

Capsaicin Nanoparticles as Therapeutic Agents against Gliomas

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Abstract

Capsaicin is an alkaloid molecule with outstanding biological activity. Several reports have shown that capsaicin exerts significant antitumoral effects in several cancer cell lines, including gliomas. However, its application has been very limited due to its hydrophobicity, low affinity, and short life span. Gliomas are a heterogeneous group of brain malignant tumors with increasing prevalence worldwide. Standard therapy against these tumors generally includes resection by surgery, radiation, and chemotherapy or their combination. However, elicitation of tumor resistance to chemical or radiation treatments remains one of the main challenges to be resolved, particularly in the case of glioblastomas. Nanotechnology is an innovative approach to the treatment of Central Nervous System diseases and especially to gliomas treatment. Indeed, the use of nanotherapeutic formulations offers several advantages over the conventional methods of drug delivery therapy. In this review, we analyzed the current literature regarding the development of capsaicin-loaded nanoparticles as a promising approach for the treatment of malignant brain tumors.

Keywords: Nanoencapsulation, Capsaicin, Glioblastoma, Drug delivery

Introduction

Natural products continue to be a key resource in the treatment of diseases. According to Newman and Cragg [1], 49% of the small molecules approved for cancer treatment from the 1940s to 2014 are natural products or molecules derived therefrom. Among them, capsaicin (CAP) (8 methyl-*N*-vanillin-trans-6-nonemaide) stands out as a hydrophobic molecule with great therapeutic potential and low cost. The

CAP molecule comprises three distinct regions, each with a specific biological relevance, i.e., the aromatic hydrophilic region (vanillin), the dipolar amide region, and the hydrophobic region (octanylic chain) [2, 3]. It possesses a highly reactive methoxy phenol group [4]. According to Yang *et al.*, [5] CAP forms specific chemical interactions with the Ca²⁺ channel through hydrophobic attractions, suggesting dynamic conformational transitions that may be responsible for bioactivity. Numerous additional reports have

documented the underlying molecular action mechanisms [6, 7] and its potential in the treatment of a variety of cancers [8]. This review addresses the use of CAP nanoparticles for the treatment of gliomas.

Capsaicin and the Central Nervous System (CNS)

CAP is a specific agonist of the Transitory Receptor Potential Vanilloid (TRPV-1), a tetrameric membrane protein with four identical subunits and a member of the family of Transient Receptor Potential (TRP) Receptors [9]. TRPV1 is expressed in the sensory neurons, as well as in numerous non-neuronal tissues including the blood vessels. Nagy *et al.*, [10] showed that TRPV-1 is expressed extensively in the brain and the epidermis, among other tissues. CAP is well known for its ability to act through the intracellular union to the TRPV1 receptors in order to deploy excitatory, desensitizing, and neurotoxicity effects [11].

The description of the structure-activity relationship of CAP and several of its analogs suggest that the vanillin region and the amide linkage are essential for the pharmacological activity on TRPV1 and thus, to display its excitatory capacity, while it is presumed that the aliphatic chain is essential for maximum potency.

CAP binds with a strong affinity to the intracellular S2-S4 of TRPV1 protein, mediated by interactions between the vanilloid group and the benzene ring [6]. In order to understand the configuration of the linkage between CAP and its interaction with TRPV1, Yang *et al.*, [5] developed an innovative hybrid approach combining the computational coupling and the functional studies. According to their work, the TRPV1 and CAP structures obtained from cryoTEM imaging clearly show conformational reorganizations near the union site, with CAP linked in a “tail-up, head-down” configuration. Binding is mediated by both hydrogen bonds and van der Waals interactions. Upon binding, CAP stabilizes the open state of TRPV1 by “pull-and- contact” with the S4-S5 linker (Figure 1). In contrast, the vanilloid and amide groups form specific interactions that bind the ligand to the receptor [12].

TRPV1 is one of the main receptors involved in pain sensation, thus it is considered a therapeutic target against pain and inflammation and has been widely used [13-15]. According to Abdel-Salam *et al.*, [15] CAP stimulates the vagal afferent fibers involved in the signaling of the immune system to the brain. The application of painful stimuli on the skin by CAP results in the activation of the medial thalamic pathway to the frontal lobe, including the area of the prefrontal cortex [16]. TRPV1 has also been identified in the

