^{131}I-Chlorotoxin
^{131}I-TM-601

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Chemical name:	^{131}I-Chlorotoxin
Abbreviated name:	^{131}I-TM-601
Synonym:	Cltx
Backbone:	Peptide
Target:	Matrix metalloproteinase 2 (MMP2) receptor
Mechanism:	Ligand-receptor binding
Method of detection:	SPECT
Source of signal:	^{131}I
Activation:	Not required
Studies:	✓ Rodents ✓ Humans

Click here for the protein sequence [http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=266221] of Chlorotoxin.

Background

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=ShowDetailView&TermToSearch=17335414&ordinalpos=5&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum]]

Chlorotoxin (CTX) is a neurotoxin comprising 36 amino acids and is isolated from the venom of *Leiurus quinquestriatus*, a scorpion of the Buthidae family. A characteristic feature of the peptide is the four disulfide bonds that give it a tight tertiary structure and a single tyrosine residue that can be iodinated. Originally, CTX was described as a calcium channel blocker (1). Later, selective and specific binding of CTX to glioma cells was demonstrated by immunochemical techniques, and radiolabeled CTX was shown to bind only to tumor cells in a mouse xenograft glioma tumor model (1). On the basis of *in vitro* studies, CTX was discovered to bind only to malignant (glioma) and not normal (glial) cells, indicating that the toxin had a binding specificity for embryologically related tumors, particularly those of neuroectodermal origin (2). Using a recombinant form of the toxin, it was shown that CTX binds to and reduces the activity of a matrix metalloproteinase (MMP) that is associated with and indirectly regulates functioning of the chloride channels on cell membranes (3, 4). Deshane et al. showed that isoform 2 of the MMP (MMP2) was the specific cellular receptor for CTX and that the ligand did not bind to the other MMPs also expressed on the glioma cells (4). In other *in vitro* studies it was shown that CTX reduced the migration ability of glioma cells through tight extracellular spaces in the brain tissue by inhibition of the MMP2, because this prevented the cells from shrinking and releasing from the extracellular matrix (5).

The MMP2 enzyme activity was observed to be significantly elevated in malignant glioma cells compared to low-grade glioma and normal brain tissues, and the upregulation correlated with the malignant progression of human gliomas *in vivo* (6). In the same study, which used immunohistochemical techniques, MMP2 was shown to be localized only in the cells and vasculature of malignant glioma tumors. These observations explained the specificity and selectivity of CTX binding to the malignant glioma tumors observed by the various investigators. Currently, resection of the tumor is the only effective treatment for glioma, but the tumor often reappears at the same spot or close to it because not all neoplastic cells can be completely removed during surgery.

Because of its specificity and selectivity for glioma cells, a synthetic version of CTX, TM-601, was generated either by production in *Escherchia coli* or by chemical synthesis (2, 4, 7). The peptide has been radiolabeled with radioactive iodine (^{131}I) to obtain ^{131}I-TM601 and used for preclinical and clinical imaging and radiotherapy of malignant glioma (8, 9). The iodinated peptide is currently being evaluated in clinical trials [http://www.clinicaltrials.gov/ct/search;jsessionid=984B9FFECC1840A9E42C96520F968618?term=TM-601] in the United States for the treatment of solid tumors or recurrent high-grade gliomas in humans (10).

Synthesis

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=DetailsSearch&Term=131I-TM-601]]

Three different methods are available for the production of CTX. This neurotoxin can either be isolated from *L.quinquestriatus* venom, or it may be produced and purified from *E. coli* by recombinant DNA and protein purification techniques (4, 7). A synthetic peptide version of CTX (TM-601) is available commercially (2). CTX is also available commercially as a freeze dried product,

Purification of CTX from scorpion venom was described by DeBin et al. (7). Crude venom was dispersed in deionized water using a Potter-Elvehjem tissue grinder. Insoluble materials in the suspension were removed by centrifugation, and the soluble portion was saved for further processing. The solution was filtered through a 10-kDa cut-off filter by centrifugation, and the filtrate was collected, vacuum-centrifuged, and reconstituted in trifluoroacetic acid (TFA). The TFA solution was batch-purified on a Waters C18 Sep-Pak cartridge, and the active component was eluted in TFA-containing acetonitrile, vacuum-centrifuged to dryness, and reconstituted in TFA for reverse-phase high-pressure liquid chromatography (RP-HPLC). All preparative RP-HPLC was performed on a Vydac TP-54 C18 column for batch purification of the various peptides. The peptides purified with RP-HPLC were repurified by ion-exchange HPLC on a polysulfoethyl aspartamide cation-exchange column, and the CTX peptide was eluted with a linear gradient of sodium chloride in phosphate buffer that contained acetonitrile (pH 4.0). Only fractions containing the center of the peaks that showed biological activity were pooled and retained from the various HPLC runs. The investigators did not provide a purification table for the scheme, and the final yield of purified CTX is not available in the publication.

