Research progress of anti-glioma chemotherapeutic drugs (Review)

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Abstract. Glioma is the most common primary intracranial malignancy in the central nervous system. At present, the most important treatment option is surgical resection of the tumor combined with radiotherapy and chemotherapy. The principle of operation is to remove the tumor to the maximal extent on the basis of preserving brain function. However, prominent invasive and infiltrative proliferation of glioma tumor cells into the surrounding normal tissues frequently reduces the efficacy of treatment. This in turn worsens the prognosis, because the tumor cannot be completely removed, which can readily relapse. Chemotherapeutic agents when applied individually have demonstrated limited efficacy for the treatment of glioma. However, multiple different chemotherapeutic agents can be used in combination with other treatment modalities to improve the efficacy while circumventing systemic toxicity and drug resistance. Therefore, it is pivotal to unravel the inhibitory mechanism mediated by the different chemotherapeutic drugs on glioma cells in preclinical studies. The aim of the present review is to provide a summary for understanding the effects of different chemotherapeutic drugs in glioma, in addition to providing a reference for the preclinical research into novel chemotherapeutic agents for future clinical application.

Contents

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1. Introduction

Glioma is a malignancy that originates from glial cells. According to the World Health Organization (WHO) in 2016, gliomas can be classified into four grades, of which grade I and II are low-grade, whereas grade III and IV are high-grade (1). In 2021, WHO updated the molecular biomarkers of different tumor types, recommended the use of Arabic numerals for scoring, emphasized the importance of grading within tumor types, and brought more benefits and meaningful guidance for clinical practice (2). Glioblastoma (GBM) accounts for ~60% of all high-grade gliomas. High-grade gliomas can be observed in individuals of all ages, but are particularly common in men (3-5). Low-grade gliomas will typically relapse after initial treatment. Following recurrence, low-grade gliomas can transform into high-grade gliomas (6-8). The incidence of glioma accounts for ~40% of all malignancies in the central nervous system tumors, which is highly invasive and infiltrative (9). In addition, it has high recurrence rates and low 5-year survival rates, which poses a serious threat to human health (10). The median survival time of patients with glioma is only 15 months (11), whereas the 5-year survival rate is <10% (12). The etiology and mechanism underlying the pathogenesis of glioma remain unclear, but it has been demonstrated to show complex molecular characteristics, such as increases in chromosomes 7 and 19, loss of chromosomes 10 and 13, amplifications of EGFR and mouse double minute 2 homolog, mutations in PTEN, neurofibromatosis type 1, platelet-derived growth factor receptor α1, isocitrate dehydrogenase 1 and deletion of cyclin dependent kinase inhibitor 2A (13,14). Ionizing radiation exposure is also considered to be one of the causes of glioma (14,15).

At present, the standard treatment option for glioma is surgical resection combined with radiotherapy and chemotherapy (16). However, the long-term therapeutic efficacy is limited, where the main reasons are as follows: i) Due to

the invasiveness of this malignancy, local resection of the tumor only removes the primary tumor but cannot remove the surrounding tissues where the tumor has already invaded (17); ii) the chemotherapeutic agent cannot reach the tumor due to the blood-brain barrier (BBB), which causes systemic toxicity and side effects, reducing the maximum efficacy (18,19); and iii) tumor cells develop internal resistance to the agent (20), thus exerting varying degrees of resistance to different types of chemotherapeutic drugs, minimizing the effect of chemotherapy. Therefore, major obstacles remain before glioma can be completely cured (20). The invasiveness of GBM is caused by the signal pathway of tyrosine kinase receptor (21). Tyrosine kinase inhibitors (TKIs) and signal pathways (PI3K/AKT, Wnt/β-catenin, Hedgehog and NF-κB) serve important roles in the progression of GBM (22-24). TKIs selectively inhibit tumor growth and regulate cell proliferation, migration, apoptosis and angiogenic factors by activating intracellular signal transduction (25). Treatment strategies for glioma includes surgery, radiotherapy, chemotherapy, targeting and immunotherapy (25,26). However, chemotherapy agents when applied individually exert limited therapeutic effects (3). As such, different combinations of chemotherapeutic agents can be applied together to overcome drug resistance and reduce their toxic adverse effects (27). Drug delivery systems, such as nanoparticles, liposomes and polymer micelles can all transport chemotherapeutic drugs through the BBB to improve efficacy and enhance the targeting effects (27,28). Therefore, chemotherapy remains to be a basic component of multimodal therapeutic method for malignant glioma treatment (29). Postoperative application of chemotherapeutic drugs in patients with glioma serves an important role in preventing postoperative recurrence. Considering the existence of the BBB in the central nervous system, the ideal chemotherapeutic drug should be small in molecular weight, lipophilic, non-protein, and able to spread to the brain stroma through the BBB (30). The main obstacle to effective glioma chemotherapy is currently drug resistance development and the occurrence of toxic side effects. Therefore, there is demand for the development of novel agents and drug delivery systems to alleviate these two challenges for clinical application. A systematic literature search for eligible studies through the electronic search engine PubMed was performed. The keywords for searches were as follows: 'Glioma', 'glioblastoma' and 'chemotherapeutic drugs'. Studies published in the past 10 years were searched using these keywords. The prevent review discusses the potential mechanism and application of various chemotherapeutic agents for glioma treatment.

