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Chlorotoxin: A Scorpion Venom-Derived Peptide for Targeted Glioma Therapy and Imaging

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ABSTRACT

Glioblastoma multiforme (GBM) is a fatal brain cancer that has a dismal prognosis because of the blood-brain barrier (BBB) and the invasiveness of the tumors that make them difficult to treat. Chlorotoxin (ClTx) is a 36-amino acid peptide of scorpion Leiurus quinquestriatus venom that has become a highly specific targeting agent of gliomas. Its action is binding to matrix metalloproteinase-2 (MMP-2), chloride channel ClC-3, and Annexin A2, which prevents tumor cell invasion, migration, and survival.

This review identifies the multifunctional uses of chlorotoxin in the treatment of glioma. Chlorotoxin conjugates such as Tumor Paint (ClTx-Cy5.5) and Tozuleristide (BLZ-100) can be used in diagnostics to allow visualization of tumors and delineation of the margins during surgery. Chlorotoxin is a perfect targeting ligand to use in therapy, which improves the delivery of chemotherapeutic drugs (e.g., doxorubicin, temozolomide) across the BBB through functionalized nanocarriers such as liposomes and polymeric nanoparticles. This is a focused strategy that enhances antitumor activity and reduces systemic toxicity. Also, chlorotoxin has been demonstrated as a promising targeted radiotherapy (131I -TM -601) and a fusion conjugate with cytotoxic proteins (e.g., gelonin) to selectively kill tumor cells.

The positive safety profile and tumor-specific targeting of chlorotoxin-based agents with the help of the initial clinical trials highlight their great potential to transform the diagnosis and treatment of glioma. Chlorotoxin represents a promising paradigm shift toward precision neuro-oncology, offering new hope to enhance patient outcomes.

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Introduction

Glioblastoma multiforme, the deadliest primary tumor of the central nervous system, with a five-year survival rate of less than 10 %, is regarded as one of the most significant problems of modern medicine. [1,2] Its aggressive nature, ability to invade normal brain tissue, and the presence of the blood-brain barrier that restricts therapeutic access are the primary barriers to effective treatment .[3] Traditional treatment methods, such as surgery, radiotherapy, and chemotherapy, though partially effective, fail to prevent the recurrence of the disease entirely. [2]

In this regard, the development of new therapeutic approaches that can specifically attack tumor cells is of vital importance. Chlorotoxin, a peptide found in scorpion venom, has attracted the attention of researchers in recent years as an emerging option in the field of cancer .[4,5] This peptide has shown great potential in the treatment and diagnosis of cancer by binding to specific receptors on the surface of cancer cells.[6]

Past research has revealed that chlorotoxin has versatile biological impacts. This peptide is not only used as a targeting agent in the oncology field, but it has been shown that it can also have direct antitumor effects, inducing apoptosis in cancerous cells of the nervous system.[7,8]

Chlorotoxin has been applied in different areas in the management of glioblastoma. In imaging, conjugates of this peptide can be used to accurately determine tumor boundaries in surgery.[9,10] This peptide has been utilized in the field of therapy to selectively deliver chemotherapeutic agents, radiotherapy agents and new drug delivery systems to tumor cells.[10,11] The possibilities of this peptide in the creation of theranostic systems with the potential to diagnose and treat at the same time have also been explored.[12]

In this comprehensive review, we have collected and analyzed reports presented in recent years regarding the clinical potential of chlorotoxin in the treatment of glioblastoma, and have also presented the future prospects for the use of this peptide in neurooncology.

Molecular Structure and Physicochemical Properties of Chlorotoxin

Chlorotoxin is a 36-amino acid cationic peptide that has a molecular weight of 4007 Daltons (Table 1).[4] The high structural stability of this peptide is due to the presence of four disulfide bonds between cysteine residues (Cys2-Cys19, Cys5-Cys28, Cys16-Cys33, Cys20-Cys35), which creates a compact globular core (Figure 1).[6] This topography offers great resistance to proteolytic degradation. The other characteristic of chlorotoxin is that it has an alkaline isoelectric point (pI: 9.5), which can be explained by the large proportion of lysine and arginine amino acids. This is a high positive charge that probably contributes to the basic electrostatic interaction with negatively charged cell membrane constituents.[13]

