Drug Delivery and Translational Research
https://doi.org/10.1007/s13346-020-00747-y

ORIGINAL ARTICLE

Nose-to-brain delivery: exploring newer domains for glioblastoma multiforme management

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Abstract

Glioblastoma multiforme (GBM) is the most common and aggressive form of the primary brain tumors in humans. The intricate pathophysiology, the development of resistance by tumor cells, and the inability of the drugs to effectively cross the blood-brain and blood-tumor barriers result in poor prognosis for GBM patients, with a median survival time of only 1 to 2 years. Nose-to-brain delivery offers an attractive, noninvasive strategy to enhance drug penetration or transport novel drug/gene carriers into the brain. Although the exact mechanism of intranasal delivery remains elusive, the olfactory and trigeminal nerve pathways have been found to play a vital role in circumventing the traditional barriers of brain targeting. This review discusses the intranasal pathway as a novel domain for delivering drugs and nanocarriers encapsulating drugs/genes, as well as stem cell carriers specifically to the glioma cells. Considering the fact that most of these studies are still in preclinical stage, translating such intranasal delivery strategies from bench to bedside would be a critical step for better management and prognosis of GBM.

Keywords Glioblastoma multiforme · Nose-to-brain delivery · Nanocarriers · Blood-brain barrier · Brain targeting

Introduction

Epidemiology

Gliomas refer to the primary brain tumors consisting of a varied clutch of neoplasms derived from numerous different cell ancestries, primarily, glial cells. Gliomas can ensue anywhere in the central nervous system (CNS), primarily, the brain. It accounts for 30% of all the brain and CNS tumors and counts to about 80% of malignant brain tumors [1–3]. These are the utmost typical form of malignant tumors in adults and still remain as one of the prime challenges to the brain cancer research fraternity with a scanty progress in the patient survival rate over the last few decades [4, 5]. Although there have been prominent advances in the field with regard to malignant glioma in adults and children, a lot is still to be achieved.

Tumors of the brain and CNS have always been classified on the basis of morphology and, recently, immunohistochemically, but with less emphasis on the underlying molecular pathogenesis. The World Health Organization, in 1979, classified brain tumors according to grades; the same was amended in the year 2007 and currently serves as a notable means of grading classes of tumors according to their biological behavior. A summarized classification of the tumors has been depicted in Fig. 1.

Currently, the standard therapy for glioma is utmost safe resection and the same is followed by chemotherapy or radiotherapy or both, and/or photodynamic therapy. However, due to the infiltration and invasive nature of glioma, it is quite difficult to achieve total resection without damage to normal brain tissue; therefore, the survival rate and quality of life of the patients are affected [6–8]. Thus, there prevails a great need to develop new methods for preoperation planning and intraoperation navigation defining boundaries of gliomas in order to achieve maximum gross total resection and, thereby, improved patient survival rate and quality of life.

Current management

Diagnosis

GBM diagnosis is a multidisciplinary exercise for clinicians. The clinical symptoms include seizures, headaches, focal neurological deficits correlating to the site of the tumor, e.g., aphasia, and motor/sensibility discrepancies. In addition,

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Published online: 27 March 2020