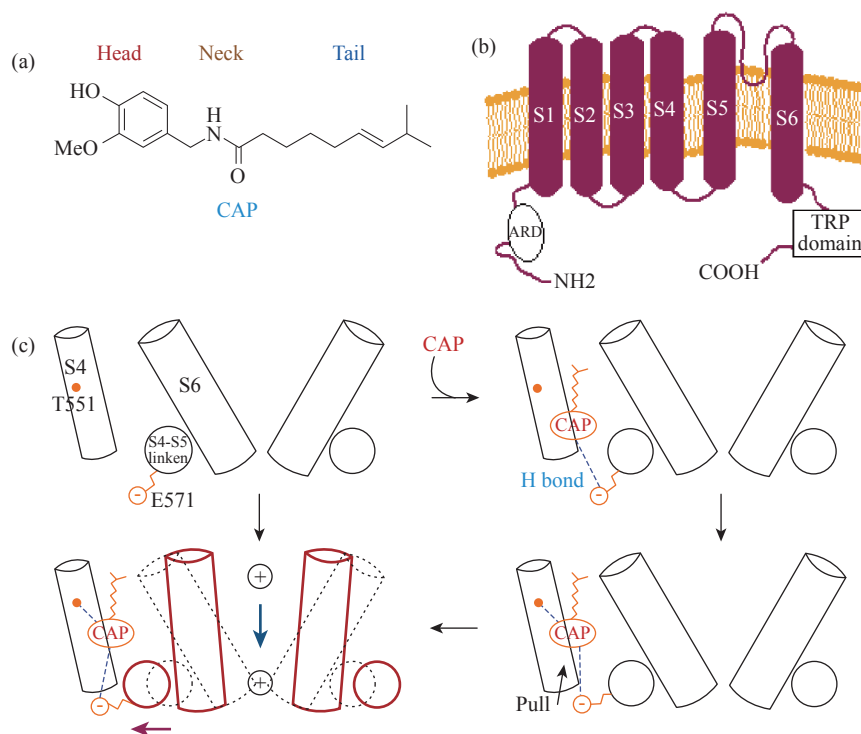


Fig. 1 (a) Chemical structure of capsaicin. (b) Schematic diagram of a TRPV1 subunit; figures taken from [5], with some modifications (c) Schematic diagram summarizing capsaicin binding and activation of the TRPV1 channel; figure reprinted with permission from [5, 12].

hippocampus, striatum, hypothalamus, and cerebellum in the brain of humans and rats [17]. According to Kauer and Gibson [18] the activation of TRPV1 in the CNS improves synaptic transmission and plasticity as well as memory formation in the hippocampus. In experimental diabetes models, Araya *et al.*, [19] showed that peripheral and central TRPV1 play a key role in heat hyperalgesia, while Xu *et al.*, [20] studied the beneficial effect of CAP on preventing Alzheimer's disease via tau changes in the hippocampus of type 2 diabetes rats, which could be related to TRPV1 activation. Activation of TRPV1 by CAP has also been reported to stimulate the spontaneous release of glutamate in the locus coeruleus [21]; moreover, microinjection of CAP into the substantia nigra has been used to induce degeneration of mesencephalic dopaminergic neurons [22]. Farrell *et al.*, [23] identified regional brain activations evoked by CAP inhalation. These regions include the insula, mid cingulate cortex, prefrontal, parietal, and premotor regions and the cerebellum. In the brainstem, CAP produced activations in respiratory-related regions of the dorsal pons and lateral medulla (Figure 2).

TRPV1 can become reactive under stress conditions and glial cell lesions, thus showing an increase in hypertrophy, cytokine production, and secretion, as well as changes in gene expression. In addition to the neurons, TRPV1 is also found in astrocytes and microglia and the emergent studies have implied TRPV1 in various aspects of glial function [25]. Also, when associated with increases in intracellular calcium, the overactivation of TRPV1 may become toxic to cells. Ho *et al.*, [26] have shown high levels of intracellular calcium, as well as subsequent mitochondrial damage and apoptosis induced by CAP. Several reports have shown that CAP exerts significant antitumoral effects in several cancer cell lines, i.e., pancreas [27], lung [28], prostate [29],

breast [30], colorectal [31], stomach [32], bladder [33], osteosarcoma [34], nasopharyngeal carcinoma [35], cholangiocarcinoma [36], melanoma [37], fibrosarcoma [38] and in human glioblastoma [11, 39, 40]. The molecular basis for such effects has not been completely elucidated, however, some are related to activation CAP receptors or regulating other signaling pathways. The anticancer mechanisms are mainly related to anti-proliferation, induction of apoptosis and autophagy, anti-angiogenesis, and anti-metastasis [41]. A major aspect of CAP action is its high specificity to cancer cells while allowing healthy cells to thrive unaffected [42].