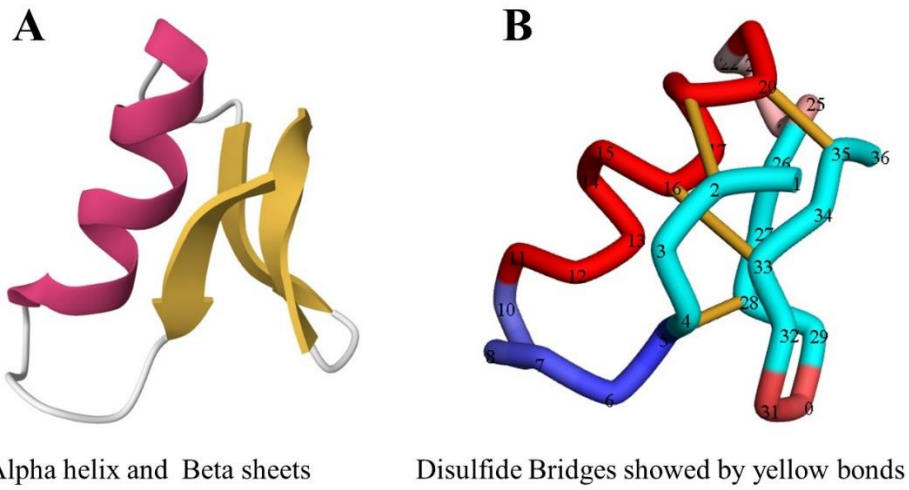

Figure 1: (A): was generated using the Mol Viewer web tool by extracting the structure from the protein data bank (PDB) entry 1CHL. This panel shows the overall protein structure with emphasis on the alpha-helix region (colored pink) and beta-sheet regions (colored yellow). (B): was created using the same PDB code and visualized with Discovery Studio software. This panel highlights the peptide's structural stability by illustrating the disulfide bridges (colored yellow) formed between cysteine residues within the protein.

Table 1: Characteristics of Chlorotoxin.

Feature	**Description**
Name	Chlorotoxin
Source	Venom of the deathstalker scorpion (*Leiurus quinquestriatus*)
Amino Acid Sequence	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
Molecular Weight	4007 Da
Isoelectric Point (pI)	9.52
Secondary Structure	One α-helix (residues 11–21), two β-sheets (N- and C-terminal)
Disulfide Bridges	4 bonds: Cys2–Cys19, Cys5–Cys28, Cys16–Cys33, Cys20–Cys35
Net Charge	Positive (due to Lys, Arg residues)
Key Molecular Targets	MMP-2, ClC-3 chloride channel, Annexin A2
Biological Functions	Inhibits cell invasion & migration, induces apoptosis, anti-angiogenic
Applications	Tumor imaging (BLZ-100, MRI, SPECT), targeted drug delivery, radiotherapy, theranostics
Clinical Status	Phase I trials completed for imaging (BLZ-100) and targeted radiotherapy (^{131}I-TM-601)

Molecular Targets of Chlorotoxin

It has been shown that chlorotoxin has a number of molecular targets, which point to its multifaceted mechanism of action. The main functional receptor of chlorotoxin is known to be matrix metalloproteinase-2 (MMP-2). The peptide selectively attaches to the active isoform of MMP-2 and acts in two independent manners: blocking the catalytic activity of the enzyme and causing internalization of the enzyme, and hence, decreasing its surface expression. Moreover, the glioma chloride channel, namely, ClC-3 is also known to be a significant target of chlorotoxin. Its suppression causes the loss of cell volume control, therefore, suppressing cell migration and invasion. Annexin A2 has also been suggested as a possible receptor of chlorotoxin based on preliminary studies; however, this interaction is not yet fully validated and requires further confirmation. Annexin A2 is hyper-expressed on the surface of glioblastoma cells and may be involved in the uptake and internalization process of chlorotoxin. These various molecular targets combined give a strong basis to the wide range of use of chlorotoxin in the diagnosis and treatment of brain tumors.[13-15]