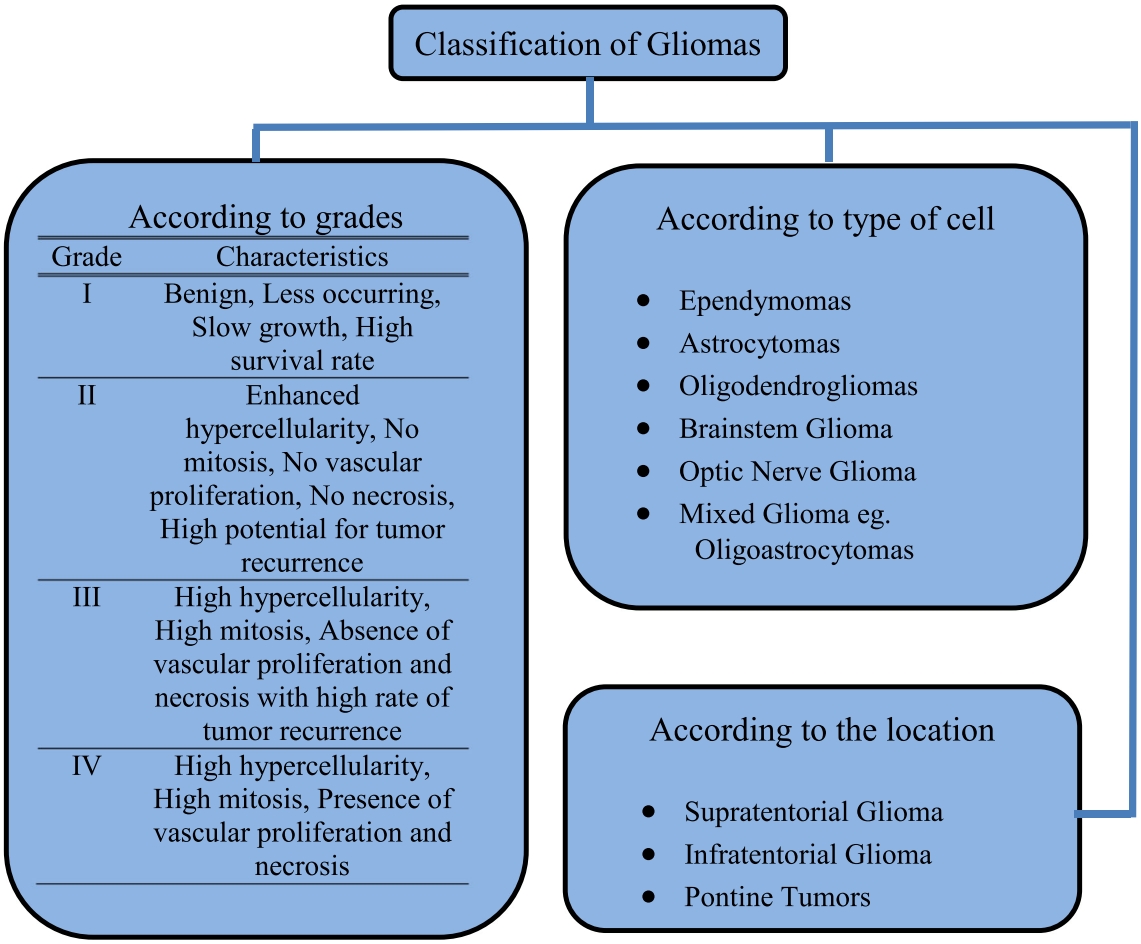

Fig. 1 Classification of gliomas

cognitive dysfunction is enormously prevalent in malignant gliomas. Magnetic resonance imaging (MRI) is generally the first test to confirm the presence of gliomal tumor in patients. MRI scans provide preliminary and basic information, i.e., the location of the tumor, its size, and the associated boundaries [9–11]. However, a high-quality MRI image can further help in operative settings and provide other vital information to surgeons. With the advancements in the imaging technology, newer methods for tumor imaging have appeared as single imaging modality fails to provide the information needed by the clinicians. This led to the concept of multimodal imaging which utilizes information generated by more than one imaging modalities, combines them, and provides copious and precise information about the tumor thereby improving the quality of surgery, understanding the prognosis, and precise grading of glioma [12–14]. Despite the aforementioned advantages, MRI has certain disadvantages such as lack of efficiency under the absenteeism of damage to blood-brain barrier (BBB) and trouble in identifying abnormal imaging as tumor recurrence/progression/pseudo-progression [15]. With an aim to recompense the aforementioned downsides of conventional MRI examination, positron emission tomography –computed tomography (PET-CT), centered in tumor metabolic imaging, is used. When united with the anatomical information of conventional MRI, PET-CT affords an important basis for treatment of sensitive glioma and patients' prognoses [16–23].

Standard of care

Upon diagnosis of glioma, generally the immediate choice of the clinicians is surgery. However, the intention of the surgery is to minimize the symptoms from the mass effect, establishing histological diagnosis and increasing efficacy of the adjunct/adjuvant therapies [24]. In the recent years, the adjuvant chemotherapy including procarbazine, lomustine, and vincristine (PCV regimen) has been successful in advancing progression-free survival but not overall survival rate [25, 26]. The contemporary standard of care for recently diagnosed glioma patients is grounded upon the study in the randomized clinical phase 3 trial done in the year 2005 [25]. The therapy included concurrent administration of temozolomide (TMZ) along with radiotherapy, and further administration of TMZ continued as adjuvant (maintenance) treatment, post-radiation cycle, for 5 days a week for 4 weeks. In clinical practice, TMZ

is generally administered up to 12 cycles if it is well tolerated by the patient.

In addition to the parenteral TMZ, a supportive local chemotherapy showed effectiveness in a prospective phase 3 trial. The trial involved surgical implantation of carmustine (BCNU)-containing polymer wafers straight into the surgical cavity, followed with radiotherapy [27–29]. Similarly, anaplastic gliomas have been generally treated by postoperative radiotherapy or chemotherapy alone [30]; similar results were also showed by the NOA-04 trial [31].

Nose-to-brain delivery: a noninvasive pathway to the brain

In the recent decades, intranasal drug administration has gained prominence owing to its practical importance, fast onset of action, and noninvasive approach to deliver drugs to brain and systemic circulation. Among the drugs which are preferred intranasally over other routes, special emphasis is given to those with BBB permeability issues, low stability and absorption in the gastrointestinal tract, and intense first-pass metabolism, specially molecules such as proteins, peptides, and high polar substances. In disparity to its clear gains, intranasal delivery has some limitations that include low bioavailability of proteins, peptides, and other highly polar molecules owing to the mucociliary clearance and enzymatic degradation. Specifically, proteases and aminopeptidases that can cleave peptides at their N- and C-termini or attack internal peptide bonds can result in the degradation of proteins and peptides either within the lumen of the nasal cavity or at the mucosal membrane [32]. There is also limitation in terms of the volume of drugs that can be sprayed in to the nasal cavity, and further, frequent use of this route of administration can cause mucosal irritation and/or nasal mucosa damage and even allergies [33–35]. However, the advances in formulation strategies such as utilization of bioadhesive polymers, penetration and absorption enhancers, and use of prodrugs and enzymatic inhibitors help to minimize the shortcomings of the intranasal delivery [36].

The pathway of nose to brain

The nose is divided by the midline septum into two cavities, each 12 cm long with 13 ml volume and surface area 150 cm^2. Each nostril is divided into 3 regions, namely the vestibular region, the respiratory region, and the olfactory region, which serves as the physiological site for intranasal transport to the brain. In the respiratory region, the mucous secreting goblet cells and the ciliated epithelial cells are involved in the mucociliary clearance mechanism that helps in eliminating foreign substances. As the blood supply to the respiratory epithelium is relatively greater, it serves as an ideal site for systemic absorption of nasally administered drugs. The trigeminal nerve endings in the respiratory and the olfactory region convey chemosensory information to the CNS. The olfactory neurons in the olfactory region are interspersed among basal and supporting cells to form the olfactory epithelium. Drugs are transported to the brain through the nasal mucosa to the CNS via the perivascular channels in the lamina propria or via intracellular/extracellular mechanisms involving olfactory and trigeminal nerves (Fig. 2).

In the recent years, nanoparticles have been explored for intranasal delivery owing to their ability to safeguard the drug from biological and chemical degradation and prevention from p-gp efflux–mediated extracellular transport [38]. Also, bioadhesive nanoformulations provide good residence time and possess the ability of opening the tight junctions of the mucosal epithelium owing to the use of the surfactants thereby enhancing the brain delivery [39, 40]. Among the colloidal carriers, vesicular systems such as liposomes [41–44] and niosomes [45], lipidic systems such as nanoemulsions [46–48], nanostructured lipid carriers [49], solid lipid nanoparticles [50, 51], polymeric nanoparticles [52–55], and micellar systems [56, 57] are the most favorable for nasal drug delivery, especially owing to their biocompatibility. A representative list of drugs, nanocarriers, and stem cell carriers investigated for the treatment of glioma via the intranasal route of administration is given in Table 1.