2. Temozolomide

Preclinical study and combination therapy of temozolomide (TMZ). TMZ is a first line chemotherapy drug for glioma (31) and a precursor alkylating agent (32). In addition, TMZ is a small molecule and lipophilic substance that can be taken orally and readily traverses through the BBB (33,34). Data from previous preclinical studies (16,27,35-51) (Table I) showed that it can exert anticancer activity. Wang *et al* (35) treated C6 cells with TMZ (250-1,000 μM) for 24, 48 and 72 h. The results revealed that TMZ inhibited the proliferation, migration and invasion of C6 cells through the MEK/ERK signaling pathway, and downregulation of vascular endothelial growth factor (VEGF)-C expression to enhance the inhibitory effects of TMZ. The invasive growth and recurrence of gliomas are closely associated with tumor neovascularization. Tumor cells secrete a number of vascular growth factors such as VEGF to facilitate the growth of tumors (52). A previous study by Sonoda *et al* (53) have revealed that overexpression of VEGF can increase tumor volume and vascular permeability in mice. Effective inhibition of VEGF in gliomas has been previously shown to inhibit neovascularization. Bevacizumab (BEV) is an anti-angiogenic agent, such that treatment with the combination of BEV and TMZ did not weaken the effects exerted by TMZ. Grossman *et al* (54) revealed that TMZ combined with BEV treatment was able to inhibit VEGF to prolong the survival time of rats injected with U87 cells (109 days). Although TMZ can exert inhibitory effects on glioma cells, it has not been able to significantly improve the survival rate of patients with glioma (36). However, therapy using a combination of different drugs may be effective for gliomas (55). Da Ros *et al* (36) treated glioma cell lines U87, A172 and T98G with TMZ (100 and 200 μM) in combination with aldoxorubicin, a novel prodrug of doxorubicin (12 μM). The results showed that low concentrations of TMZ (100 μM) conferred no toxicity in any of the cell lines. However, higher concentrations of TMZ (200 μM) were shown to be toxic to U87 and A172 cells whereas T98G cells remained TMZ-resistant (36). Aldoxorubicin could significantly reduce the cell viability of all cell lines tested, where toxicity to T98G cells was shown to be more potent (36). Therefore, it was concluded that the addition of aldoxorubicin can enhance the killing effects of TMZ on TMZ-resistant glioma cells (36). TMZ combined with formononetin has a synergistic inhibitory effect on glioma cells (56). TMZ combined with pilocin and formononetin can inhibit the growth of glioma cells *in vitro* and tumor growth *in vivo* (57). In addition, TMZ combined with proteasome inhibitors also have certain antitumor effects. TMZ combined with alphatinib (EGFR inhibitor) has an inhibitory effect on glioma *in vivo* and *in vitro* (58). TMZ combined with bortezomib (BTZ) (proteasome inhibitor) can enhance the sensitivity of TMZ in the treatment of gliomas *in vitro* and *in vivo* (59). TMZ combined with amlotinib (a novel multi-target TKI) can synergistically inhibit the proliferation and angiogenesis of glioma cells and inhibit tumor growth *in vitro* (60). TMZ forms part of the standard chemotherapeutic strategy for the treatment of glioma. However, drug resistance to TMZ and toxic effects (myelosuppression and delayed thrombocytopenia) limit its efficacy, which highlights the importance of exploring the novel treatment options (61,62). Previous preclinical studies (63-65) applied nanoparticles as carriers in an attempt to effectively deliver chemotherapeutic drugs to the glioma region by crossing the BBB, and minimize drug resistance (62). These nanocarriers can be functionalized by some molecules to cross the BBB and specifically target glioma cells. For example, Angiopep2 (A2) has brain-targeting properties, such that its modified polymeric counterpart can penetrate the BBB through receptor-mediated transport, which is widely applied to design nanocarriers (66). Polo-like kinase 1 (PLK1) serves a key role in cell cycle regulation, where its expression in glioma tissues was found to be significantly higher compared

Table I. Preclinical studies of anti-glioma chemotherapeutics.

Author (year)	Agent	*In vivo*/*In vitro*	Combination therapy	Treatment effect	(Refs.)
Wang *et al*, 2016	TMZ	*In vitro*	-	Inhibiting proliferation, migration and invasion	(35)
Da Ros *et al*, 2018	TMZ	*In vitro*	Aldoxorubicin	Enhancing the killing effect of TMZ on TMZ-resistant glioma cells	(36)
Shi *et al*, 2020	TMZ	Both	Polymer micelle	Inhibiting cell viability and prolonging the survival time of U87 mice	(16)
Hu *et al*, 2021	TMZ	-	NF-κB and MGMT inhibitors	Downregulating MGMT and improving TMZ sensitivity	(37)
Wu *et al*, 2021	TMZ	*In vitro*	Talazoparib	Inhibiting the proliferation and downregulating MGMT and poly (ADP-ribose) polymerase	(38)
Rahman *et al*, 2019	TMZ	Both	Bortezomib	Inhibiting the proliferation, MGMT, MGMT mRNA and nuclear phosphorylation/activation p65. Prolonging the survival time of mice	(39)
Rezaei *et al*, 2020	BCNU	*In vitro*	miR-181a	Inhibiting cell proliferation and migration. Promoting apoptosis	(40)
Lu *et al*, 2016	Nimustine	*In vitro*	Hyperbaric oxygen	Inhibiting tumor growth and reducing the tumor volume	(41)
Yi *et al*, 2019	BCNU	Both	Metal nanoparticles	Inhibiting proliferation and decreasing the survival rate. Targeting efficiency is higher	(42)
Ashrafzadeh *et al*, 2020	Cisplatin	Both	OX26 monoclonal antibody and liposomes	Increasing the uptake of C6 cells. Improving the mean survival time and reducing toxicity	(43)
Zhang *et al*, 2017	Cisplatin	*In vitro*	Nanoparticles and convection-enhanced delivery	The distribution volume of the brain was higher and prolonging the survival time of rats	(44)
Thakur *et al*, 2020	Doxorubicin	*In vitro*	Exosomes	Inhibiting the proliferation	(45)
Meng *et al*, 2019	Doxorubicin	Both	Nano-micelle	Inhibiting the proliferation and inducing apoptosis. Reducing the tumor volume and increasing drug accumulation	(46)
Zhang *et al*, 2017	Doxorubicin	*In vivo*	Liposomes and vincristine	Reducing the tumor volume and prolonging the median survival time	(27)
Park *et al*, 2016	Vincristine	*In vitro*	-	Inhibiting glioma cell proliferation and glioma angiogenesis	(47)
Fu *et al*, 2019	Vincristine	Both	Nanoparticles	Increasing the toxic effect of vincristine. Reducing tumor and prolonging the mean survival time	(48)
Wu *et al*, 2016	Vincristine	Both	Solid nano-lipid particles, NLCS and TMZ	The inhibition effect of NLCS was the strongest and inhibiting tumor growth	(49)
Koosha *et al*, 2017	Topotecan	*In vitro*	A966492 and radiation	Decreasing the cell survival rate and increasing the radiation lethal effect	(50)
Sharon and Rubinstein, 2021	Topotecan	*In vitro*	CaCl2 and PLGA microspheres	Decreasing the relative survival rate of cells and increasing the toxic effect of topotecan	(51)