For the production and purification of CTX in *E. coli*, a recombinant, polyhistidine (polyHis)-linked peptide (HIS-CTX) was produced (4). To produce HIS-CTX, the CTX was cloned into a pGHBE plasmid and then subcloned into a commercially available pRSETB vector that contains the

N-terminal polyHis tag used for purification of the recombinant peptide. The polyHis-tagged peptide was purified by metal affinity chromatography under buffer conditions as described by the manufacturer.

The synthetic peptide version of CTX has been used in some studies (2). The peptide is available commercially from several vendors.

Some investigators have used a lyophilized preparation of the peptide for their studies (9, 10). Recombinant CTX expressed in *E. coli* and purified by HPLC is available commercially as a lyophilized powder.

The radiolabeling of CTX with ^{131}I was described by Hockaday et al. (9), who used lyophilized CTX and followed the Iodogen method (11). Labeling of the peptide was determined by instant thin-layer chromatography. The investigators did not provide data detailing the Rf value of the ^{131}I-CTX under these conditions, nor did they provide specific activity or yield of the labeling reaction. However, they mentioned that a labeling efficiency of >92% was required for use in humans. Shen et al. (8) labeled CTX with ^{131}I as mentioned above and purified it on a Dowex column. They reported that the amount of free labeled iodine (^{125}I or ^{131}I) in the product was <5%, as determined by thin-layer chromatography. The preparations typically had a specific activity of 7.4 MBq/nM (200 µCi/nM), but the radiochemical yield was not mentioned.

In Vitro Studies: Testing in Cells and Tissues

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=DetailsSearch&Term=131I-TM-601+in+vitro]]

No publications are currently available.

Animal Studies

Rodents

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=DetailsSearch&Term=131I-TM-601+rodentia]]

A study to determine the dosimetery of ^{131}I-CTX in athymic mice with intracranially implanted human glioma xenografts was performed by Shen et al. (8). The investigators used ^{125}I-CTX as a surrogate radiochemical to study its tissue distribution in the animals. For this, athymic mice were implanted with D54 MG human malignant glioma xenografts in the right brain. Fifteen days after implantation, the mice were intracranially injected with ^{125}I-CTX in the right brain and sacrificed at 24, 48, 72, or 96 h after the injection. Subsequently, the blood, heart, stomach, small intestine, spleen, kidneys, liver, and thyroid of these animals were collected and weighed, and the radioactivity accumulation in each organ was determined. The ^{125}I-CTX accumulated primarily in the tumors, but the radiochemical was also detected in the stomach, kidneys, and thyroid of the animals. Concentrations of the radiolabel decreased in all organs except the stomach and thyroid. The investigators suggested that the radioactivity was detected in the stomach and thyroid probably as a result of accumulated radiolabel from dehalogenation of the radiochemical. The kidneys accumulated the

radiolabel because of rapid clearance through the urinary system. Data obtained from this study was also used to estimate doses to be used in a clinical trial for patients with malignant gliomas. Details of how this was done are given in the publication (8).

Other Non-Primate Mammals

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Details-Search&Term=131-TM-601+Non-Primate+Mammals]]

No publications are currently available.

Non-Human Primates

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Details-Search&Term=131-TM-601+Non-Human+Primates]]

No publications are currently available.

Human Studies

[PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Details-Search&Term=131-TM-601+Human+]]

A phase I/II clinical trial was performed in adult patients with recurrent high-grade glioma to investigate the biodistribution and toxicity of ^{131}I-CTX (9). A lyophilized preparation of CTX was used to generate the ^{131}I-labeled peptide used in this study. Patients underwent tumor resection, and an intracavitary reservoir was implanted to deliver a single dose of the radiochemical 2 to 4 weeks after the surgery. Total-body planar gamma imaging and whole-brain single-photon emission computed tomography scans were performed at 0, 1, 2, 3, and 6 to 8 days after administration of the radiochemical. Unbound ^{131}I was eliminated from the brain within 48 h and the remaining radioactivity remained bound to the tumor for 6 to 8 days. A comparison of the tumor volumes, as determined by imaging and magnetic resonance imaging (MRI), indicated that tumor volumes determined with ^{131}I-CTX closely paralleled the T2 volumes but not the T1-weighted gadolinium contrast volumes obtained with MRI. Similar results obtained with MRI and radiolabeling indicated that the labeled peptide was present in the tumor vicinity up to 7 days after injection. From these observations the investigators concluded that ^{131}I-CTX was a suitable radiochemical to determine the extent of a primary malignant glioma.

During the study it was observed that the radiolabel rapidly penetrated the cavity wall and ~79% of the radioactivity left the site of administration within 24 h (10). The remaining label was localized primarily in the tumor cavity and some label was detected in the surrounding regions, indicating that the peptide bound tightly to the tumor. The amount of label that accumulated in the stomach, spleen, and kidneys was low, but some accumulation was observed in the bladder. This was probably because the excretion route of the ^{131}I-CTX metabolites is through the urinary tract. Compared to other organs, some uptake of ^{131}I-CTX by the thyroid gland was evident, but it was below toxic levels because the patients had already received a high dose of potassium iodide 1 day prior to and 3

days after administration of the radiopeptide. The investigators concluded that ^{131}I-CTX may have an anti-tumoral effect and recommended that a phase II study should be conducted to confirm its efficacy.

References

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