Enhancement of intranasal delivery through formulations

Based upon the application necessities, a variety of formulations have been developed to improve the delivery of pharmacological moieties to the brain. They vary from simpler formulations such as nasal solutions to novel formulations such as gel or spray systems containing nanoparticulate systems encapsulating drugs.

Nasal drug solutions

The uptake across the nasal epithelium is a complex process owing to the poor transport, possible degradation, and rapid clearance. However, despite the nanotechnological approaches as discussed in the above section, solutions of drugs sprayed in the nasal cavity have also been known to deliver pharmacological moieties to the brain. Intranasal solutions are associated with problems such as drainage and rapid clearance with low permeability being the prime concern. However, in order to improve the permeability, the use of permeation enhancers has proven to be of aid. Permeation enhancers used for improvement of intranasal permeation include surfactants such as Laureth-9 [72], Brij 35 and Brij 96 [73], bile salts and its derivatives such as sodium glycocholate [74] and sodium tauro-24,25-dihydrofusidate [75]; phospholipids such as didecanoyl-L-μ-phosphatidylcholine [76]

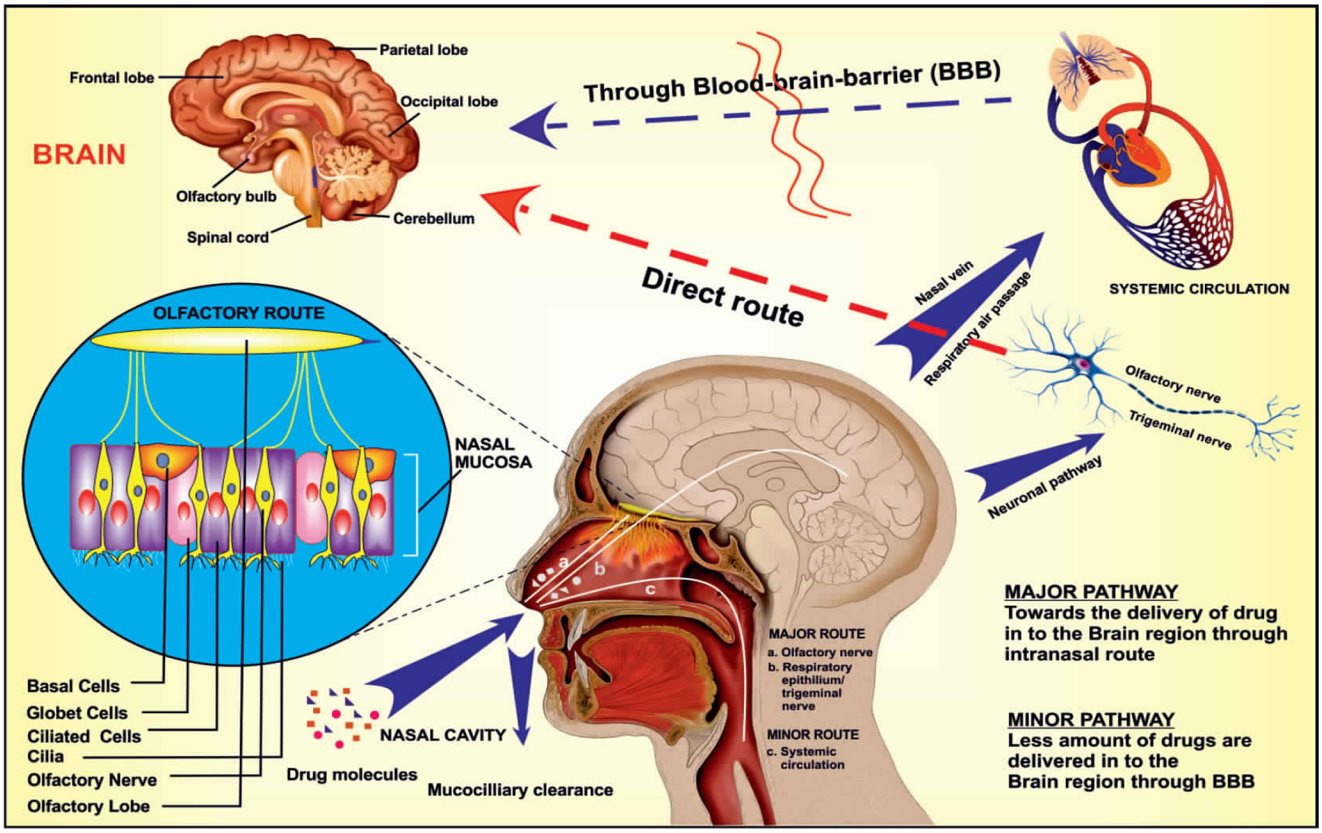

Fig. 2 Drug transport from the nasal cavity to the brain primarily through the neuronal pathway via olfactory and trigeminal sensory neurons and secondly through the systemic circulation. Reprinted with permission from Agarwal et al. [37]