CAP activity against glioblastoma

Gliomas have been classified according to their malignancy to affected cells in astrocytoma (astrocytes), oligodendrogliomas (oligodendrocytes), and ependymomas (ependymal cells). Accordingly, they are classified into various intensity categories into Low Grade (grade II) or High-Grade (Grades III and IV) Malignant Gliomas. The most aggressive form of glioma is Glioblastoma (Grade IV) which is characterized by uncontrolled tumor cell proliferation, causing necrotic areas and diffuse infiltration [43-46]. In 2016, the World Health Organization (WHO) incorporated molecular diagnostic criteria in the classification for infiltrating gliomas, including mutation of isocitrate dehydrogenase, deletion of 1p/19q chromosome, and histone mutations [47]. Glioblastoma, also known as Grade IV astrocytoma, occurs more frequently in males and it is one of the most aggressive human cancers with an average expectancy of survival of around one year after diagnosis [45, 48].

Intracellular signaling pathways for tumor growth and proliferation are key to an invasion of healthy tissue and for stimulation of blood vessel proliferation. Much effort in the drug development process has

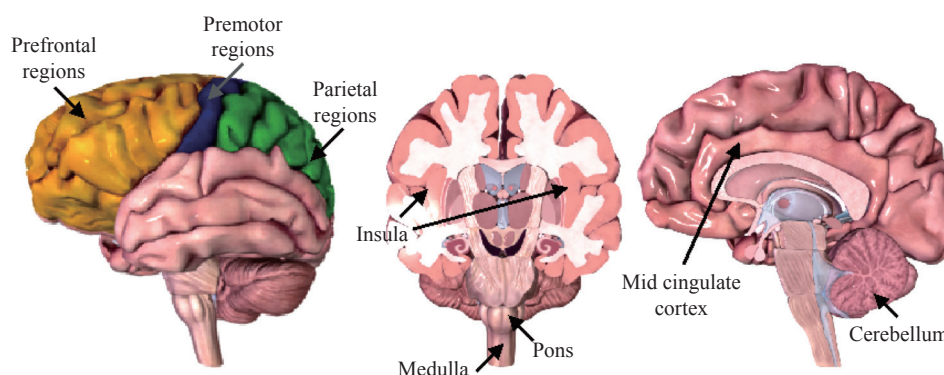


Fig. 2 Regions of the brain activated by capsaicin inhalation [23]. Images: BIODIGITAL [24].

been focused on interference with such mechanisms [49]. Many factors that influence the process of tumorigenesis, proliferation, and invasion have been identified, for example, many growth factor pathways and cytokines are involved in the phenotype of malignant gliomas. Knowledge about the role of TRP channels in brain tumor growth and progression has been evolving rapidly [50]. Angiogenesis has been widely recognized as a key event in glioma progression [51]. Indeed, neovascularization in brain tumors is highly correlated with its biological aggressiveness, malignancy degree, and clinical recurrence and outcome. It is also inversely correlated with post-

operative survival. Recently, a direct connection has been shown between the expression changes in the TRP channels during tumoral angiogenesis [52]. Given the importance of angiogenesis in glioma growth and progression, TRP channels have become an increasingly promising target for therapeutic agents capable of inducing anti-angiogenesis. In this regard, the “vascular segmentation strategy” [53] will be a very promising therapy. Nersesyan *et al.*, [54] assessed the TRPV1 expression in different glioma cell lines and determined the correlation between the expression of the protein with age and sex in tissue samples obtained from patients with glioblastoma.

Table 1 Reported Glioma receptors

Receptor	Types of receptor	Study model	Ref
Protease-activated receptor 2	(PAR) 2	U87 cells and human glioma tissue	[55]
Adenosine receptor (AR)	A ₁ , A _{2A} , A _{2B} , A ₃	Glioma cells (C6, U373MG, U87MG, U138MG, ADF)	[56]
Metabotropic P2Y nucleotide receptors	P2Y ₁ – P2Y ₁₂	C6 cells	[57]
Ionotropic receptors P2X	P2X ₇	C6 cells	[57]
Receptor tyrosine kinase	RTK	Human glioma tissue	[58]
Epidermal growth factor receptor	EGFR	Human glioma tissue	[59]
Platelet-derived growth factor	PDGFR	Human glioma tissue	[59]
Vascular endothelial growth factor	VEGFR-1, VEGFR-2	Human brain tumors, glioma cells (C6, U87MG)	[60]
Discoidin domain receptor 1	DDR1	Glioma cells (U87MG, G140), human glioma tissue	[59]
Neurotrophic tyrosine kinase receptor type 1	TrkA	Glioma cells (U251, C6-2B), human glioma tissue	[59]
Cannabinoid receptor	CB1, CB2	Glioma cells (U87MG, U373MG, C6), tissue samples	[61]
Transforming growth factor-β	TGF-β	Glioma cells (U373MG, A172), tissue samples	[62]
Hepatocyte growth factor/scatter factor	HSF/SF	Glioma cells (U251MG, U373MG, GL261)	[63]
Fibroblast growth factor receptor	FGFR	Glioma cells (HGG, SF188, KNS42), patient-derived cell lines (IN1591, IN2017, IN2356, IN2688, IN1520)	[64]
Insulin-like growth factor receptor	IGFI, IGFII, IGF-IR, IGR-IIR	Human glioma tissue, normal brain	[65]
Cytokines			
Interleukin-13 receptor	IL-13R	Human glioma tissue, normal brain	[66]
Folate receptor	FR-a	Human glioma tissue	[67]
Other receptors			
Transferrin receptor	TfR2	Glioma cells (TB10, U87MG, T98G, U251)	[68]
Integrins	α _v β ₃ , α _v β ₅	Glioma cells (U87MG, SF763)	[69]
Tenascin	TNC	Human glioma tumor	[70]
Adhesion receptors			
	CD44	Human glioma tissue	[71]
	CD90	Human glioma tumor, glioma cells (U87MG, U251)	[72]

