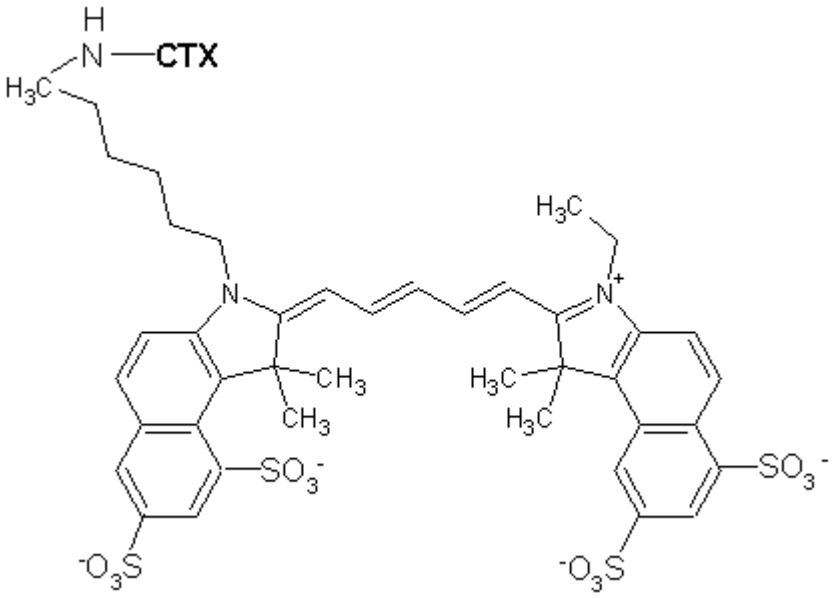
Chlorotoxin:Cy5.5

CTX:Cy5.5

The MICAD Research Team

Created: July 23, 2007; Updated: August 30, 2007.

Chemical name:	Chlorotoxin:Cy5.5
Abbreviated name:	CTX:Cy5.5
Synonym:	Tumor paint
Agent Category:	Peptide
Target:	Matrix metalloproteinase 2 (MMP2)
Target Category:	Enzyme-substrate binding
Method of detection:	Near infrared (NIR)
Source of signal:	Cy5.5
Activation:	Not required
Studies:	• *In vitro* • Rodents



Structure of Chlorotoxin:Cy5.5.

Background

[PubMed]

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 that it contains 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) that was later shown to bind specifically to

NLM Citation: The MICAD Research Team. Chlorotoxin:Cy5.5. 2007 Jul 23 [Updated 2007 Aug 30]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

isoform 2 of a matrix metalloproteinase (MMP2) associated with the channels (2). The MMP2 was shown to be upregulated in many cancers, especially glioblastomas, and there appears to be a correlation between the level of MMP2 expression and poor outcome of the disease (1-6). For most neoplastic conditions, a surgical resection of affected tissue is the most common treatment, and the precision of this technique can influence patient prognosis. The identification and removal of tumors is largely based on a surgical judgment and can be imprecise because it involves avoiding the removal of normal or apparently healthy tissue. Incomplete removal of cancer cells is a problem often encountered with brain tumors, and >80% of malignant cancers recur at the site of tumor removal (7). Clear distinction of tumor tissue from normal tissue could alleviate the problem of incomplete removal of cancer tissue by surgical resection and help improve patient prognosis. The development of agents that bind to tumor-specific molecules and their use to illuminate tumor cells during surgery would greatly facilitate the achievement of this goal.

Biocompatible fluorescent contrast agents conjugated to peptides, proteins, or antibodies, including those visible with near infrared (NIR) light, have been developed and evaluated for the optical imaging of tumors (8, 9). These agents can be used for non-invasive or intraoperative imaging because water or hemoglobin do not absorb in the NIR spectrum and allow the photons to penetrate the tissue. The use of a cyanine (Cy) dye conjugated to a variety of molecules has been explored to image various cancers (10). From these studies it was concluded that small peptides offer a greater advantage over large molecules, e.g., antibodies, because they are easy to synthesize and modify, are likely to be less immunogenic, can be rapidly cleared from circulation, and rapidly accumulate in the target area to give a high signal/background ratio.

Brain tumor imaging has been performed with NIR probes that targeted tumor microglia or required proteolytic activation (11, 12). However, the former approach does not always correlate with detection of the brain tumor margins because patients are often treated with dexamethasone, an inhibitor of microglia activation, to reduce post-operative edema. Previously, a multifunctional probe fabricated with iron oxide nanoparticles coated with covalently bound bifunctional polyethylene glycol, CTX, and Cy5.5 was developed and successfully used *invitro* to image glioma cells (13). CTX and Cy5.5 are available commercially in the United States. Fluorescence microscopy and magnetic resonance imaging showed that conjugated nanoparticles were taken up preferentially by the glioma cells. From observations made during the study, the potential use of the conjugate for real-time imaging of glioma brain tumors was suggested. Subsequently, Veiseh et al. developed and evaluated a CTX:Cy5.5 dye bioconjugate that requires no protease activation to image neoplastic tumors in a mouse model (14).