and dimyristoylphosphatidylglycerol [77]; cyclodextrins (μ, β, α) and their methylated versions [78, 79]; cationic polymers such as chitosan and its derivatives [80, 81]; and lipids such as oleic acid [82]. Jiang et al. investigated the levels of methotrexate in cerebrospinal fluid and blood of rats upon intranasal administration. It was found that plasma level upon intranasal administration was less than as compared with the intravenous administration. However, the concentration of methotrexate in the CSF was significantly higher than that upon intravenous administration. The ratio of the value of AUC_{CSF} between the intranasal and intravenous route was found to be 13.76 and the absolute bioavailability was 6.3%, whereas the drug targeting index was found to be 21.7 [83, 84]. In a similar study Shingaki et al. evaluated the outcome of acetazolamide, a cerebrospinal fluid secretion inhibitor, on intranasal transport of 5-flurouracil to the cerebrospinal fluid and subsequent uptake in the brain. When compared with the concentration of the drug reaching the brain when the dose was given intranasally to that when given intravenously, it was found that the drug reaching to the brain was better when administered via the intranasal route. Also, the plasma concentration of the drug via both routes was found to be similar [85]. Cho et al. delivered perillyl alcohol to the brain via the intranasal route in glioma-induced animal model to study its effect in TMZ-sensitive and TMZ-resistant glioma cells. It was seen that the animals treated through intranasal administration of perillyl alcohol exhibited decreased tumor growth and increased survival rate [86]. Similarly, Pineda et al. explored the intranasal route for the delivery of TMZ in glioma-bearing nude mice. It was seen that intranasal administration of the drug led to decreased tumor growth and increased life span of the animals bearing glioma [66].

In addition to drugs, delivery of oligonucleotides has also been investigated for GBM. Since they do not readily cross the BBB upon systemic administration, intranasal administration can be a noninvasive alternative to deliver these agents, which can be then utilized for precise gene upregulation and splice editing. One of the first successful instants of intranasal delivery of an oligonucleotide therapy for treating brain tumors was using GRN163, a telomerase inhibitor. Fluorescent-labeled GRN163 was found to rapidly distribute in the brain of non-tumor bearing rats while it preferentially accumulated in the intracerebral tumors. The tumor-induced rats survived significantly longer when treated with GRN163 for 12 days as compared with control animals and, further, showed no neurological symptoms or evidence of tumor at the end of 3 months [59].

Intranasal delivery of nanocarriers for GBM

Nanosystems facilitate controlled and targeted delivery circumventing BBB for effective GBM therapy. Their

Table 1 Representative list of intranasally delivered drugs/nanocarriers/stem cells for GBM

Compound	GBM model	Animals used	Intranasal dose	Efficacy	Ref.
Methotrexate	9 L rat glioma cells injected into the right frontal cortex	Male Wistar rats	2.5 mg	Decreased tumor volume	[58]
GRN163	U251MG human GBM cells injected intracerebrally	Male athymic rats (rnu/rnu, homozygous)	0.65 μmol	Increase in median survival rate	[59]
Vascular stomatitis virus	U87MG human GBM cells injected into striatum	CB17-SCID (CB17SC-M, NCr nude mice)	2.5×10^7 PFU	Olfactory bulb tumor reduction	[60]
Neural stem and progenitor cells	U87MG human GBM cells injected into right forebrain	Male NMRI-nu/nu or C57BL/6 mice	3×10^5 cells	Targeted migration to intracerebral glioma	[61]
Monoterpene perillyl alcohol	Recurrent GBM patients with at least 3 relapses	Humans	440 mg/day	Increase in median survival rate	[62]
Kaempferol	--	Wistar rats	100 μg	5-fold increase in brain distribution	[63]
Bortezomib	U251 human glioma cells injected intracranially	Athymic nude mice	1 mg/kg	Increase in median survival rate	[64]
Temozolomide	TG16, TG1N, and TG20 human glioma cells injected intracerebrally	Swiss nu/nu mice	7 mg/kg	Increase in median survival rate	[66]
Stem cells	Stereotaxic implantation of U251 human glioma cells	Athymic nu/nu mice	62,500–125,000 cells/μL	Improved survival rate	[67]
Farnesylthiosalicylic acid	Stereotaxic implantation of RG2 cells	Cyclosporine-treated Wistar rats	500 μM	Significant decrease in tumor area	[68]
Anti-Gal-1 siRNA	GL261-WT/GL 261-BFP tumor cells injected intracranially	C57BL/6J mice	--	Up to 50% reduction in Gal-1 in tumor-bearing mice	[69]
miR17 or miRNA	GL26-Luc cells injected intracranially	C57BL/6J mice (H-2b)	20 μg	Delayed brain tumor growth	[70]
Camptothecin	C6 glioma cells injected intracranially	Sprague-Dawley male rats	1.2 mg/kg	Improved survival rate	[71]

required attributes include biocompatibility, biodegradability, and *in vitro* and *in vivo* stability. The size of the brain-targeted nanocarriers is one of the main deciding factors for efficient delivery. It has been reported that colloidal nanoparticles having size less than 20 nm are transported extracellularly to the brain upon intranasal administration. In the case of intracellular transport to brain, the mechanism of endocytosis depends on the size of nanocarriers. Nanocarriers of size less than 200 nm are internalized via clathrin-dependent endocytosis, whereas particles of size 100–200 nm enter the cells via caveolae-mediated endocytosis. The exact mechanism of transport of nanocarriers via the nasal pathway is still unclear; however, both paracellular and transcellular transport of nanosystems to the brain have been observed [87, 88]. Further, in the case of intranasal delivery, nanoformulations should not aggregate upon contact with mucus, and the mucociliary clearance should be minimal. The material properties and the related surface chemistry significantly influence the capability to avoid mucociliary clearance while particle size, charge, and shape of the carrier do not seem to contribute critically [89, 90]. Lipid-based nanocarriers like liposomes and polymeric nanocarriers have been reported to exhibit minimal mucociliary clearance. In addition, it is imperative that the intranasally delivered carriers do not induce irritation or damage to the nasal mucosa [91]. Several nanocarriers have been explored for the delivery of neuroprotective therapeutics against neurodegenerative diseases like Alzheimer's, Parkinson's diseases in addition to antipsychotic drugs and drugs for migraine, and stroke [84]. The following sections discuss the different nanosystems investigated for glioma therapy.