Brito *et al.*, [73] described the role of TRPV1 in the normal physiology and physiopathology of various body organs. These authors highlight that, drugs such as CAP, which targets this channel, could be of clinical importance. It is through the activation of TRPV1 that CAP seems to exert many of its beneficial effects, since the TRPV1 receptors are found in numerous types of tissues [73, 74].

Tumoral angiogenesis is a physiological response to hypoxia in response to an increase in tumor mass and also the result of critical mutations which activate a transcriptional program leading to angiogenesis [75]. Calcium (Ca^{2+}) is an important secondary messenger and its entry through the plasma membrane affects angiogenesis [53]. Several reports indicate that angiogenic growth factors such as the Vascular Endothelial Growth Factor (VEGF) and the Fibroblastic Growth Factor (FGF) can activate the TRP channels at the transcriptional and post-transcriptional levels, causing an increase in the endothelial Ca^{2+} , which in turn modulates the transduction signaling pathways which regulate angiogenesis [76]. According to Kale *et al.*, [77] the ion channels are implicated in oncogenesis. In this context, numerous *in vivo* experiments targeted to various ion channels in cancer models illustrate the great potential of this approach [78, 79].

TRPV1 is expressed in a wide variety of glial cells, especially in microglia and astrocytes [25]. Glial cells carry out immunologic activities in response to biochemical challenges through the secretion of proinflammatory cytokines and chemokines [80]. The inflammation modulates the expression and activity of TRPV1 in the CNS [81]. Amantini *et al.*, [11]

suggest that the molecular characterization of the TRPV1 channel in glioma tumors could be a useful indicator for their evolution and prognosis as well as a key target for new therapeutic approaches. Thus, CAP offers great potential from a theoretical and experimental viewpoint. Indeed, several studies have reported the effects of CAP on human glioblastoma (Table 2). Gil and Kang [40] assessed the effect of CAP on inhibition and cell differentiation in human glioma cells. Their results indicated that CAP induces apoptosis through the induction of mitochondria-mediated caspase cascades via down-regulation of Bcl-2 and up-regulation of Bax. It was also observed that CAP increases the levels of caspase-3 and -9. It is well known that caspase-3 activation plays a role in the induction of apoptosis and is frequently preceded by the expression of Bcl-2 and Bax via the mitochondrial pathways. It was also shown that CAP induces terminal differentiation through the expression of genes related to such differentiation.

On the other hand, Jeon *et al.*, [82] showed that CAP induces inhibition of human glioma cell viability. Apoptosis mechanisms were related to the mitochondrial (Bcl-2/Bax) and activation of the MAPK pathways (Kinase Protein Activated by Mitogens). This protein is involved in processes leading to the death of various cancer cell lines. A different study showed that CAP can induce apoptosis and autophagy in glioblastoma cells. In this case, the authors verified the role of apoptosis and autophagy in U251 cells after capsaicin treatment by using the autophagy inhibitor 3-methyladenine. The results showed that inhibition autophagy could increase the expression of P53 (a suppressor of tumor growth) and Puma-a in U251