Synthesis

[PubMed]

The synthesis of CTX:Cy5.5 was described by Veiseh et al. (14). Both CTX and the Cy5.5 dye (as an ester) were purchased from commercial sources. The Cy5.5 dye was dissolved in anhydrous dimethyl formamide and mixed with a CTX solution in bicarbonate buffer (pH 8.5) at a ratio of 3:1. The conjugation was allowed to proceed for 1 h at room temperature in the dark. Excess dye was removed by dialysis against phosphate-buffered saline (PBS) for 18 h at 4°C. The samples were diluted in PBS to various appropriate dilutions and filter-sterilized for use in the different studies. Quality of the conjugated product was monitored by mass spectroscopy. The yields, purity, and Cy5.5/CTX ratio of the conjugated product were not provided in the publication.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

For *in vitro* evaluation of CTX:Cy5.5, 9L glioma cells and primary fibroblasts (as controls) were exposed to the same concentration of the bioconjugated agent under identical conditions and the binding was visualized by NIR fluorescence (NIRF) microscopy (14). All the 9L glioma cells exposed to the probe emitted a signal in the NIRF spectrum, and a minimal signal was detected with the fibroblasts. It was also observed that the MMP2 inhibitor 1,10-phenanthroline blocked the binding of CTX:Cy5.5 to the 9L glioma cells, which indicated that the CTX:Cy5.5 bioconjugate bound specifically to the metalloproteinase.

In another study to show that MMP2 is specifically involved in the binding of CTX:Cy5.5, MCF7 cells known to express low levels of MMP2 and bond CTX:Cy5.5 poorly compared to the other cell lines were transfected with a plasmid encoding the MMP2 (14). Cells expressing the MMP2 were sorted from the untransfected cells and an increased expression of the proteinase in these cells was confirmed by indirect immunofluorescence, Western blot analysis, and gelatinase assays. Although no signal/noise ratios were provided by the investigators, from image analysis it was clear the MMP2-transfected cells showed higher CTX:Cy5.5 binding compared to control cells transfected with the control plasmid (which contained no MMP2 insert). This again confirmed that CTX:Cy5.5 bound specifically to MMP2.

Animal Studies

Rodents

[PubMed]

The *in vivo* activity of CTX:Cy5.5 was investigated non-invasively by injecting the bioconjugate into mice bearing 9L glioma xenografts (14). The NIRF signal was significantly higher in all xenografts compared to the non-neoplastic tissue at all time points through day 14. Also, mice bearing 9L glioma cell flank xenografts showed a significantly increased NIRF signal compared to that observed in the brain at all time points tested. To investigate the specificity of the CTX:Cy5.5 signal from the tumors, the

probe was coadministered with CTX to mice bearing the xenografts. With the use of biophotonic imaging, CTX was observed to block the binding of CTX:Cy5.5 to the tumors. In another study, Cy5.5 alone was administered to the mice bearing xenografts and, for comparison, another group of the same mice received the bioconjugate alone. The NIRF signals obtained from the tumors of these two groups of mice were compared. With the use of imaging it was observed that mice injected with the probe had a significantly higher tumor signal/noise ratio (from ~15-fold higher at day 1 to four-fold higher at day 4 after the injection) compared to mice injected with Cy5.5 alone. A similar distinction was observed between tumors and normal tissue in a brain xenograft model. This distinction showed that CTX:Cy5.5 bound specifically to the neoplastic tissue and did not bind to the surrounding, normal, healthy tissue.

Imaging studies were also conducted to determine whether CTX:Cy5.5 illuminated cancer foci of MMP2-positive tumors that were not of neuroectodermal origin (14). Transgenic mice that expressed the SV40T gene in the prostate epithelium (15) were injected with CTX:Cy5.5. The probe was observed to illuminate the primary prostate cancer as well as metastatic tissue in the lungs. It was also reported that the microscopic foci of cancer cells in the lymphatic channels and the lymph nodes of the animals were easily detected under simulated surgical conditions. A similar study was performed with mice bearing Rh30 rhabdomyosarcoma xenografts. The investigators observed that CTX:Cy5.5 targeted the tumor specifically compared to the normal surrounding tissue in this model as well. Presence of cancer cells in the tumors or absence of cancer cells in the normal tissue, as distinguished by the use of CTX:Cy5.5, was also confirmed by pathological examination. These studies demonstrated that CTX:Cy5.5 could help clearly distinguish cancerous tissue from normal tissue under simulated operating conditions.