Lipid-based intranasal delivery systems for GBM

Some of the studies evaluating intranasally administered nanocarriers for GBM treatment involved in vitro assessment of these nanocarriers in GBM cell lines followed by nasal mucosa permeation studies and pharmacokinetic or brain distribution analysis. One of the initial studies involving intranasally administered nanocarriers reported the formulation of hexadecylphosphocholine-based liposomes stabilized with soya lecithin and loaded with paclitaxel. These mucoadhesive liposomes of size 100–200 nm and zeta potential − 25 mV exhibited a slow and sustained release of paclitaxel in simulated nasal fluid and a sudden release in simulated cerebrospinal fluid. These nanocarriers were found to be endocytosed by the glioma cells through clathrin-mediated pathway, and an improved therapeutic efficacy was observed in chemoresistant U87MG glioma cells owing to the synergistic anticancer action of paclitaxel and miltefosine. The ability of these liposomes to cross BBB was analyzed by artificial membrane permeability assay and further demonstrated by *in vivo* brain

uptake studies upon intranasal administration in Wistar rats. [92]

Another lipid-based nanocarrier, prepared using high-pressure homogenization and ultrasonication, was used to augment brain targeting of TMZ via nasal route of administration. To the mixture of vitamin E, melted Gelucire and TMZ, hot surfactant solution consisting of Tween 80/Transcutol (6:4) was dispersed. The primary emulsion thus obtained was ultrasonicated, homogenized, and cooled to form TMZ nanostructured lipid carriers. The formulation was optimized using a four-factor, three-level Box−Behnken design to achieve a small particle size, maximum loading efficiency, and optimum drug release. The *in vitro* drug release studies revealed a sustained release of the drug from the nanocarriers while the *ex vivo* transport analysis revealed greater permeation in nasal mucosa. The pharmacokinetic and brain distribution studies carried out in Wistar rats showed enhanced brain targeting efficiency of intranasally administered nanocarriers as compared with intranasally/intravenously administered drug dispersion. The higher brain accumulation of radiolabelled TMZ nanocarriers upon intranasal administration as compared with those administered intravenously was also demonstrated using Gamma scintigraphy study [93]. In a similar study, nanostructured lipid carriers of the quercetin having size of 118.2 nm and zeta potential of − 20 mV were developed. Briefly, pre-emulsion was prepared by mixing hot aqueous surfactant solution (polaxomer188 and soya lecithin) to the melted lipid phase consisting of glyceryl mono stearate and Capmul GMO. Further, this pre-emulsion was then subjected to 10 cycles of high-pressure homogenization at 600 bar. The *in vitro* drug release and the *ex vivo* nasal permeability studies revealed sustained release and higher diffusion of quercetin from the nanostructured lipid carriers than pure drug suspension. The treatment with nanocarriers did not change the mucosal structure or nasal epithelium with negligible inflammation or necrosis. The nanocarriers had higher cytotoxicity than a standard drug, adrenomycin, as evidenced by the *in vitro* cell growth assay in astrocytoma-glioblastoma cell line (U373MG). The biodistribution studies following nasal administration in Wistar rats showed higher quercetin concentration in the brain for nanocarriers as compared with plain quercetin suspension. This could be attributed to the transcellular as well as paracellular transport of nanocarriers via olfactory neurons in the olfactory membrane [94]. Similarly, kaempferol-loaded mucoadhesive nanoemulsion is prepared by high-pressure homogenization and characterized for their morphology and mucoadhesive strength. The oil phase is consisted of medium-chain triglycerides and egg lecithin, while the aqueous phase contained polysorbate 80 and water. Histopathological studies established the safety of the nanoemulsions for the nasal mucosa. Further, in comparison with the kaempferol drug solution, the developed formulation enhanced the drug content in rat's brain by 5 times following intranasal administration and was found to reduce C6 glioma cell viability through induction of apoptosis [63]. In a recent study, Labrasol-Transcutol-based mucoadhesive microemulsion system and in situ gels of HPMC K4M and Poloxamer 407 loaded with teriflunomide exhibited enhanced nasal permeation and *in vitro* cytotoxicity. These nanocarriers were also deemed safe with reduced risk of liver and kidney toxicity as per the histopathological evaluation. The biodistribution studies of these nanocarriers revealed rapid drug delivery and two-fold increase in brain uptake upon intranasal administration [95].

Polymer-based intranasal delivery systems for GBM

Polymeric nanocarriers were also investigated for intranasal delivery of therapeutics. Methotrexate-loaded polylactic acid (PLA) nanoparticles were prepared using solvent evaporation technique, and Carbopol 934 was added to the nanoparticle dispersion to form thermosensitive gels. These nanoparticles were non-irritants to the nasal mucosa and exhibited increased residence time. While the *in vitro* cytotoxicity of the nanocarriers in U373 MG cells was similar to that of the pure drug, the pharmacokinetic and brain distribution pattern confirmed the passage of the drug into the brain parts, viz., the cerebellum and cerebrum [96].

Studies involving pharmacodynamic analysis of intranasal nanocarriers for GBM therapy are limited. A host of studies involving cell penetrating peptide, Tat analog-modified methoxy poly(ethylene glycol) (MPEG)/poly(ε-caprolactone) (PCL) amphiphilic block copolymer–based micelles, were carried out by Kanazawa et al. Coumarin-loaded MPEG/PCL-Tat micelles of about 100-nm size were prepared, and the *in vitro* cellular uptake in C6 glioma cells was studied. The blood concentration and brain distribution of coumarin following intravenous and intranasal administration were compared. Further, the intranasally administered Tat-modified micelles showed high brain distribution after 4 h as compared with non-modified micelles with minimal distribution in non-targeted tissues. One important observation from these studies is that preferential accumulation of these carriers in the tumor site of the brain compared with other parts was less. Thus, it is possible that the enhanced permeability and retention effect is not connected with this route of administration [97]. The potential of MPEG/PCL-Tat micelles against brain tumor was evaluated by encapsulating an anticancer drug camptothecin (CPT) in them and administering intranasally to the C6 glioma-bearing rats. These micelles permitted high accumulation of the drug in C6 cells, significantly inhibited the tumor growth, and increased the median survival time of glioma-bearing rats from 18.2 to 32.6 days [71]. MPEG/PCL-Tat copolymer was also mixed with siRNA solution to form polymer-siRNA complexes, which were then analyzed for