Table 2 *In vitro* studies evaluating the action mechanisms of CAP in gliomas

Cell lines	Effective dose (mM)	Anti-cancer mechanisms	Ref
A-172	200-250	Induced apoptosis by the reduction in the basal generation of ROS	[86]
C6	50 - 200	Induced apoptosis by formation of peroxynitrite.	[39]
U373, U87, FC1, FLS	50	Induced apoptosis mediated by TRPV1 and requires p38 MAPK activation.	[11]
A-172	50 - 100	Induced apoptosis through down-regulation of Bcl-2 and activation of caspase-3 and terminal differentiation.	[40]
U251MG, U87MG, SNU-444, U251MG	200	Induced apoptosis mediated TRAIL via DR5 upregulation and surviving down-regulation.	[46]
U87MG	200	Induced apoptosis mediated via p-38 MAPK and mitochondrial (Bcl-2/Bax) pathway.	[82]
U251	100	Induced apoptosis, autophagy, and activation of the expression of P53.	[83]
U251	200	Induced apoptosis via ROS and Ca^{2+} mediated mitochondrial pathway.	[85]

cells, thus concluding that CAP could induce apoptosis by autophagy inhibition [83]. A further study by Kim *et al.*, [46] in different cell lines of malignant glioma showed that CAP is a potent sensitizer of apoptosis induced by the TRAIL ligand (Tumor Necrosis Factor Related Apoptosis Inducing Ligand), a potent apoptotic stimulator; tumor cells are significantly more sensitive to such apoptosis than normal cells [84]. On the other hand, Xie *et al.*, [85] showed that the CAP and di-hydro CAP apoptotic effects in U251 cells are associated with the generation of Reactive Oxygen Species (ROS), increased concentrations of calcium (Ca^{2+}), mitochondrial depolarization, the release of cytochrome C in the cytosol resulting in the activation of Caspase-3 and -9, and these effects were further confirmed by observations of the anti-tumor effects of CAP and di-hydro CAP *in vivo* in a U251 cell murine tumor xenograft model. Qiao *et al.*, [39] studied the role of peroxynitrite on C-6 glioma cells apoptosis; they found that CAP stimulated an increase in superoxide and nitrite causing an increase in peroxynitrite inside the glioma cells concomitant to the increase in the levels of nitrotyrosine proteins. Table 2 summarizes the *in vitro* mechanisms and doses of CAP reported in the literature, while Figure 3 shows the main molecular target of CAP in glioma cells.

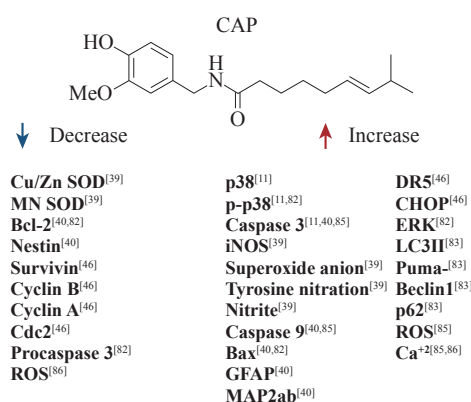


Fig. 3 Molecular target of CAP in glioma cells.

In addition to the activity in glioblastoma cells, it has been shown that CAP has a notable effect on the integration of cells tight junctions and permeability [87, 88]. Previous *in vivo* studies demonstrated that the use of CAP may result in the reversible opening of the Blood-Brain Barrier (BBB), which is a key limiting factor in the bioavailability of therapeutic agents to the brain [89]. The BBB is a physical and enzymatic barrier responsible for preserving brain homeostasis. It selectively inhibits the permeabilization of high molecular weight compounds with a negative charge

and low lipid solubility [90]. Only a few chemical moieties have been found to trespass the BBB [91]. *In vitro* studies using murine models have shown that CAP can open the tight junctions of the endothelial monolayer in a dose-dependent reversible manner [89, 92, 93]. This opens the possibility that CAP is used not only as a therapeutic agent but also as a candidate for the development of drug vehicles. However, its use remains limited due to its hydrophobicity, low affinity, and short life span [94].

Standard therapy against gliomas includes surgery, radiation, and chemotherapy or their combination. However, elicitation of tumor resistance to chemical or radiation treatments remains one of the main challenges to be resolved, particularly in the case of gliomas [43, 95]. A further complication stems from the collateral harm on healthy cells given the low specificity of the therapeutic agents. Thus, a major area of research interest is the achievement of more effective drug systems with less collateral damage [96]. A major tool to achieve this goal is the use of nanotechnology. Indeed, new formulations based on nanoparticles allow more specific drug delivery on cell ligands so that accumulation of the therapeutic agent reaches minimum inhibitory concentrations at a faster pace and with high specificity toward cancer cells [97]. In addition, it is possible to design nanosystems to carry specific chemical conformations able to recognize specific cell receptors resulting in a higher drug load accumulation inside the tumor with diminished secondary effects [96, 97].