TMZ, temozolomide; MGMT, O6-methylguanine-DNA methyltransferase; NLCS, nano-lipid carriers; BCNU, carmustine.

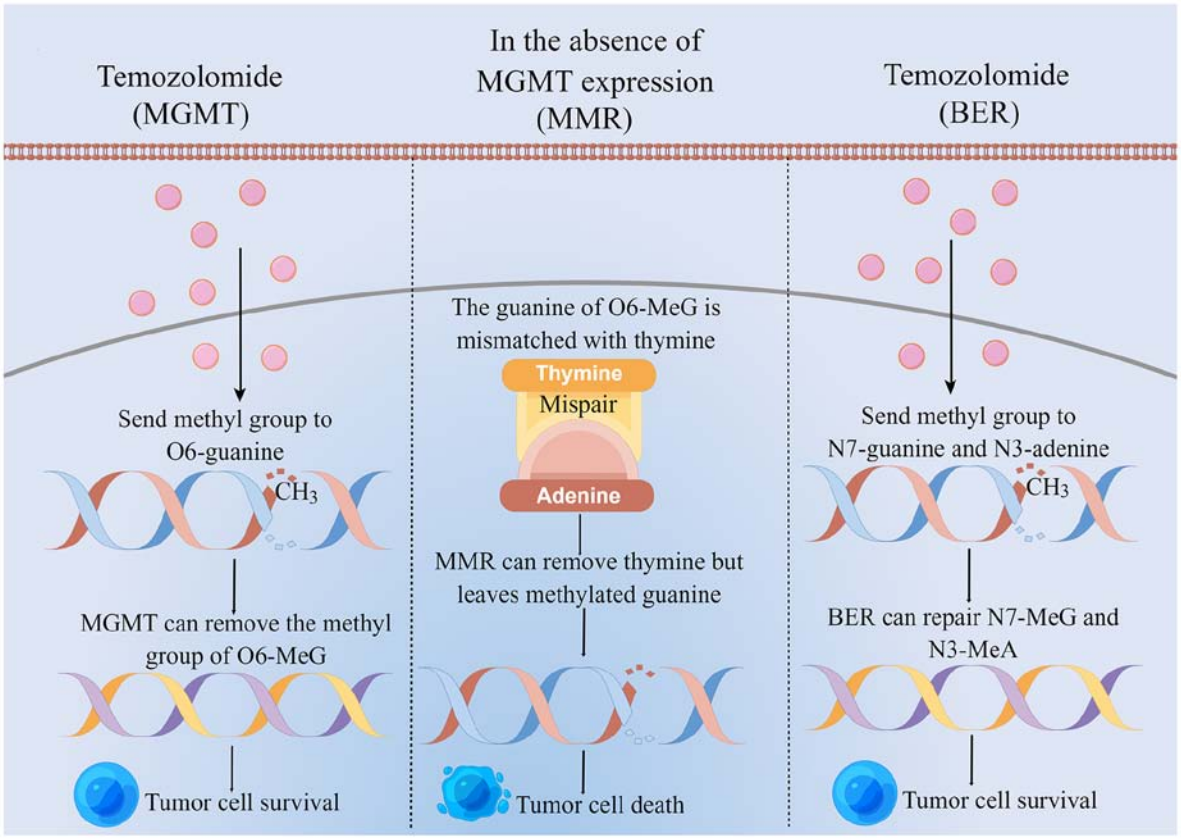

Figure 1. DNA repair mechanism of TMZ against tumor resistance. MGMT, O6-methylguanine-DNA methyltransferase; O6-MeG, O6-methylguanine; N7-MeG, N7-methylguanine; N3-MeA, N3-methyladenine; CH_3, methyl; MMR, mismatch repair; BER, base excision repair.

with that in corresponding normal brain tissues. Inhibition of PLK1 activity can lead to cell cycle arrest and increase apoptosis of glioma cells. Shi *et al* (16) previously prepared a polymer micelle modified by A2 (A2PEC). These micelles in turn encapsulated TMZ and small interfering RNA (siRNA) targeting PLK1 (TMZ-A2PEC/siPLK), which were used in U87 and LN-299 glioma cells. The results of these previous *in vitro* experiments showed that TMZ-A2PEC/siPLK could effectively reduce cell viability to a greater extent compared with that mediated by TMZ alone. In addition, results from *in vivo* experiments revealed that TMZ-A2PEC/siPLK could significantly prolong the survival time of mice injected with U87 cells (47.5 days). The degree of weight loss was also the slowest in the TMZ-A2PEC/siPLK, which also exhibited the least systemic toxic effects. TMZ-A2PEC/siPLK is an option for glioma therapy because it can reverse drug resistance while minimizing the toxicity of TMZ (16). This strategy of drug co-delivery using nanocarriers and molecular targeting has the potential of reversing chemotherapeutic drug resistance, reducing toxic effects and improving treatment efficacy.