their distribution in brain after intravenous and intranasal administration in anesthetized rats. In an attempt to elucidate the mechanisms that promote transfer to brain via the nasal pathway, the distribution of fluorescent-tagged siRNA was analyzed in the transmucosal pathway as well as the olfactory and trigeminal nerves. It was observed that the nucleic acids are transported to the olfactory bulb and brainstem via the olfactory and trigeminal nerve pathways, and then to other brain tissues (Fig. 3) [98]. The same group also studied the intranasal co-delivery of siRNA and CPT using MPEG-PCL-Tat micelles. The cellular uptake, *in vitro* transfection efficiency, and cytotoxicity induced by Raf-1 gene silencing of the siRNA-CPT/MPEG-PCL-Tat micelles were studied in C6 glioma cells. The cell viability was found to decrease with an increase in uptake of siRaf-1. Further, *in vivo* therapeutic efficacy assessed in glioma-bearing rats showed that intranasal delivery of these micelles for 7 days significantly enhanced the delivery of siRNAs and CPT to the brain and prolonged the mean survival period. In addition, no neuronal toxicity or damage to nasal mucosa was observed [99, 100].

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles modified with Ephrin type-A receptor 3 (EPHA3) tyrosine kinase antibodies were also developed using an emulsion-solvent evaporation method to deliver TMZ intranasally. The antibody-conjugated nanoparticles showed higher cellular uptake and cytotoxicity in C6 glioma cells as compared with unmodified nanoparticles. *In vivo* fluorescence imaging in glioma-bearing rats showed higher distribution of nanoparticles in the brain with minimal accumulation in other organs after nasal administration as compared with intravenous route after 4 h (Fig. 4). Further, anti-EPHA3 nanoparticles resulted in a better anti-glioma effect with 1.52-fold longer median survival time [101].

Van Woensel et al. used chitosan nanoparticles to deliver siRNA in mice for studying its effect on Gal-1 for treatment of GBM and observed that after nasal administration, siRNA were detected in hindbrain by rapidly passing through olfactory pathway. They concluded that chitosan nanoparticle, by virtue of its mucoadhesive properties, is an excellent formulation for delivery of biological active agents to target the brain tumor [69]. The same group also studied the effect of intranasal siGal-1 delivery in the tumor microenvironment of GBM that will act synergistically to immuno/chemotherapy regime in improving the survival of tumor-bearing mice [102].

Hybrid nanosystems for GBM management

In another study, polymer/lipid hybrid nanoparticles composed of a cationic lipid 1,2-dioleoyl-3- trimethylammonium-propane

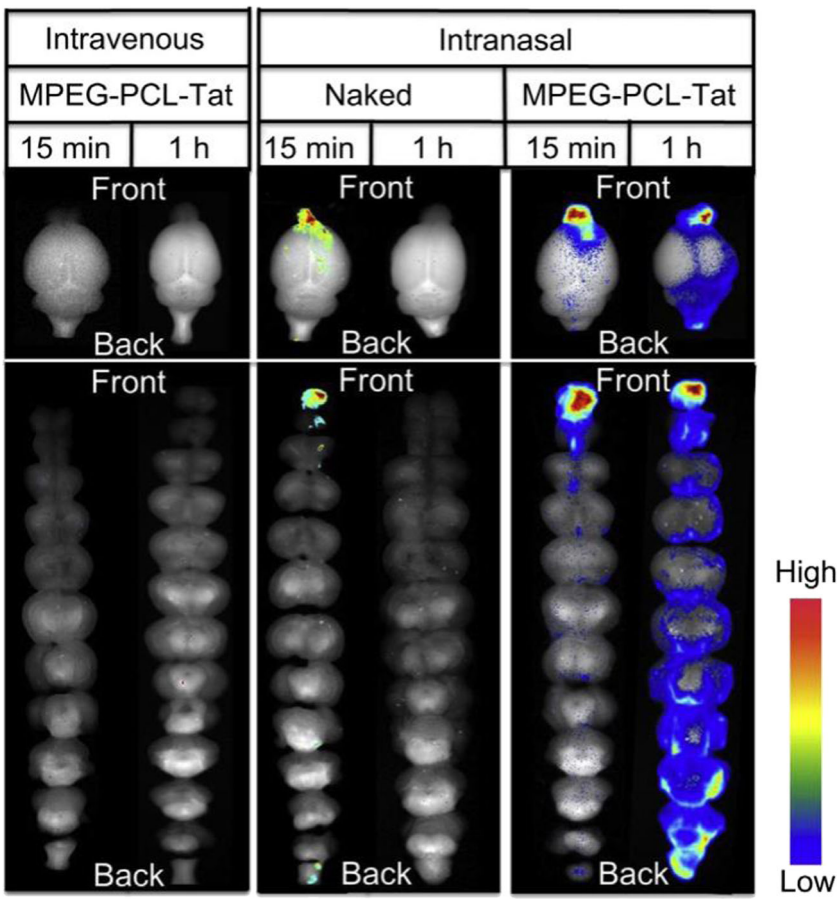

Fig. 3 Dynamics of siRNA/MPEG-PCL-Tat complex in brain tissue following intranasal or intravenous administration. Reprinted with permission from Kanazawa et al. [95]

