Introduction

The evolution of toxin production has enabled organisms to adapt to ever changing environments and increase species fitness for survival. These developed toxins are used for protection and apprehension of prey. Science and biotechnology has tapped into the mode of action of toxins from organisms such as snakes, spiders, scorpions, cone snails, marine corals, etc. and discovered the increasing potential for human use. Current research and development of scorpion toxins has proved to be successful in the treatment of cancers. There has been advanced achievements in the use of scorpion toxins in the treatment and early detection of malignant glioma brain tumors.

There are approximately 80,000 patients diagnosed with gliomas each year throughout the world. Gliomas are the most common type of brain tumors and have a high mortality rate with most sufferers dying within twelve to eighteen months from diagnosis. The rate of tissue infiltration and invasiveness of these tumors render them inoperable. Researchers have found a chemical in the yellow Israeli scorpion’s (*Leiurus quinquestriatus*) venom that possesses capabilities of inhibiting the actions of the glioma tumors. This chemical has been identified as chlorotoxin and is used in treatment of gliomas and in the identification of glioma cells.

Characterization of Chlorotoxin
Arachnid venom is characterized by its ability to induce paralysis by blocking the ion channels involved in the generation of action potentials leading to muscle contraction. Scorpion venoms are sodium, potassium, chloride, and ryanodine channel selective peptides. *Leiurus quinquestriatus*, commonly known as the yellow Israeli or death stalker scorpion, possesses venom that is the most toxic of all scorpions. This chemical contains “histamines, enzymes, enzyme inhibitors, and the potent neurotoxins chlorotoxin and charbydotoxin”(Neurophysiologywordpress.com).

Chlorotoxin is a small peptide toxin consisting of thirty-six amino acids with four disulfide bonds (Figures 1-2) "that give it a tight tertiary structure and a single tyrosine residue that can be iodinated" or treated with iodine. (http://www.ncbi.nih.gov/bookshelf/br.fcgi?book=micad&part=CTXCy55) The disulfide bonds play an important role in the folding structure and function of some proteins, especially those secreted to the extracellular matrix. They assist the protein in stabilizing the tertiary structure by holding proteins together with strong bonds, increasing hydrophobic interactions, and increasing the concentration of protein residues consequently decreasing concentration of water molecules. Chlorotoxin affects cells by blocking chloride channels within the cell. Chlorotoxin amino acid sequence is significantly homologous to other insect toxins (Debin, 1993).

**Figure 1. Amino acid sequence of chlorotoxin**

H - Met - Cys - Met - Pro - Cys - Phe - Thr - Thr - Asp - His - Gln - Met
- Ala - Arg - Lys - Cys - Asp - Asp - Cys - Cys - Gly - Gly - Lys - Gly -
- Arg - Gly - Lys - Cys - Tyr - Gly - Pro - Gln - Cys - Leu - Cys - Arg -
NH2 (Disulfide bridge: 2 - 19,5 - 28,16 - 33,20 - 35)

http://www.anaspec.com/products/product.asp?id=30976&_kk=chlorotoxin&_kt=63515ae0-35f0-42d6-ae69-6e1b6b2949a7&gclid=COPy8--rk54CFWpd5QodDI1UpQ
**Gliomas**

Gliomas are the most widespread of brain tumors. These particular cancerous cells have a unique invasive ability that allows them to infiltrate the brain and produce inoperable tumors. Gliomas originate from glial support cells within the brain. These support cells function in the nutrition and support of the neurons and other cells. The mechanism by which these tumors become invasive is by the “modification of receptor-mediated adhesive properties of tumor cells, degradation and remodeling of extracellular matrix by tumor-secreted metalloproteinases, and creation of an intercellular space for invasion of tumor cells” (Deshane, 2003). The gliomas utilize special chloride channels that are not found in healthy cells to spread into small tightly packed spaces in the brain. The introduction of extracellular components takes place at the “confrontation zone” between the glioma cells and the healthy brain tissue (Deshane, 2003). Molecules that enhance the tumor cells ability to move throughout the brain are developed within the extracellular matrix. The interactions between the gliomal cells and the extracellular matrix occur through integrins within the tumor cells. “In addition to these interactions with the extracellular brain environment, invading glioma cells undergo dramatic shape and cell volume changes allowing them to make their way through the narrow extracellular spaces” (2003). In order for successful invasion to occur, fluid composed of chloride ions and metalloprotease enzymes must be secreted. The metalloproteases are enzymes that belong to a protease group that breakdown or digest proteins. Once invasion
takes place, the tumor grows and compresses the normal brain cells against the internal wall of the skull. This pressure on the healthy tissue prohibits proper functioning of the brain and high incidence of paralysis.