Nanoparticles against Glioblastoma

As noted above, the use of nanotherapeutic formulations offers significant advantages over the conventional methods of drug delivery, i.e., faster accumulation of the drug in the cancer tissue thus increasing its therapeutic effect in a shorter time, decrease in systemic toxicity, and the possibility of surface functionalization to achieve a higher degree of specificity according to the molecular binding site. Also, nanoparticles can circulate throughout the body without risk of stroke and penetrate most cell compartments without difficulties [98, 99]. Numerous investigations have shown that it is possible to control the distribution profiles of anticancer drugs by trapping in submicronic colloidal systems, thus increasing the antitumoral effects while reducing secondary systemic

damages [100]. Additionally, nanoparticles provide the possibility of delivering the drug to the brain as they increase the drug concentration inside or at the luminal surface of the BBB cells, compared to what can be achieved after the systemic administration of a free drug. This favors passive diffusion of the drug and subsequent uptake by target cells. There are several mechanisms by which nanoparticles can be transported to the brain, endocytosis is one of the main ones [101, 102].

Nanotechnology is an innovative approach to the

treatment of CNS diseases and, particularly, for the treatment of gliomas. Indeed, several research reports have been published on nanoparticles for brain drug delivery [102]. Among the strategies, lipid-based carriers, polymer-based carriers, and inorganic carriers are the most widely studied. The physicochemical properties of such systems, as well as their *in vitro* stability and, in some cases, the assessment of their biological properties, have been described. Table 3 presents examples of biopolymer-based nanoparticle systems developed for the treatment of malignant

Table 3 Biopolymer-based nanoparticle systems against glioblastoma

Biopolymer	Drug	Effects	Ref
PLGA	Transferrin	The nanoparticles were found to be highly absorbed and endocytosed by the cells using an energy-dependent process.	[103]
Angiopep-PEG-PLC	Paclitaxel	The nanoparticles enhanced accumulation in the Glioma bed and infiltrating margin of intracranial U87MG glioma tumor-bearing <i>in vivo</i> model were observed by real-time fluorescence image.	[104]
N-isopropyl acrylamide vinylpyrrolidone and acrylic acid	Curcumin NanoCure™	The nanoparticles can inhibit malignant brain tumor growth through the modulation of cell proliferation, survival, and stem cell phenotype.	[105]
Glycol chitosan, Dextran sulfate	Methotrexate	The physicochemical properties of resulting particles were investigated, evidencing the contribution of these nanoparticles for brain targeting.	[106]
Glutathione-PEG	Doxorubicin	<i>In vitro</i> BBB Transwell™ study showed significantly higher permeation of the doxorubicin-loaded NPs compared with the free doxorubicin solution through the coculture of rat brain endothelial (RBE4) and C6 astrocytoma cells.	[107]
Hyaluronic acid/chitosan	Curcuminoid	The electrostatic complex showed stronger dose-dependent cytotoxicity against C6 glioma cells and higher uptake in C6 glioma cells.	[108]
mPEG-PLC	Capsaicin	The nanoparticles can cross the blood-brain barrier and showed a remarkable inhibition on the growth of U251 cells	[94]
PLGA	Loperamide	The nanoparticles can efficiently cross the BBB, with high crossing efficiencies when their surface is functionalized with an active targeting moiety.	[109]
PLGA	Paclitaxel and curcumin	The nanoparticles can efficiently penetrate the BBB and enhance brain delivery efficiency, as demonstrated in both <i>in vitro</i> and <i>in vivo</i> studies. The system exhibited improved glioma therapy efficacy yet with reduced adverse toxicities.	[110]
Chitosan	Temozolomidechlorotoxin and biotin	The nanoparticles exhibited sustained stability in cell culture media for two weeks and can cross the BBB and deliver temozolomide into avascular region of the brain.	[111]
MPEG-PCL	Honokiol and doxorubicin	The nanoparticles could efficiently suppress glioma cell proliferation and induce cell apoptosis <i>in vitro</i> .	[112]
Cerium Oxide		The nanoparticles had a cytotoxic effect on anaplastic astrocytoma (grade III glioma) cells while the same concentration did not show cytotoxicity on microvascular endothelial cells used as stromal cell models.	[113]
Anti-melanotransferrin and apolipoprotein E	Doxorubicin	The nanoparticles improved the permeability of Dox across the BBB and enhanced the efficiency in inhibiting the multiplication of U87MG cells.	[114]
PLGA	Doxorubicin	The results demonstrated that doxorubicin-loaded PLGA nanoparticles are efficiently internalized into the human glioma cells via clathrin-mediated endocytosis, then accumulate in lysosomes and release the doxorubicin	[115]
Lactoferrin-hyaluronic acid/chitosan	Curcuminoid	The results showed that nanoparticles were preferentially taken up by brain capillary endothelial cells. After crossing the BBB, the nanoparticles remained largely intact and were more effective in targeting glioma cells (C6).	[116]
Transferrin-DSPE-PEG2K	Temozolomide and bromodomain	Tumor-bearing mice treated with nanoparticles loaded with temozolomide and the bromodomain inhibitor JQ1 have decreased tumor burden and prolonged survival.	[117]

glioma brain tumors.