Mechanism of Invasion and Metastasis Inhibition

The anti-invasive property of chlorotoxin is mainly explained by the inhibition of the MMP-2 activity.[13] This enzyme helps tumor cells to be infiltrated and invaded by breaking down the elements of the extra-cellular matrix (ECM). Chlorotoxin inhibits the degradation of ECM by binding to and inhibiting this enzyme, therefore, greatly diminishing the invasive potential of glioma cells in different models. [13,16,17] Also, the anti-invasive effect could be caused by inhibition of chloride channels such as ClC-3, which interferes with the cell shape changes required to move through tissue.[14]

Other Receptors and Cellular Signaling

Even though the main known targets are MMP-2, ClC-3, and Annexin A2, there is evidence that chlorotoxin can bind to other receptors. The peptide is capable of binding to large protein complexes in the cancer cell membrane, which suggests that there is a multi-component signalosome on the cell surface. [6] The exact downstream signaling pathway of chlorotoxin is not known yet but it has been observed that this peptide may cause changes in actin cytoskeleton organization and blockage of its dependent processes.[18] In addition, some chlorotoxin conjugates [8] induce apoptosis, indicating that the peptide is capable of triggering cell death eventually.

Imaging Applications

Specific binding of chlorotoxin to glioma cells makes it a useful tool in the development of advanced imaging systems. Such systems allow accurate diagnosis, margin delineation of tumors in surgery and monitoring of response to treatment. [4,6,9,10,12]

Fluorescence and Near-Infrared Imaging

This technology is one of the most viable uses of chlorotoxin. The chlorotoxin-Cy5.5 conjugate, which is a tumor paint, has been shown to be able to visualize tumor margins and small metastatic lesions in animal models.[9] The technology was further developed to BLZ-100 (Tozuleristide), in which chlorotoxin is conjugated with the indocyanine green (ICG) dye. The efficacy and safety of this conjugate in glioma patients have been confirmed in phase I clinical trials.[10,19] Also, the use of IRDye 800CW has shown that chlorotoxin can target brain tumors.[20]

Magnetic Resonance Imaging (MRI)

Chlorotoxin is conjugated to superparamagnetic iron oxide nanoparticles (SPIONs) in this area to be used as a contrast agent. It has been demonstrated in several studies such as by Sun et al. and Veiseh et al. that these chlorotoxin-functionalized nanoprobes are capable of crossing the blood-brain barrier and specifically accumulating in glioma tumors.[12,21,22] The targeted glioma cells using SPIO-FITC-cltx nanoparticles were also confirmed by Meng et al. .[23] Subsequent advancement of this technology has given rise to the development of silica nanoparticles that comprise of magnetite nanoparticle clusters that have demonstrated great potential in the detection of brain tumors .[24]

Nuclear Imaging (SPECT)

Chlorotoxin has been conjugated with gamma-emitting radioisotopes to be used in Single-photon emission computed tomography (SPECT) imaging. It has been demonstrated that ^{111}I-TM-601 can be successfully used as an imaging and dosimetry agent in studies involving ^{131}I-TM-601. [25-27] Other promising findings in SPECT imaging of glioma have also been reported by Qiao et al. using ^{131}I-BmK CT .[28] Moreover, Zhao et al. created a theranostic platform based on polyethylenimine, which allows performing SPECT imaging and drug delivery at the same time .[29]

Therapeutic Applications

The distinctive targeting ability of chlorotoxin to glioma cells has facilitated the emergence of various and novel therapeutic approaches that are mainly based on three main approaches.[30-32]

Targeted Radiotherapy

Chlorotoxin has also been utilized as a targeting moiety to internal radiotherapy by conjugating it with radioisotopes like Iodine-131 to form the therapeutic agent ^{131}I-TM-601.[25,27] The preclinical trials have shown that this conjugate has selective and long-term retention in glioma tissues after intracavitary administration of a high local radiation dose to the tumor and spares the surrounding normal brain tissue .[25] These encouraging preclinical safety and efficacy data resulted in a Phase I/II clinical trial in patients with recurrent high-grade glioma that validated the safety and tolerability of ^{131}I-TM-601 and its particular long-term accumulation at the tumor site. [27] It has been noted to induce antitumor responses and improved survival in a group of patients, which is encouraging. [27]