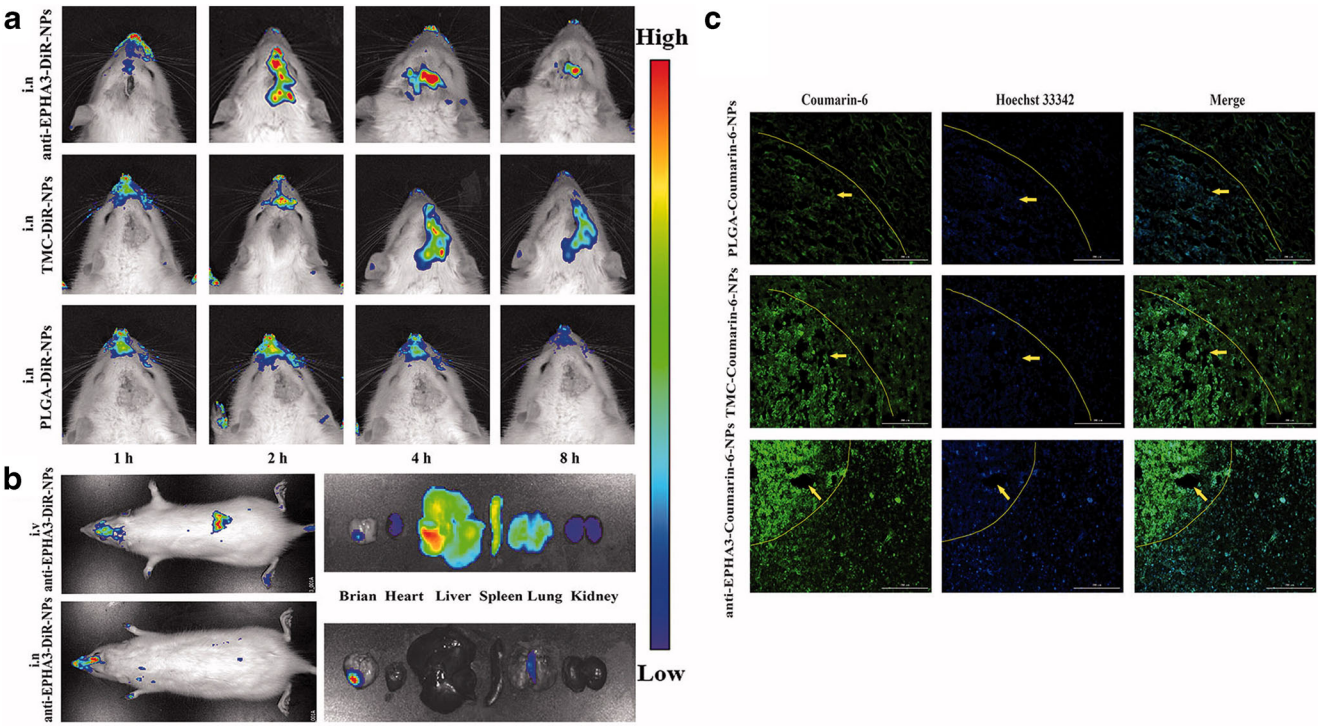

Fig. 4 (A) *In vivo* fluorescence imaging at predetermined time points after intranasal administration of dye-loaded PLGA nanoparticles. (B) Excised tissues imaging of anti-EPHA3-modified dye-loaded PLGA nanoparticles at 4 h after intranasal and intravenous administration. (C) Fluorescence microscopy images of the brain, acquired 4 h after intranasal administration of coumarin-6-loaded PLGA nanoparticles to glioma-bearing rats. Green, coumarin-6; blue, Hoechst 33342 (nuclei); yellow arrows point to the tumor site (Reprinted with permission from Chu et al. [98])

(DOTAP) and PLGA-PEG were developed to deliver farnesylthiosalicylic acid to GBM. *In vitro* cytotoxicity was analyzed using glioma RG2 cell line while *in vivo* biodistribution and antitumor efficacy were studied in RG2 tumor-bearing rats. A higher accumulation of nanocarriers was observed in the olfactory bulb and brain post-intranasal administration as compared with intravenous route. Further, intranasal administration of multiple doses was found to be as effective as intravenous administration in reducing the tumor. In addition, the drug content in the liver and spleen was found to be 10-fold lower for intranasal route, confirming it to be a safer route of administration with minimal nonspecific accumulation and side effects [68]. In another study, grapefruit-derived nanovectors developed using lipids extracted from edible plant exosomes and coated with folic acid and polyethylenimine (PEI) were used to carry miR17, which downregulates MHC1 gene in cancer cells resulting in activation of natural killer cells and tumor growth inhibition. These nanocarriers rapidly delivered miR17 to the brain upon intranasal administration, significantly prolonged the survival, and delayed the tumor growth in GL26 tumor-induced mice [70].

In few other studies, polymer-coated metal/alloy nanoparticles were employed for intranasal delivery to the brain. For instance, polyvinyl alcohol/PEI/fluorescein isothiocyanate complex–coated magnetite nanoparticles were synthesized by co-precipitation and loaded with carmustine for magnetically targeted delivery to the brain following intranasal administration. These nanocomplexes possessed a time-dependent loading of the drug and maximum release of 75.8% of the loaded drug. They showed enhanced uptake, internalization, and superior cytotoxicity towards human glioblastoma (HG) cells in the presence of an external magnetic field [103]. In another study, FePt alloy nanoparticles were synthesized and modified with hyaluronic acid and lactoferrin to provide CD44 specificity for targeting brain tumor and enhance tumor accumulation. *In vitro* release profile and the magnetic field and laser-triggered hyperthermia effect were studied followed by cell viability studies in U87MG cells to analyze the nanosystem's capability as a multimodal therapeutic platform. The *in vitro* photothermal, chemo-photothermal, and chemo-magnetophotothermal cytotoxicity analysis of these nanoconjugates revealed significant difference in cell viability suppression. The leaching of Fe and Pt contents from the nanoconjugate further enhanced the therapeutic effect due to generation of reactive oxygen species (ROS). Lactoferrin conjugation enabled enhanced olfactory uptake and accumulation of drug in brain and tumor as evidenced by the mucus penetration study, *ex vivo* transport across nasal mucosa, and the nasal penetration study in Wistar rats [104].