Study on targeted therapy for reversing TMZ resistance. The unique stability and solubility of TMZ render it viable for oral administration and gives it the ability to pass through the BBB. It can be activated without liver metabolism and can spontaneously transform into the active alkylating agent monomethyl triazene 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MITC) under physiological conditions (67,68). The main mechanism of TMZ for treating glioma is to first deliver its methyl group to tumor cell DNA, causing DNA methylation and DNA damage in tumor cells. This in turn inhibits tumor cell proliferation, while inducing cell apoptosis (62,69). TMZ destroys the DNA of tumor cells by delivering the active methyl groups to O^6 and N^7 position of guanine and the N^3 position of adenine to form cytotoxic O^6-methylguanine (O^6-MeG), N^7-methylguanine (N^7-MeG) and N^3-methyladenine (N^3-MeA) (70). By contrast, cytotoxicity induced by TMZ is mainly caused by O^6-MeG. TMZ-resistant DNA repair systems mainly include O^6-methylguanine-DNA methyltransferase (MGMT), mismatch repair (MMR) and base excision repair (BER) (Fig. 1) (62). MGMT is a DNA repair protein that removes the methyl group from O^6-MeG and binds it to its own cysteine residue, so that the tumor cells survive (71,72). In the absence of MGMT expression, O^6-MeG mismatches with thymine, where MMR removes thymine leaving behind the methylated guanine, which reduces the efficiency of DNA mismatch repair and produces double strand breaks, in turn inducing eventual cell death. BER can repair N^7-MeG and N^3-MeA so that the tumor cells survive (73-75). MGMT is the most important factor for TMZ resistance in GBM and can be regulated by various factors, including transcription factors, epigenetic elements (such as methylation, phosphorylation, histone acetylation and microRNA expression from the MGMT promoter), the protein kinase A, and the MAPK/JNK and NF-κB signaling pathways (76). Both O^6-benzylguanine (O^6-BG) and O^6-(4-bromophenyl) guanine (O^6-BTG) are MGMT inhibitors. Although both held promise as a treatment option for glioma, they can cause damage to normal tissues,

causing adverse events (71). Hu *et al* (37) revealed that receptor-interacting protein 2 (RIP2) could activate the NF-κB signaling pathway, upregulate the expression of MGMT and increase the resistance of glioma cells to TMZ. By using NF-κB and MGMT inhibitors combined with TMZ on TMZ-resistant glioma cell lines T98G and U87MG in addition to RIP2-overexpressing cells, it was found that MGMT expression was downregulated whereas the inhibition of NF-κB/MGMT signaling can improve TMZ sensitivity. The RIP2/NF-κB/MGMT signaling pathway serves an important role in mediating TMZ resistance. Poly (ADP-ribose) polymerase (PARP) is an enzyme that regulates BER. PARP1-mediated BER/single strand break repair is a key process for the repair of N^7-MeG and N^3-MeA. Wu *et al* (38) previously documented that the PARP inhibitor talazoparib (25 nM) significantly inhibited the proliferation of MGMT-positive glioma globular-forming cells induced by TMZ. In addition, the protein expression levels of MGMT and PARP activation induced by TMZ were decreased. PARP can regulate the activity of MGMT through BER, whereas PARP inhibitors can improve the sensitivity of TMZ for the treatment of gliomas. The *in vitro* results from a study by Rahman *et al* (39) demonstrated that pre-treatment with 10 nM 26S proteasome inhibitor BTZ combined with TMZ could significantly inhibit the proliferation of glioma cells while reducing the expression levels of MGMT, MGMT mRNA and nuclear phosphorylation/activation of the p65 subunit. Subsequent *in vivo* experiments revealed that BTZ + TMZ treatment could significantly reduce the tumor volume and prolong the survival time of the mice (the median survival time was 114 days). BTZ + TMZ was therefore proposed to consume MGMT and block NF-κB/p65 signaling transduction to improve the sensitivity of TMZ (39). Riluzole is a metabolic glutamate receptor 1 (mGluR1) inhibitor. It has been previously reported that riluzole can inhibit the progression of GBM by inhibiting the PI3K/AKT/mTOR signaling pathway. PI3K inhibitors can also inhibit the expression of MGMT by inhibiting the PI3K/AKT/NF-κB and mGluR1/PI3K/AKT/mTOR signaling pathways. TMZ combined with riluzole was found to significantly inhibit the proliferation of the MGMT-positive cell lines T98G and GL261, but not in the MGMT-negative cell line U87. In addition, riluzole was also able to downregulate MGMT expression induced by TMZ. TMZ combined with riluzole could significantly reduce the average maximum cross-sectional area and volume of the tumors in mice injected with GL261 cells, suggesting TMZ + riluzole as a potential treatment (77). The expression level of MGMT is associated with the efficacy of treatment. Overexpression of MGMT is an important mechanism underlying TMZ resistance. Understanding this mechanism while developing novel treatment methods would be beneficial for improving the sensitivity to TMZ.