Precise Drug Delivery Systems

Chlorotoxin has been widely used to functionalize different nanocarriers to allow direct delivery of chemotherapeutic agents to tumor locations only.[18,30,32] Liposomes that were functionalized (chlorotoxin-modified doxorubicin-loaded liposomes) showed a high level of cellular uptake and antitumor cytotoxicity in glioma cell lines in comparison to their non-targeted counterparts. [30] More sophisticated approaches have also been designed such as a dual-targeting system in which the chlorotoxin was co-decorated with an ApoE-derived peptide mApoE on liposomes to act synergistically to both cross the blood-brain barrier and target tumors.[32] Polymeric nanoparticles made of such materials as poly(lactic-co-glycolic acid) or PLGA and functionalized with chlorotoxin have been utilized to deliver drugs such as temozolomide [33] and the natural compound morusin [18] in a controlled manner. These specific nanoparticles augmented drug concentration in the tumor augmented cancer cell toxicity and minimized systemic side effects.[18,33] There are other nanocarriers such as polyethylenimine PEI-

based complexes that have been developed as theranostic vectors that can co-deliver therapeutic agents such as doxorubicin and imaging agents .[29]

Fusion Therapeutics

An extremely novel approach is the genetic or chemical fusion of chlorotoxin to therapeutic proteins that are not inherently cell-penetrating to form hybrid molecules that combine the high specificity of chlorotoxin with potent cytotoxic toxins. [8,31,34] Gelonin-CTX Gel-CLTX was a fusion protein that was made by attaching chlorotoxin to gelonin a ribosome-inactivating protein.[31] This chimeric was selectively endocytosed by glioma cells resulting in powerful inhibition of protein synthesis and cell death.[31] The other one is the Cytochrome c-CTX Cytc-CTX hybrid protein that was engineered to take advantage of the pro-apoptotic activity of cytochrome c.[8] The soluble fusion protein was produced successfully and was capable of selectively inducing apoptosis in glioma cells.[8] The same was done with bacterial protein cytotoxic necrotizing factor 1 CNF1 CNF1 forming the CTX-CNF1 fusion.[34] This recombinant protein specifically targeted glioma cells in vivo and greatly prolonged the life of glioma-bearing mice when it was administered systemically.[34] Moreover new bicyclic peptides based on chlorotoxin have been designed with enhanced stability and binding affinity that can be used as the future scaffolds in therapeutic development.[35]

Theranostic Approaches

The combination of diagnostic and therapeutic capabilities into one integrated platform is a paradigm shift in precision neuro-oncology where chlorotoxin has become an important targeting ligand to build advanced theranostic nanoplatforms.[12,29,36] These new systems permit real-time tracking of drug delivery and therapeutic response with simultaneous delineation and targeted treatment of tumours.

A number of advanced chlorotoxin-based theranostic systems have shown impressive preclinical efficacy. One of the best examples is the superparamagnetic iron oxide nanoparticles functionalized with chlorotoxin and loaded with therapeutic agents to form a multimodal system that offers contrast to magnetic resonance imaging and delivery of drugs to tumor sites. [12] The other innovative method involved the use of chlorotoxin-conjugated polyethylenimine-based nanocomplexes which were radiolabeled with ^{99}mTc to be used in single-photon emission computed tomography imaging and at the same time deliver chemotherapeutic drugs such as doxorubicin to glioma cells.[29] More recently redox-responsive nanoparticles have been designed that combine chlorotoxin-mediated targeting with regulated drug release systems; in one example chlorotoxin-conjugated cross-linked chitosan-PEG copolymer nanoparticles loaded with O ^{6} benzylguanine were used to target and release drugs into orthotopic tumors and significantly increased survival in a combination with temozolomide chemotherapy.[37] Also chlorotoxin has been used in gene therapy vectors where it has been used to target tumors and track tumors as demonstrated by chlorotoxin-labeled magnetic nanovectors that can deliver green fluorescent protein encoding DNA to glioma cells and can be visualized using magnetic resonance imaging.[36]

Chlorotoxin is strategically incorporated into these theranostic platforms and greatly increases their accumulation and penetration across the blood-brain barrier in tumor cells and reduces off-target effects. This synergistic integration of accurate diagnostic potential and specific therapeutic intervention has an unprecedented potential in the personalized management of glioma that has the potential to transform the treatment approaches by offering the possibility of image-guided surgery and real-time adjustment of therapy.