Intranasal delivery of stem cells for GBM

Neural stem/progenitor cells (NSPC) and mesenchymal stem cells (MSC) have recently gain potential for treatment of

glioma by intranasal delivery. NSPCs have been reported to rapidly migrate to malignant glioma via olfactory pathway and carry biological active genes products targeting tumor to the brain with reduced systemic exposure. Upon intravenous infusion, it has been shown that the majority of MSCs and NSPCs become entrapped in microvasculature of the pulmonary circulation owing to their larger size and are distributed to the liver, spleen, kidney, and bone marrow within 48 h. Further, most of the studies involving MSCs and NSPCs to deliver prodrug activators, viral vectors, and nanoparticles opt for the invasive intratumoral route of administration. Thus, intranasal delivery of these cells utilizing their inherent migratory capacity to sites of brain trauma can be an effective and noninvasive alternative. However, the exact pathways of intranasal delivery of stem cells and the reason for their inherent migration to diseased regions of the brain are not yet elucidated [61, 105, 106].

In one of the studies involving stem cells, MSCs expressing TNF-related apoptosis-inducing ligand (TRAIL) were administered intranasally to mice bearing intracranial U87 glioma xenografts. The penetration of the MSCs into the brain was tracked using live animal imaging, MRI, and histological evaluation. Further, the MSC improved the median survival of irradiated glioma-bearing mice in comparison with non-irradiated and irradiated control mice, thereby demonstrating nasal delivery of stem cell–based therapeutics as a possible adjuvant to radiotherapy [107]. In yet another study, the multikinase inhibitor, sorafenib, was used to prime MSCs and upon administering intranasally to nude mice bearing intracerebral U87MG xenografts reduced tumor angiogenesis but not tumor volume. The modest therapeutic effect was attributed to the pro-tumorigenic properties of MSCs, which may limit the action of sorafenib. Thus, it is imperative that more research is necessary to validate the use of MSCs for delivery of therapeutic agents for GBM [108]. In an interesting study by Ahmed et al., the intracranial administration of adenovirus-loaded NSPCs enhanced the survival of glioma-bearing rats much better than virus-loaded MSCs indicating that the therapeutic efficacy of carriers might be closely linked to the similarity between the origin of the carrier and the malignancy [109]. Thus, NSPCs may be a better carrier for stem cell–based therapy of GBM.

Genetically modified NSPCs that carry an enzyme to convert prodrug 5-flurouracil to active drug fluorouracil are under phase I clinical trial in humans (NCT01172964), and three other clinical trials involving NSPCs for therapy of high-grade gliomas are currently recruiting participants (NCT03072134, NCT02015819, NCT02192359) [105]. In a recent study, methimazole, a FDA-approved compound, was employed to delay the nasal clearance and improve the penetration of oncolytic virus loaded NSPCs to the olfactory epithelium for *in vivo* brain tumor targeting and therapy. A single dose of methimazole before intranasal administration of NSPCs delayed their clearance for at least 24 h and resulted in significant survival benefits in glioma-bearing mice [110]. Many questions need to be probed before putting these stems cell carriers in clinical use, for instance, the mechanisms of differentiation, proliferation, and treatment of disease, possible side effects, and the origin of stem cells. The results of some of the early clinical trials will provide a clearer picture regarding the efficacy of intranasally delivered NSPCs in GBM therapy.

In addition to stem cells, viruses and bacteriophages have also been employed in selectively killing glioma cells. For example, a vesicular stomatitis virus strain VSVrp30a upon intranasal administration infected the olfactory neurons and gained access to the CNS through the olfactory nerve. The viruses possessed high degree of selectivity to the tumor cells without infecting the surrounding areas and eliminated the tumor cells in the olfactory bulb [60]. When the clinical trials involving intratumoral or intravenous injection of an oncolytic rat H-1 parvovirus in recurrent glioblastoma patients were going on [111], another study was carried out for analyzing the intranasal delivery of the same in tumor-induced rats. It was observed that the viral replication–associated regulatory proteins, responsible for oncolytic property, were exclusively expressed in the tumor tissues. Although significant prolongation of survival was observed in the rats, the oncolytic activity observed in rats was not conspicuous [112]. Similarly, Ff filamentous bacteriophages administered intranasally accumulated primarily in the olfactory bulb, penetrated the brain, and reduced tumor growth, thereby extending the median survival of GL261 tumor-induced mice [113]. Thus, the potential of nose-to-brain transport is now clearly evident, and with the knowledge from the initial clinical trials involving intranasally delivered therapeutic entities in addition to the advancements in targeted nanocarrier systems and nasal delivery devices, a new therapeutic regime involving intranasal administration can be designed for not only GBM but for several other brain-related disorders.

Conclusion

A growing number of strategies to combat GBM are being proposed with the discovery of novel delivery routes and molecular targets on glioma cells and the BBB. Intranasal route of administration holds great promise as a direct, noninvasive alternative for the brain drug delivery. Nasal ciliary elimination, cellular excretion mechanism, enzyme metabolism, efflux by transport proteins expressed in nasal mucosa, and blood vessel absorption are some of the factors limiting the absorption of drugs. Some transporter inhibitors, vasoconstrictors, or enzyme inhibitors can be employed to improve the stability of drugs and extended their halftime in nasal cavity. The use of nanocarriers modified with targeting moieties drugs is the most promising strategy to selectively target

drug into brain thereby increasing the chances of glioma therapy and reduction in systemic toxicity. However, most of the studies are limited to preclinical evaluations and none of them has completed its phase 3 clinical trials. Stem cell therapy and oncolytic viral therapy also provide a powerful new platform for treating malignancies; however, it is still in its infancy and needs to address many obstacles to become successful. Further, the development of both increasingly specific therapeutics and better delivery systems that can tackle drug resistance in glioma is the need of the hour to achieve dramatic enhancements in prognosis of GBM patient outcomes.

Funding information We would like to acknowledge University Grants Commission, India, for providing D.S. Kothari Postdoctoral Fellowship to Sreeranjini Pulakkat and Department of Atomic Energy-Board Of Research In Nuclear Sciences (DAE-BRNS) for providing research fellowship to Prashant G. Upadhaya.

Compliance with ethical standards

Conflict of interest Prashant G. Upadhaya, Sreeranjini Pulakkat, and Vandana B. Patravale declare that they have no conflict of interest.

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