3. Nitrosourea-based chemotherapy

Targeted therapy and other combinatorial therapy using nitrosourea-based chemotherapeutic drugs. Nitrosourea-based chemotherapeutic drugs, including nimustine (ACNU), carmustine (BCNU), lomustine (CCNU) and ranimustine can pass through the BBB. ACNU is a second-line chemotherapeutic agent for the treatment of gliomas (78,79). Nitrosourea-based chemotherapeutic agents act by inducing cytotoxicity in malignant gliomas, where they can induce tumor cell death and changes in biological behavior. These drugs mainly target tumor cell DNA by first alkylating the bases of tumor cell DNA, which then form inter-chain crosslinks. This causes high cytotoxicity, induces DNA double-strand break and prevents DNA replication and transcription to finally result in cell apoptosis and necrosis (80). Nitrosourea chemotherapeutic drugs have myelosuppression and hematological toxicity (80,81). Due to the limited efficacy of chemotherapeutic drugs when applied alone, previous studies that investigated the effects of multiple combination therapies revealed that the resultant efficacy is superior compared with that in chemotherapy alone (30,80,82). O^6-alkylguanine-DNA alkyltransferase (AGT)/MGMT is the main component that determines the efficacy and drug resistance of nitrosourea. O^6-BG is an inhibitor of AGT that can effectively inhibit the activity of AGT. By contrast, O^6-BTG can exert superior inhibitory effects on AGT, and has since entered clinical trial testing, but it lacks a specific target and induces serious myelosuppressive toxicity (83,84). Tumor cell death is dependent on the inhibition of glycolysis. The glycolysis inhibitor 3-bromopyruvate combined with BCNU has been shown to significantly enhance the cytotoxicity of BCNU while reducing intracellular ATP and glutathione levels in human glioma cell lines SF763 and SF126. Glycolysis inhibitors are expected to become an effective agent for reversing nitrosourea resistance (84). Rezaei *et al* (40) previously treated U373 glioma cells with microRNA (miR or miRNA)-181a combined with BCNU. The results showed that miR-181a mediated downregulation of AKT1, suggesting that miRNA can inhibit glioma cell proliferation by regulating the PI3K/AKT signaling pathway. In addition, miR-181a significantly reduced the semi-inhibitory concentration of BCNU from 169.8 μM in BCNU alone to 83.25 μM in the combined treatment group. The combined use of miR-181a and BCNU was also shown to significantly inhibit cell migration compared with that in BCNU alone. In addition, miR-181a could potentiate apoptosis induced by BCNU (40). Hyperbaric oxygen is a non-invasive form of therapy that can inhibit glioma cell proliferation and inflammatory cell infiltration. Lu *et al* (41) used hyperbaric oxygen combined with ACNU to treat mice injected with the human glioma SU3 stem cells, which significantly inhibited tumor growth and reduced tumor volume, where the tumor inhibition rate was ≤76.29%. The tumor weight of the mice treated with combined therapy was found to be significantly lower compared with that in mice treated with either drug alone, suggestive of a synergistic effect. Subsequent tumor histopathological results showed that after hyperbaric oxygen combined with ACNU treatment, the degree of necrosis and inflammatory cell infiltration were reduced. However, toxicity associated with nitrosourea-based chemotherapy and resistance to nitrosourea are key factors that minimize their efficacy. To address the toxicity issue, Yi *et al* (42) used the synthesized BCNU and loaded them into metal nanoparticles prior to treating glioma U251 cells, which was found to inhibit proliferation while significantly decreasing its survival rate. In the mouse model, this nano-compound was shown to readily deliver the drugs to the brain without causing damage to the normal brain tissue. In addition, it tended to preferentially accumulate in glioma cell masses, to a greater degree

compared with the accumulation of BCNU alone. The results of subsequent wound healing assays *in vivo* revealed that the synthesis of BCNU and metal nanoparticles is beneficial to wound tissue regeneration and promote wound healing, implicating them to be another potential treatment option (42). Nitrosourea chemotherapeutic drugs have been applied for the treatment of gliomas before the emergence of TMZ (81). Over the past decade, previous studies have shown that nitrosourea chemotherapy can be used for the remedial treatment of TMZ resistance (85).