Blood-Brain Barrier (BBB) Penetration

Crossing the blood-brain barrier is one of the key problems with the treatment of brain tumors, and chlorotoxin has already shown some peculiarities in this respect. The exact mechanism through which chlorotoxin passes this barrier is multifaceted and complicated, and different studies have suggested a number of strategies to maximize this process.

One of the most effective methods to increase the penetration of chlorotoxin across the blood-brain barrier has been dual-targeting systems. In a pioneering study by Formicola et al., Doxorubicin-loaded liposomes were functionalized with both chlorotoxin and an ApoE-derived peptide (mApoE). This dual-functionalization produced a synergistic effect: mApoE promotes receptor-mediated endocytosis via engagement of the low-density lipoprotein receptor (LDLR) on brain endothelial cells, whereas chlorotoxin facilitated basolateral exocytosis, thereby enabling the nanocarrier to traverse the endothelial cell layer and enter the brain parenchyma.[32] Additional studies have confirmed the ability of chlorotoxin-based systems to cross the blood-brain barrier and target glioma cells. [38,39]

An orthogonal method is the low-dose radiotherapy that temporarily enhances blood-brain barrier permeability. It has been demonstrated that low dose (2 Gy) whole-brain irradiation 20 hours prior to treatment with chlorotoxin-functionalized polymer nanoparticles containing silver greatly enhanced the expression of chlorotoxin receptors, such as MMP-2 and ClC-3, and the accumulation of nanoparticles in the tumor and even in disseminated glioblastoma cells.[38]

Interestingly, it has been shown that chlorotoxin per se has an intrinsic capacity to penetrate the blood-brain barrier. Chlorotoxin-functionalized multimodal nanoprobes have been used to study the ability of this peptide to cross the intact blood-brain barrier and target brain tumors in genetically engineered mouse models.[12] This inherent feature, along with improvement measures, creates a niche of chlorotoxin to deliver therapeutic and diagnostic agents to the central nervous system.

Clinical Investigations and Safety Profile

The chlorotoxin-based agents have shown good safety and pharmacokinetics profiles in clinical trials in humans (Table 2). Phase I trial of ^{131}I-TM-601 in targeted radiotherapy has proven its safety in recurrent high-grade glioma patients, with specific tumor retention and promising antitumor effects with no dose-limiting toxicities.[27] Equally, the Phase I trial of BLZ-100 (Tozuleristide) as a fluorescence-guided surgery showed that it was safe up to 30 mg doses, with a rapid clearance and good visualization of tumors of various glioma grades.[10]

Pharmacokinetic experiments indicate that there are regular trends across species and chlorotoxin-based agents have been found to exhibit rapid blood clearance and selective tumor accumulation. Preclinical studies of radiolabeled chlorotoxin analogs demonstrated good biodistribution with ideal tumor-to-normal tissue ratios, [25,28] and clinical studies indicated that the analogs were cleared rapidly through the body but retained tumor targeting properties.[10] These properties aid the therapeutic window of diagnostic and therapeutic uses of neuro-oncology.

Table 2: Summary of Phase I Clinical Trials of Chlorotoxin-Based Agents in Glioma