(http://neurophilosophy.wordpress.com/2006/06/28/a-potential-scorpion-venom-based-treatment-for-gliomas/)

**Figure 3.** Glioma tumor causing shift of tissue increasing compression of internal wall

![Image of glioma tumor with glioma label](http://www.urmc.rochester.edu/neuroslides/slide064.html)

**Crossing the Blood Brain Barrier**

Glioma tumors prove to be difficult to treat with chemical therapies due to the selectivity and protective characteristics of the blood brain barrier. The blood brain barrier functions as a highly selective gateway for nutrients and gases to exchange between the blood and brain. It inhibits the entry of microbes, immune response cells, large molecules, and many drugs. The barrier is made of capillaries that function in waste removal and nourishment. “Each capillary vessel is bound by a single layer of endothelial cells, connected by ‘tight junctions,’ thereby making it very difficult for most molecules to exit the capillaries and permeate into the brain”(NCI, 2005). A substance must be
“recognized” by the barrier to be permitted to cross. Nanoparticles seem to cross the barrier depending on the size of the particle, what it is composed of, and it molecular structure. Some large tumors can disrupt the integrity of the blood brain barrier allowing penetration by nanoparticles. Once penetration has been achieved, the nanoparticle can distribute the attached substance and detect or inhibit cancer cells. The chlorotoxin-bound nanoparticle used in detection imaging is a “15 nanometer” particle that is capable of crossing the blood brain barrier. (NCI, 2005)

**Use of Chorotoxin in Identification and Treatment of Gliomas**

A synthetic form of chlorotoxin has been developed and proved to reduce the spread of glioma tumors. When attached to a poison, chlorotoxin was found to destroy glioma tumors with no adverse affects to the healthy brain cells (Figure 4).

**Figure 4. Chlorotoxins affect on brain tumors**

![Figure 4. Chlorotoxins affect on brain tumors](http://www.sfn.org/index.aspx?pagename=publications_rd_toxins)

Chlorotoxin specifically binds to the surface of glioma cells and impairs their ability to invade the healthy brain (Deshane, 2003). "Glioma cell migration and invasion into fetal brain aggregates is significantly reduced by chlorotoxin"(Ullrich, 1998). Chlototoxin has been shown to inhibit chloride channels in epithelial cells and chloride fluxes across glioma membranes. Matrix Metalloproteinase -2 (MMP-2) has been identified as the receptor for chlorotoxin. Metalloproteinases are involved in the hydrolysis of the extracellular matrix and remodeling of normal tissues (Visse, 2003). "MMP-2 belongs to
a superfamily of zinc-dependent endopeptidases and is "secreted as a latent zymogen." Zymogens are a group of compounds that are inactive precursors of enzymes and require some change before activation occurs (http://wordnetweb.princeton.edu/perl/webwn?s=zymogen). Tumor cells use MMPs to degrade and remodel the matrix of healthy cells and destroy the chloride channels within the membrane and therefore allow “metastatic spread” of the tumor (Brown, 1995). "Human cells rarely express MMP II, but glioma cells, which are constantly reconstructed, express MMP II almost all the time”(www.the scientist.com). Chlorotoxin binds to the matrix metalloproteinase II complex. The enzymatic role of MMP-2 in glioma cells is inhibited with the introduction of chlorotoxin, consequently reducing its expression. Metalloproteinase-2 is expressed in other tumor types that are involved in the remodeling of tissues but plays a more significant role as a matrix metalloproteinase in gliomas. (Deshane, 2003). Harold Sontheimer, a neurobiology researcher at the University of Alabama, Birmingham states cockroach cells express the MMP-2 complex which may explain why insects are so vulnerable to the effects of the Leiurus quinquestriatus venom (www.thescientist.com).