An excellent review on the nanoparticle drug delivery systems to target high-grade gliomas has been recently provided by Frosina [118]. In this review, two chitosan-based nanosystems are described. The first one was reported by Fang, *et al.*, [111] who used chitosan to protect temozolomide (TMZ) from degradation and increase its penetration to gliomas by using the tumor-targeting peptide chlorotoxin which binds to glioma initiating cells. These nanoparticles had an average diameter of less than 100 nm and exhibited sustained stability in cell culture media for two weeks. According to the research carried out by Saboktakin *et al.*, [106] methotrexate loaded glycol-chitosan and dextran sulfate nanoparticles represent a good alternative for brain tumor treatment. Yang *et al.*, [108] formed electrostatic complexes preparing an aqueous mixture with chitosan and hyaluronic acid. Such complex was found to present a higher uptake in C6 glioma cells. Hernández-Pedro *et al.*, [119] detailed the development of a nanovector made with a supra-paramagnetic iron oxide core covered with polyethylene glycol (PEG) grafted with chitosan and polyethanolamine for the treatment of gliomas. Specificity for the tumor was achieved with siRNA and chlorotoxin peptide. Kievit *et al.*, [120] developed a nanofiber system based on chitosan-polycaprolactone to study the migration of glioblastoma and the topographical change induced by its growing cells. This nanofiber substrate offers a new tool to study the effect of anti-migratory agents on malignant cells. Irani *et al.*, [121] developed an innovative tertiary system comprising TMZ chitosan loaded and gold nanoparticles which were incorporated onto polyurethane nanofibers. This system proved effective in inhibiting the growth of U87 glioblastoma cells *in vitro*. Also, Muzzarelli and Muzarelli [122] prepared methotrexate-loaded *O*-carboxymethyl chitosan nanoparticles and assessed the release of the anticancer agent in various media. These authors have developed a wide variety of nanosystems using covalently bound bioactive onto chitosan matrixes. On the other hand, improved release through convection has been explored by Saltzman [123] for delivery of chemotherapeutic agents for the treatment of gliomas in animal models, thus achieving significantly longer survival periods. The best results were obtained when target cells where glioma stem cells.

Castro [124] proposed the development of high-density lipoprotein synthetic nanoparticles to administer chemotherapeutic agents to gliomas in

animal models. Such nanoparticles are transported through the cell surface receptor SR-B1 and can induce glioblastoma tumor regression. Kuo and Hsu, [114] reported on the use of colloidal solid-lipid cationic nanoparticles with melanotransferrin and apolipoprotein E (ApoE) on their surface to transport the anti-mitotic drug doxorubicin through the BBB for glioblastoma treatment. Nanoparticles were applied to endothelial microvascular cells, astrocytes, and U87MG cells with good results. The best encapsulation results for sustained release were obtained with 10% stearyl-amine for sustained release and optimal drug delivery. Chitosan-blended nanoparticles offer many advantages for the delivery of CAP to gliomas as they can overcome the BBB by adsorptive transcytosis, a key transport mechanism for positively charged molecules [125].

Glioblastoma can also be inhibited by blocking the CK2 protein (Kinase 2 Protein) and the overexpression of the EGFR and the EGFRvIII (Epidermal Growth Factors). When such therapy approach is followed by the stimulation of anti-tumor immunity by antibodies against Antigen 4 (CTLA-4), associated with cytotoxic lymphocytes or the Lymphocyte Surface Receptor (CTL), the efficacy of the nanoconjugates treatment will be increased [126].

Additionally, a multicomponent, flexible chain nanoparticle system composed of three iron oxide nanospheres and a liposome loaded with a chemotherapeutic agent was developed by Karathanasis [127]. This system provides a linear chain assembly to facilitate the release of the agent on the walls of the glioma blood vessels. The application of a low external radiofrequency field thereafter allows the rapid release of the chemotherapeutic agent due to the rapid mechanical disruption of the liposome and its beneficial on-site action.

Capsaicin Nanoencapsulation

From the above discussion, it is clear that CAP can be tumor prevention and anticancer molecule with significant effects. However, its application has been very limited due to its hydrophobicity which causes low solubility in physiological fluids and a short half-life [94]. The following section describes some reports in which several CAP-loading nanosystems have been developed and evaluated to find suitable carriers to prolong the drug retention of CAP in the blood circulation and allow active targeting of specific

cancer cells for enhanced, precise delivery and target specificity [41].