Parameter	**BLZ-100 (Tozuleristide)**	**^{131}I-TM-601**
Clinical trial phase	Phase I	Phase I
Number of patients	17 adults	18 adults (17 GBM, 1 anaplastic astrocytoma)
Administration route	Single intravenous injection	Intracavitary (via catheter)
Dose range	3, 10, 20, 30 mg	0.25, 0.50, or 1.0 mg TM-601 (each with 10 mCi ^{131}I)
Dose-limiting toxicity (DLT)	None	None
Maximum tolerated dose (MTD)	Not reached	Not reached
Adverse events	No drug-related serious adverse events	Only minor adverse events
Pharmacokinetics	Serum $t_1/_2$ ~30 min (doses ≥9 mg)	Clearance within 24-48 hours; long-term tumor retention
Imaging / efficacy outcomes	Fluorescence signal in high- and low-grade tumors (greater intensity in high-grade)	At day 180: 4 stable disease, 1 partial response; 2 patients disease-free >30 months
Conclusion	Safe up to 30 mg; useful for fluorescence-guided surgery	Well tolerated; promising antitumor activity
Reference	[10]	[27]

Comparison to Other Targeting Ligands

The choice of the right targeting ligands is a key factor in the design of successful molecular therapies of glioma. This comparative analysis clarifies the relative location of chlorotoxin in the existing spectrum of targeting moieties, which offers a subtle analysis of its unique features and therapeutic consequences.

The comparative evaluation shows that each of the targeting modalities has unique therapeutic profiles (Table 3). Chlorotoxin has outstanding molecular specificity in neuroectodermal malignancies, where multimodal targeting features and demonstrated clinical efficacy in the treatment of gliomas.[4,10,13,27] RGD-based systems provide a high level of tumor targeting flexibility with strong preclinical records, although with relatively low specificity to neural tumors.[40,41] Targeting by transferrin receptor offers a superior blood-brain barrier penetration via physiological transport systems, but it is mainly used as a delivery vehicle, and does not have intrinsic therapeutic advantages.[42]

Chlorotoxin is a unique multimodal target therapeutic with a high-affinity target, which is exploited by the strategic integration of chlorotoxin in neuro-oncological applications. Although alternative ligands offer useful properties to particular uses, the combination of molecular specificity, functional flexibility, and clinical success of chlorotoxin makes it dominant in targeted therapies in neuroectodermal tumors.[43]

Table 3: Comparative Analysis of Targeting Ligands for Glioma Therapy.

Targeting Ligand	Key Features	Advantages	Limitations	Clinical Status	Reference
Chlorotoxin	• Targets MMP-2, ClC-3, Annexin A2 • 4 kDa peptide • Scorpion venom-derived	• High specificity for neuroectodermal tumors • Multimodal targeting capability • Direct anti-invasive effects • Demonstrated clinical success in Phase I trials	• Primarily effective for neuroectodermal tumors • Smaller size may reduce circulation time • Limited to specific tumor types	Phase I clinical validation (BLZ-100 for fluorescence-guided surgery; ^{131}I-TM-601 for targeted radiotherapy)	4,10,13,27
RGD Peptides	• Targets integrins αvβ3/αvβ5 • Synthetic peptides (0.5-2 kDa) • Broad tumor targeting	• Extensive preclinical validation • Good tumor penetration capability • Versatile for various cancer types	• Lower tumor specificity compared to chlorotoxin • Broader integrin targeting increases off-target effects • Limited clinical translation in neuro-oncology	Extensive preclinical data	40,41
Transferrin (TfR)	• Utilizes natural iron transport system • Antibodies or protein conjugates (~80 kDa) • Efficient BBB crossing	• Established delivery platform • Natural targeting mechanism • Proven BBB penetration capability	• Functions primarily as delivery vehicle without therapeutic effects • Immunogenicity concerns with repeated use • Limited to single receptor targeting	Limited clinical translation in neuro-oncology	42

11. Conclusion

In conclusion, chlorotoxin has proven to be a highly versatile and specific targeting agent in the treatment and imaging of glioma. Its capacity to bind to numerous molecular targets on tumor cells as well as penetrate the blood-brain barrier has permitted the creation of novel diagnostic and therapeutic platforms. Since the development of fluorescence-guided surgery with BLZ-100, targeted drug delivery systems, and novel fusion proteins, chlorotoxin-based platforms have demonstrated substantial potential in improving glioma treatment. Further development of these strategies, especially with the use of sophisticated theranostic tools and combination therapies, is likely to improve the accuracy and efficacy of treatment of patients with this difficult disease.

Author Contribution

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Ethical Approval

Not applicable. This manuscript is a review article and does not involve any human or animal subjects.

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