The tissue distribution of CTX:Cy5.5 was also studied (14). The unbound bioconjugate was fairly evenly distributed through the mouse body, except in the renal collecting system, up to 96 hours after administration. The renal collecting system had a high NIRF signal compared to the other organs because the bioconjugate is excreted in the urine.

From these studies, the long duration (up to 14 days) of probe activity allowed tumor imaging even after the peak circulating levels had dropped significantly. The clear distinction of tumors from normal tissues led the investigators to conclude that CTX:Cy5.5 could be a good probe for the detection of neoplastic cells during surgery to remove tumors from human patients.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

References

1. Soroceanu L., Gillespie Y., Khazaeli M.B., Sontheimer H. Use of chlorotoxin for targeting of primary brain tumors. Cancer Res. 1998;**58**(21):4871–9. PubMed PMID: 9809993.
2. Deshane J., Garner C.C., Sontheimer H. Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. J Biol Chem. 2003;**278**(6):4135–44. PubMed PMID: 12454020.
3. Sakakibara M., Koizumi S., Saikawa Y., Wada H., Ichihara T., Sato H., Horita S., Mugishima H., Kaneko Y., Koike K. Membrane-type matrix metalloproteinase-1 expression and activation of gelatinase A as prognostic markers in advanced pediatric neuroblastoma. Cancer. 1999;**85**(1):231–9. PubMed PMID: 9921997.
4. Kanayama H., Yokota K., Kurokawa Y., Murakami Y., Nishitani M., Kagawa S. Prognostic values of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 expression in bladder cancer. Cancer. 1998;**82**(7):1359–66. PubMed PMID: 9529029.
5. Davidson B., Goldberg I., Kopolovic J., Lerner-Geva L., Gotlieb W.H., Ben-Baruch G., Reich R. MMP-2 and TIMP-2 expression correlates with poor prognosis in cervical carcinoma--a clinicopathologic study using immunohistochemistry and mRNA in situ hybridization. Gynecol Oncol. 1999;**73**(3):372–82. PubMed PMID: 10366463.
6. Chambers A.F., Matrisian L.M. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst. 1997;**89**(17):1260–70. PubMed PMID: 9293916.
7. Barker F.G., Chang S.M., Gutin P.H., Malec M.K., McDermott M.W., Prados M.D., Wilson C.B. Survival and functional status after resection of recurrent glioblastoma multiforme. Neurosurgery. 1998;**42**(4):709–20. PubMed PMID: 9574634.
8. Bremer C., Ntziachristos V., Weissleder R. Optical-based molecular imaging: contrast agents and potential medical applications. Eur Radiol. 2003;**13**(2):231–43. PubMed PMID: 12598985.
9. Ntziachristos V., Bremer C., Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol. 2003;**13**(1):195–208. PubMed PMID: 12541130.
10. Tung C. Fluorescent peptide probes for in vivo diagnostic imaging. Biopolymers. 2004;**76**:391–403. PubMed PMID: 15389488.
11. Kircher M.F., Mahmood U., King R.S., Weissleder R., Josephson L. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. Cancer Res. 2003;**63**(23):8122–5. PubMed PMID: 14678964.

12. Ntziachristos V., Tung C.H., Bremer C., Weissleder R. Fluorescence molecular tomography resolves protease activity in vivo. Nat Med. 2002;**8**(7):757–60. PubMed PMID: 12091907.
13. Veiseh O., Sun C., Gunn J., Kohler N., Gabikian P., Lee D., Bhattarai N., Ellenbogen R., Sze R., Hallahan A., Olson J., Zhang M. Optical and MRI multifunctional nanoprobe for targeting gliomas. Nano Lett. 2005;**5**(6):1003–8. PubMed PMID: 15943433.
14. Veiseh M., Gabikian P., Bahrami S.B., Veiseh O., Zhang M., Hackman R.C., Ravanpay A.C., Stroud M.R., Kusuma Y., Hansen S.J., Kwok D., Munoz N.M., Sze R.W., Grady W.M., Greenberg N.M., Ellenbogen R.G., Olson J.M. Tumor paint: a chlorotoxin:cy5.5 bioconjugate for intraoperative visualization of cancer foci. Cancer Res. 2007;**67**(14):6882–8. PubMed PMID: 17638899.
15. Huss W.J., Maddison L.A., Greenberg N.M. Autochthonous mouse models for prostate cancer: past, present and future. Semin Cancer Biol. 2001;**11**(3):245–60. PubMed PMID: 11407949.