4. Platinum chemotherapy

Platinum chemotherapeutic agents include cisplatin, carboplatin and oxaliplatin. It has been reported that the exposure of glioma cells to the platinum family of chemotherapeutic drugs can induce tumor cell death. The potential mechanism underlying this effect may be the induction of single-strand or double-strand breaks in the DNA (86). Cisplatin and carboplatin are broad-spectrum agents that cannot be taken orally. Instead, they need to be injected intravenously because they are not soluble in water and are not stable. In addition, they are polar macromolecules, meaning that they cannot pass through the BBB easily (87). This results in low antitumor efficacy, high risks of systemic toxicity and drug resistance. Cisplatin therapy can cause nephrotoxicity and gastrointestinal toxicity, while carboplatin therapy can cause myelosuppression and ototoxicity (88). After carboplatin and cisplatin are injected intravenously, the permeability of BBB to carboplatin and cisplatin is limited, leading to the uneven distribution of these drugs to the tumor area, where the antitumor effect is poor. However, although arterial infusion can greatly increase the accumulation of drugs in the tumor cell masses, it is considered to be highly toxic (89). To improve therapeutic efficacy and reduce systemic toxicity, chemotherapeutic drugs can be packaged into liposomes, peptide/protein conjugates, polymers, polymer micelles and nanoparticles (90). Transferrin receptor (TR) is a potential biological vehicle for drug delivery in the brain. Furthermore, OX26 is a mouse monoclonal antibody that can target TR in rats. Ashrafzadeh *et al* (43) previously packaged the OX26 monoclonal antibody into the targeted polyethylene glycol cisplatin liposomes (TPL-Cispt). Results from the cell uptake assay *in vitro* showed that TPL-Cispt delivery resulted in significantly higher cell uptake, specifically increasing uptake by C6 cells 1.43-fold. Subsequent results from *in vivo* experiments showed that the mean survival time (MST) of rats treated with TPL-Cispt was 45 days, which represents a 1.7-fold increase. In addition, the toxicity normally induced by cisplatin was shown to be reduced. Therefore, polyethylene glycol liposomes carrying cisplatin was concluded to improve the treatment efficacy of brain tumors with reduced toxicity. However, lipid nanoparticles are typically large in diameter and adhere to the extracellular matrix, meaning that they cannot penetrate brain tumors efficiently. Zhang *et al* (44) previously manufactured a type of brain-penetrating nanoparticles encapsulating cisplatin and delivered it by convection-enhanced delivery (CED). CED (Fig. 2) is a minimally invasive, locally targeted drug delivery method. Using stereotactic technology, the therapeutic agents in the catheter are directly injected into the tumor around the BBB to achieve the purpose of accurate targeted therapy (91-93). The diffusion rate of brain-penetrating nanoparticles containing cisplatin in healthy rats and rats injected with F98 glioma cells was found to be significantly higher compared with that in rats treated with cisplatin alone (44). In addition, the distribution volume of brain-penetrating nanoparticles containing cisplatin in the brain was found to be 14 times higher compared with that in the cisplatin-alone group. After the brain-penetrating nanoparticles containing cisplatin and cisplatin alone were delivered separately through CED, the distribution volume of brain-penetrating nanoparticles containing cisplatin in the brain was found to be 29 times higher compared with that in the cisplatin-alone group. In terms of survival, the targeted delivery of brain-penetrating nanoparticles of cisplatin using CED can significantly prolong the survival time of rats with superior tolerance (44). In conclusion, although platinum-based chemotherapeutic agents are highly effective especially *in vitro*, the BBB and occurrence of systemic toxicity limit their efficacy in treating glioma, which require optimization using alternative drug delivery systems.

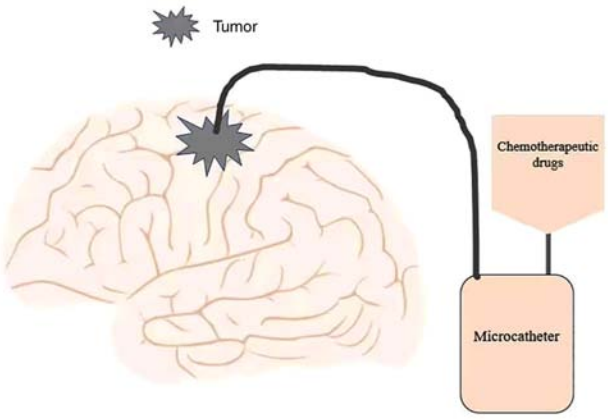

Figure 2. Schematic diagram of convection-enhanced delivery.

5. Other chemotherapy agents

Doxorubicin (DOX). DOX is an anthracycline antibiotic chemotherapy agent and is one of the most extensively used antitumor drugs (94). Its main mechanism of action is by quickly entering the nuclei of tumor cells and inhibiting the proliferation of tumor cells by binding to DNA and blocking DNA replication (95,96). DOX has been found to inhibit the proliferation of glioma cells. After the glioma cells were treated with 10 and 100 nM of DOX for 7, 10 and 14 days, the cell survival rate was decreased in a dose- and time-dependent manner, which was the lowest at the dosage of 100 nM DOX for 14 days. However, DOX treatment combined with TMZ conferred no synergistic effects (97). DOX cannot pass through the BBB easily, which limits its use in gliomas. However, exosomes can cross the BBB and serve as a potential carrier for the delivery of chemotherapeutic drugs for glioma. Thakur *et al* (45) previously loaded DOX into exosomes derived from SF7761 glioma stem cells and U251 glioma cells using a microfluidic device, before treating SF7761 and U251 cells with DOX-containing exosomes. The results showed that DOX-containing exosomes can inhibit the proliferation of cells more effectively compared

with that treated with DOX alone. Over the past number of years, to improve the permeability of BBB to chemotherapeutic drugs, studies into novel drug delivery systems have accelerated. Meng *et al* (46) used a borneol-modified nano-micelle delivery system to encapsulate and deliver DOX. Results from proliferation experiments *in vitro* showed that borneol-modified DOX nano-micelles inhibited the proliferation and migration of C6 glioma cells while inducing C6 cell apoptosis to a higher degree, where the magnitude of these inhibitory effects was greater compared with those mediated by DOX alone. The results of tumor experiments *in vivo* revealed that borneol-modified DOX nano-micelles significantly reduced the tumor volume, bleeding and necrosis in mice compared with those treated with DOX alone. There were no clear side effects following measurements of the weight of the mice. In addition, borneol-modified DOX nano-micelles significantly improved the transport efficiency of DOX across the BBB, which was rapidly accumulated in the brain tissues. However, large doses or long-term use of DOX can cause cardiomyocyte toxicity, ultimately leading to heart failure. To minimize this toxic adverse effect, it has been proposed that DOX liposomes can be used for targeted delivery, where the combination of DOX liposomes with other drugs conferred superior antitumor activity (94,96). Vincristine can mediate cardioprotective effects against DOX-induced cardiomyocyte toxicity and chemical and hypoxic oxidative stress. T7 is a heptapeptide ligand of the transferrin receptor that can bypass the BBB and target glioma directly. ^{D}A7R is a D-peptide ligand of VEGF receptor 2 overexpressed on angiogenesis, which has exhibited promising homing characteristics towards glioma. Zhang *et al* (27) previously applied T7- and ^{D}A7R dipeptide-modified liposomes to co-deliver DOX and vincristine into mouse glioma tumors, which significantly reduced the tumor volume, reduced the tumor proliferation rate and significantly prolonged the median survival time (34 days). Although DOX confers anti-glioma effects, it cannot pass through BBB at its effective therapeutic dose range. Therefore, it is necessary to develop novel drug delivery systems into the brain to improve its efficacy (98,99).

