then changed to acetate buffer (pH 6.5) using an NAP-5 column. The DTPA-hEGF conjugate was prepared and purified using procedures similar to those detailed above.

To obtain ^{111}In-labeled hEGF, the radiometal was mixed with either DTPA-hEGF or Bz-DTPA-hEGF in ammonium acetate buffer (pH 6.0), and the mixtures were incubated at room temperature for 1 h (18). The labeled conjugates were purified with the use of a NAP-5 column, and the labeled products were eluted in phosphate-buffered saline (PBS). Analysis of the labeled products was performed with the use of a SPEC RP-HPLC C_{18} column.

Stability of the labeled hEGF conjugates was studied by mixing the PBS solutions of the labeled conjugates with calf serum and leaving the mixture at 37°C for 24 h. After incubation, analysis of the labeled products was performed with the use of a SPEC RP-HPLC C_{18} column.

The ^{111}In labeling efficiency values for DTPA-hEGF and Bz-DTPA-hEGF were reported to be 89 ± 7% and 87 ± 4%, respectively (18). The radiochemical purity of the product was reported to be >96% as determined with RP-HPLC C_{18} column analysis. The maximum

specific activity of the labeled peptides was reported to be 66 GBq/μmol (1.78 Ci/μmol). The serum stability of both conjugates was similar, and the transchelation of radioactivity to the blood proteins was reported to be 5.3% and 4.6% with ^{111}In-Bz-DTPA-hEGF and ^{111}In-DTPA-hEGF, respectively, at 37°C after 24 h.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Sundberg et al. studied the binding and internalization of ^{111}In-DTPA-hEGF and ^{111}In-Bz-DTPA-hEGF with U343 cultured cells (18). The cells were exposed to the labeled peptides at 37°C for up to 24 h. The cells were harvested at different time points, washed with cold serum-free medium, and treated with ice-cold glycine buffer (pH 2.5) to remove any non-specific membrane or receptor-adhering radioactivity that has not been internalized. The cells were then treated with 1 M sodium hydroxide for at least 90 min and counted in a sodium iodide crystal to determine the amount of internalized radioactivity. Cellular internalization of both conjugates was reported to be rapid because the percent radioactivity within the cells was determined to be higher than the membrane-bound fraction (~45% *versus* ~10% for ^{111}In-DTPA-hEGF, and ~60% *versus* ~15% for ^{111}In-Bz-DTPA-hEGF at 8 h). To determine specificity of ^{111}In-DTPA-hEGF and ^{111}In-Bz-DTPA-hEGF binding, the cells were treated with the labeled conjugates in the presence of excess unlabeled EGF. Between the two conjugates, ^{111}In-Bz-DTPA-hEGF was reported to have faster cell-binding kinetics. Presence of excess unlabled EGF almost eliminated the binding and internalization of both the labeled conjugates, indicating that the two labeled peptides had a high specificity for the EGFR.

Saturation studies with ^{111}In-DTPA-hEGF and ^{111}In-Bz-DTPA-hEGF showed that the U343 cells had a maximal binding (total number of receptors) of 0.09 pmol/10^5 cells and a dissociation constant (K_d) of 2.7 and 2.0 nM, respectively (18).

Animal Studies

Rodents

[PubMed]

Tolmachev et al. compared the biodistribution of ^{111}In-DTPA-hEGF and ^{111}In-Bz-DTPA-hEGF in normal mice (19). The animals (n = 3/time point) were injected with the respective conjugates through the tail vein and euthanized at 0.5, 1, 4, and 24 h after injection. The blood and the main organs were then collected from the animals. Another set of animals (n = 3/labeled conjugate) were injected with 100 μg unlabeled hEGF, and the two labeled conjugates were respectively administered to the two groups of animals 0.5 h later. The animals were subsequently euthanized 0.5 h after administration of the labeled conjugates, and the various organs were collected as before. Radioactivity from both labeled conjugates was reported to clear rapidly from the blood with <1% remaining in circulation at 30 min. At any given time point, the amount of radioactivity remaining

in circulation with ^{111}In-Bz-DTPA-hEGF was two-fold that of ^{111}In-DTPA-hEGF. Among the organs, radioactivity from both the labeled conjugates was detected primarily in the liver (~10 to 17.5% of the injected dose/g tissue (% ID/g)) and kidneys (~10 to 35% ID/g). In other organs, the accumulated radioactivity was ~0.5–3% ID/g with both labeled compounds. Treatment of the animals with unlabeled EGF followed by administration of the labeled conjugates resulted in a reduced accumulation of radioactivity in the liver, but an increased uptake of the label was observed in the kidneys during the same time. Also, an increased level of radioactivity was observed in the blood with both conjugates when the animals were pre-injected with unlabeled EGF. Compared with ^{111}In-Bz-DTPA-hEGF, a significantly higher amount of ^{111}In-DTPA-hEGF was reported to accumulate in the kidneys and bones of the animals. The area under the curve ratios for ^{111}In-DTPA-hEGF *versus* ^{111}In-Bz-DTPA-hEGF were determined to be 2.1 and 2.6 for the kidneys and bones, 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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