Mantellero *et al.*, [128] used *in vitro* studies with Franz diffusion cells to study the CAP release kinetics using different solid formulations. Results showed a higher CAP release from the nanoencapsulated formula when compared to other formulations, while Amruthraj *et al.*, [129] prepared CAP-capped silver nanoparticles that were found to be compatible with blood cells in hemagglutination tests. Furthermore, Xing *et al.*, [130] reported the formulation of gelatin-acacia nanocapsules loaded with CAP through complex coacervation with hydrolysable tannins. Such nanocapsules had a mean diameter of 300-600 nm and were able to allow a 20.81% CAP load, with 81.17% efficiency. Sharma *et al.*, [131] used solid-lipid nanoparticles to encapsulate CAP which exhibited a mean size of 100 nm, and prolonged release of the bioactive compound was observed for a period of 14 h. Bejrappa *et al.*, [132] prepared capsicum oleoresin loaded nanocapsules in a gelatin matrix to determine the effect of the freezing process in the absence or presence of excipients, on the stability of such nanocapsules during freeze-thawing and freeze-drying procedures. Zhu *et al.*, [133] prepared capsaicin-loaded liposomes (52.2 ± 1.3 nm mean diameter) for oral administration. The *in vivo* pharmacokinetic study and irritation tests showed an enhanced bioavailability and reduced inflammation in a murine gastric mucosa model. This same research group formulated a polyvinylpyrrolidone (PVP) / sodium cholate /phospholipid micellar system for oral administration which significantly improved the CAP oral bioavailability and showed a reduced irritation on the gastric mucosa [134, 135]. Peng *et al.*, [136] prepared CAP-loaded methoxy poly(ethyleneglycol)-poly(ϵ -caprolactone) nanoparticles aimed at improving solubility and bioavailability and reducing the side effects of CAP, while Cirino *et al.*, [137] evaluated the effectiveness of liposomes to deliver CAP to protect rodent bladder tissue against CAP-induced inflammation.

Studies in our laboratories [138] have addressed the effect of the degree of *N*-acetylation of chitosan on the biophysical properties, colloidal stability, and encapsulation efficiency of CP in chitosan-based nanocapsules. This system comprised an oily core, lecithin, and a polymer layer and it proved to be a promising platform for the effective administration of peptides, lipophilic drugs, and vaccines. Additional research on this same system by Kaiser *et al.*, [87]

compared the cytotoxicity of free CAP versus the chitosan-CAP nanocapsules on epithelial cell lines (MDCK-C7) and their effect on the integrity of the tight junctions and cell permeability. This study demonstrated that when CAP is encapsulated, it can be applied at a concentration of 500 μ M without compromising the viability of MDCK-C7 cells as compared to the free molecule. Giri *et al.*, [139] evaluated the effectiveness of CAP-loaded nanoliposomes in protecting the liver from oxidative stress. The phospholipid vesicle (nanoliposomes) had a mean diameter of 277.7 nm. In this study, they observed that free CAP produced mild protection, while liposomal CAP exerted a significant effect on reducing liver oxidative stress. Jiang *et al.*, [94] evaluated the ability of the CAP-loaded methoxy polyethylene glycol-poly(caprolactone) (mPeg-PLC) nanoparticles to cross the BBB and the uptake of these nanoparticles in the glioma cells and its ability to inhibit cell proliferation in human glioblastoma. The efficacy of the CAP-loaded nanoparticles against tumor cells was significantly higher than the CAP by itself, especially at low concentrations ($p < 0.05$). After the absorption of CAP-loaded nanoparticles in tumor cells by endocytosis at low concentrations, CAP was released slowly into the cytosol resulting in increased efficiency. Recently, we evaluated the antiproliferative activity of CAP-loaded nanoemulsions and chitosan nanocapsules in two glioblastoma cell lines (H4, U118MG). The CAP-loaded nanosystems exhibited a significant reduction in cell viability compared to their unloaded counterparts [140]. Although more research is required to understand better the mechanisms of action and the mechanisms for drug delivery at the active site, these nanosystems hold promise in preclinical and clinical applications [102].

Conclusions

The advantages of nanobiotechnological approaches in the development of suitable carriers to extend CAP retention in the blood, thus facilitating active targeting to specific cancer cells can provide new therapeutic solutions for the treatment of CNS diseases and to the treatment of gliomas. Nanoparticles will continue to play a significant role in drug delivery, and numerous research reports have been published on specific nanosystems for brain drug delivery. In turn, different CAP-loaded nanosystems are successful in improving bioavailability, increasing half-life, and

reducing irritation at research level. Although more research is required to better understand the underlying mechanisms of action and the mechanisms for drug delivery at the active site, these nanosystems hold promise in preclinical and clinical applications.

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Conflict of Interests

The authors declare that no competing interest exists.

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