Inhibition of enzyme function is the method by which chlorotoxin renders gliomas ineffective. In order for successful tumor invasion to occur, there must be an "efflux of chloride ions from the cancerous cells" (Ullrich, 1998). Low-conductance glioma chloride channels mediate the outflow of chloride ions from the tumor cells. The chlorotoxin selectively binds to the glioma chloride channels and inhibit their function, preventing the passage and invasion of glioma cells. The chloride channels' balancing ability is disrupted and the glioma cell is prevented from shrinking and traveling to other areas in the brain (University of Washington, 2009).

In addition to its ability to inhibit glioma invasions, chlorotoxin has proven to be successful in the early identification of glioma cells. “Synthetic and fluorescently labeled forms of chlorotoxin retain the properties of the natural molecule; chlorotoxin conjugated to nanoparticles has been used to help in the detection of gliomas by neuroimaging” (University of Washington, 2009). The Fred Hutchinson Cancer Research Center in
Seattle used the affinity of chlorotoxin for brain tumor cells and attached a fluorescent marker to the peptide (Figure 5). Within hours after injection, animal tumors glowed. He states that ‘the problem is so many cells go undetected’. Chlorotoxin spotted as few as 2,000 cancer cells, 500 times more powerful than magnetic resonance imaging. Using chlorotoxin, surgeons can spot tumor cells with infrared light. (www.thescientist.com)

**Figure 5. Nanoparticle Chlorotoxin**

![Schematic diagram for synthesis of nanoparticle-chlorotoxin (NPC) and NPC-Cy5.5 conjugates. NPC-Cy5.5 is able to bind to and fluorescently illuminate glioblastoma tumors.](Reprinted with permission from ref. 4; Copyright 2005 American Chemical Society)

TransMolecular, Inc. has synthesized the chlorotoxin peptide and attached iodine-131 as a radioactive isotope. The synthetic radio-labeled peptide, known as TM-601, is injected into the bloodstream where it “homes in on glioma cells, delivering its cargo of radioactive atoms. These atoms then decay, destroying the cancerous cells in the process while leaving adjacent healthy cells unaffected. TM-601 acts like a ‘smart missile’ because the glioma cells it targets divide rapidly, and therefore have properties similar to those of invertebrate cells. Invertebrates, which constitute the prey of the scorpion, have rapidly dividing cells, which gives those organisms the capacity for quick regeneration of damaged tissue”(University of Washington, 2009). There have been no reported side-effects observed with the use of the synthetic chlorotoxin and is eliminated as waste product in the urine and sweat. Scorpion venom evolved to attack rapidly dividing cells in order to achieve rapid effects of envenomation and this evolved adaptation has proved useful in biotechnology.
Conclusion
The mode of action for scorpion venom has been identified as ion channel blocking. This venom has evolved to affect rapidly dividing cells in prey. This characteristic of *Leiurus quinquestriatus*, also known as the death stalker and the Israeli yellow scorpion, has made it a useful tool in the early identification and treatment of glioma tumors in humans. Gliomas are generally inoperable and lethal. The small peptide, Chlorotoxin, produced by this scorpion binds to the matrix metalloproteinases in the glioma cells inhibiting their function and consequently inhibiting their invasion of the extracellular matrix. Chlorotoxin bound to nanoprobes enable early identification of glioma tumors by fluorescence. These nanoprobes are 500 times more effective than current magnetic resonance imaging techniques.
References


http://neurophilosophy.wordpress.com/2006/06/28/a-potential-scorpion-venom-based-treatment-for-gliomas/

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