Vincristine. Vincristine is a type of drug that functions by destroying the stability of microtubules and interferes with the metabolism of tumor cells by acting on the tubulin cytoskeleton of tumor cells (100). Therefore, anti-angiogenesis and antitumor activities (47) have been reported. Park *et al* (47) reported that vincristine can inhibit glioma cell proliferation in a dose-dependent manner while inhibiting glioma angiogenesis under hypoxic conditions, which was proposed to be mediated at least in part by the inhibition of hypoxia-inducible factor-1α (HIF-1α). Vincristine is a microtubule-targeting drug that is also commonly used in gliomas. Microtubule-associated protein 2 (MAP2) is a microtubule-stabilizing protein. Knocking down MAP2 expression can significantly inhibit the migration and survival of glioma cells *in vitro*, in addition to inducing apoptosis and increasing the sensitivity of glioma cells to vincristine (101). Vincristine has a large molecular weight and therefore cannot pass through the BBB easily, which can cause peripheral neuropathy and systemic toxicity (102). The existence of BBB and the blood-brain tumor barrier (BBTB) render the accurate targeting of chemotherapeutic drugs challenging. Fu *et al* (48) designed a peptide nanoparticle carrier dual-modified with T7 and reticulon 4 receptor (NGR) based on encapsulated solid lipid nanoparticles from red blood cells (T7/NGR-RBCSLNs) to encapsulate vincristine for the treatment of gliomas. T7 was found to penetrate the BBB and target glioma cells. NGR is a polypeptide ligand of CD13 that is overexpressed during angiogenesis and has demonstrated promising homing characteristics towards glioma. In addition, it can penetrate the BBTB and target glioma cells. The results of cytotoxicity testing *in vitro* showed that T7/NGR-RBCSLNs encapsulated with vincristine could significantly increase the toxic effects of vincristine on C6 cells. Subsequent tumor experiments *in vivo* found that the tumor in the T7/NGR-RBCSLNs-encapsulated vincristine group was significantly reduced, where the relative tumor inhibition rate was the highest and the MST was significantly prolonged (36 days). Vincristine combined with the double-targeting delivery (T7/NGR-RBCSLNs) showed the optimal anti-glioma effects *in vivo* and *in vitro* in the brain. The efficacy conferred by treatment with only a single chemotherapeutic drug is limited whereas treatment with a combination of drugs can improve efficacy without worsening systemic toxicity. Therefore, overcoming drug resistance using other drug delivery systems is required to enhance the efficacy of chemotherapy. Wu *et al* (49) used solid nano-lipid particles and nano-lipid carriers (NLCS) to enclose vincristine and TMZ. Subsequent results from the *in vitro* cytotoxicity assay showed that the inhibitory effect of NLCS-vincristine + TMZ on U87 glioma cells was the most potent, which was significantly higher compared with that mediated by either drug alone. The results of this previous tumor study *in vivo* showed that NLCS-encapsulated vincristine and TMZ significantly inhibited tumor growth, where the tumor inhibition rate was >80%. These results were consistent both *in vivo* and *in vitro*. This dual-drug administration provides a novel idea for the clinical intervention of glioma.

Topotecan. Topotecan is a water-soluble, semisynthetic camptothecin analog that has a small molecular weight and can pass through the BBB (103). It has been approved for use in different types of tumors as topoisomerase I inhibitors by Serwer *et al* (104). It is toxic to glial cells and relatively non-toxic to normal brain tissue (105). Topotecan can form stable topoisomerase I and DNA cleavage complexes *in vivo* and *in vitro*. During the process of DNA replication in tumor cells, topoisomerase I can be embedded in DNA, resulting in DNA damage and fragmentation, which finally promote the death of tumor cells (104). Topotecan has significant reported antitumor effects according to various preclinical studies (106). Small ubiquitin-like modifier (SUMO) modification is a unique form of protein post-translational modification that is key for the maintenance of cell function under stress or adverse conditions. However, blocking SUMO-1-3 binding in glioma cells can inhibit DNA synthesis, proliferation and survival of glioma cells. Topotecan (1 and 10 μM) significantly inhibited SUMO binding in glioma cells, decreased cyclin-dependent kinase 6 and HIF-1α expression and changed the cell cycle and metabolic profile (103). At present, the most important strategy used to treat glioma is to maximize surgical resection combined with radiotherapy and chemotherapy. However, the adverse

Table II. Clinical trials of anti-glioma chemotherapy drugs.

Clinical trials number	Status	Agent	Phase	Combination therapy	Research purposes
NCT00272870	Terminated	TMZ	I	O6 Benzyl guanine	Overcoming TMZ resistance and hematologic toxicity
NCT05052957	Not yet recruiting	TMZ Carmustine	II	O6 Benzyl guanine	Treatment
NCT00687765	Completed	TMZ	I and II	BSI-201 (PARP-1 inhibitor)	Determining the maximum tolerated dose of BSI-201 and evaluating efficacy
NCT03914742	Recruiting	TMZ	I and II	BGB-290 (PARP inhibitor)	Treatment
NCT03749187	Recruiting	TMZ	I	BGB-290 (PARP inhibitor)	Evaluating the side effects and the optimal dose
NCT00994071	Completed	TMZ	I	ABT-888 (PARP inhibitor)	Treatment
NCT01644955	Completed	Carboplatin	I	Convection-enhanced delivery	Treatment and assessing maximum tolerated dose and toxic effects
NCT03603379	Completed	Doxorubicin	I	Anti-EGFR-immunoliposomes loaded with doxorubicin (C225-ILs-dox)	Pharmacokinetics
NCT00944801	Completed	Doxorubicin	I and II	Pegylated liposomal	Treatment
NCT02766699	Unknow	Doxorubicin	I	EGFR(V)-EDV-Dox	Assessment of safety, tolerability and immunogenicity
NCT02333513	Unknow	Vincristine	-	-	Treatment
NCT03927274	Recruiting	Topotecan	I	Convection-enhanced delivery	Assessing safety and tolerability
NCT02278510	Completed	Topotecan	I	Gadolinium DTPA and convection-enhanced delivery	Treatment
NCT03154996	Completed	Topotecan	I	Gadolinium and convection-enhanced delivery	Treatment

PARP, Poly (ADP-ribose) polymerase; TMZ, temozolomide.

effects caused by radiotherapy and chemotherapy are typically severe. Radiosensitization drugs can improve the efficacy of radiotherapy and reduce its side effects. Koosha *et al* (50) reported the radiosensitization effects of topotecan combined with A966492 on U87 glioma cell spheres. A966492 is a new type of PARP inhibitor, that can cross the BBB. Compared with radiation alone, topotecan combined with A966492 and 6 mV X-ray radiation significantly increased the lethal effects of radiation on U87 glioma cell spheres. Chemotherapeutic drugs can induce systemic toxicity, while local chemotherapy can reduce systemic exposure. Sharon and Rubinstein (51) divided the dose of topotecan into three equal parts and changed it daily during the 72-h incubation with U87 glioma cells to simulate slow and continuous administration. The relative survival rate of cells was significantly decreased, from 35 to 7%. After $CaCl_2$ was added, the relative survival rate of cells was decreased even further, significantly from 28 to 1.9%. Topotecan mediated synergistic effects with $CaCl_2$. Co-loading of $CaCl_2$ into topotecan poly (lactic-co-glycolic acid) microspheres increased the toxic effects of topotecan on U87 cells, where the cell viability decreased from 72 to 27%. Topotecan has not been routinely used in clinical practice due to toxicity and related pharmacokinetic shortcomings. Drug delivery systems, such as poly (lactic-co-glycolic acid) microspheres, are increasingly being used as the blueprint to develop novel cancer treatments, because this technology can regulate the biological distribution and target accumulation of chemotherapeutic drugs, thereby reducing their toxicity.

6. Advances in the clinical application of anti-glioma chemotherapeutics

In recent years, patients with glioma who received a single chemotherapy regimen are particularly at risk of drug resistance and relapse. In a previous clinical study of 27 patients treated with TMZ combined with cisplatin, the progression-free survival (PFS) was improved (107). In another clinical trial, TMZ combined with bevacizumab was used to treat 30 patients with recurrent GBM, which yielded a median overall survival (OS) of 51 weeks and a PFS of 52% at 6 months, suggesting that this treatment may be effective for patients with recurrent GBM (108). Herrlinger *et al* (109) previously compared the effects of CCNU combined with TMZ and standard TMZ therapy on the survival time of patients with GBM and MGMT methylation. The results showed that the OS and median survival time in the CCNU + TMZ treatment group were significantly higher compared with those in the standard TMZ group (109). However, in the study performed by Kim *et al* (110), the efficacy of procarbazine combined with CCNU for the treatment of patients with recurrent GBM and MGMT methylation was not optimistic due to its toxicity. Although the combination of chemotherapeutic drugs together does exert certain curative effects, drug resistance and toxicity limit efficacy. Therefore, additional prospective studies are required to evaluate the efficacy of various combinations of chemotherapeutic drugs for the treatment of gliomas. A number of clinical trials (Table II) that are evaluating the targeted treatment of GBM using chemotherapeutic drugs and other combination therapies can be found in https://clinicaltrials.gov. Amongst these studies, one of them is the evaluation of the combination of O^6-BG and TMZ for the treatment of newly diagnosed GBM (ClinicalTrials.gov identifier no. NCT00272870).

7. Conclusion

Glioma is the most common malignancy in the central nervous system with high recurrence rates and poor prognosis. Drug resistance is the main complication obstructing glioma chemotherapy and provides a significant challenge for improving the therapeutic effects. The development of novel agents and the use of drug resistance induction media and molecular signaling pathway inhibitors are some of the methods of overcoming the resistance of glioma to chemotherapeutic drugs. The combined use of drugs and nanoparticles along with other carriers has been reported to reduce drug resistance and toxicity. Therefore, it is of importance to develop novel drug delivery methods for improving drug solubility, prolonging circulation time, enhancing the targeted effects and improving the efficacy of chemotherapeutic drugs for clinical application. As information is being constantly accumulated regarding the physiology of glioma, the comprehensive application of multiple treatment methods for glioma and the treatment mechanism of chemotherapy drugs, chemotherapeutic agents serve an increasingly important role for the treatment of glioma.

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Authors' contributions

YSZ wrote the manuscript. YSZ, NC and LCW collected and analyzed data. JBH and WW critically revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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