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REVIEW

Recent Advances in the Use of Lipid-Based Nanoparticles Against Glioblastoma Multiforme

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Abstract
Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults. Although the overall incidence is less than 10 per 100,000 individuals, its poor prognosis and low survival rate make GBM a crucial public health issue. The main challenges for GBM treatment are related to tumor location and its complex and heterogeneous biology. In this sense, a broad range of nanoparticles with different sizes, architectures, and surface properties, have been engineered as brain drug delivery systems. Among them, lipid-based nanoparticles, such as liposomes, have been pointed out as promising materials to deliver antitumoral drugs to the central nervous system and thus, to improve brain drug targeting and therapeutic efficiency. Here, we describe the synthesis and general characteristics of lipid-based nanoparticles, as well as evidence in the past 5 years regarding their potential use to treat GBM.

Keywords Glioblastoma · Liposomes · Lipid-based nanoparticles · Solid lipid nanoparticles · Blood–brain barrier

Introduction

The central nervous system (CNS) is also responsible for vital functions for humans' everyday life, like consciousness, memory, and coordination. This complex system is formed by two major cell types, neurons, and glial cells. The neurons are specialized cells that transmit and receive electrical (synapsis) and chemical signals to the whole organism. The glial cells are supportive cells that do not participate in electric impulses but play an essential role in the CNS (Araque and Navarrete 2010; Kettenmann et al. 1995; Salazar-García et al. 2015).

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An astrocytoma is the hyperproliferation of abnormal astrocytes. The World Health Organization stands these neoplasms into four prognostic grades: (a) grade I tumor, (b) grade II low-grade diffuse astrocytoma, (c) grade III astrocytoma or anaplastic astrocytoma and d) grade IV astrocytoma, such as glioblastoma (GBM) (Agnihotri et al. 2013).

GBM is the most aggressive malignant tumor in the CNS and has a poor prognostic. Briefly, the GBM consists of a group of a genetically and phenotypically diverse group of tumors from which 90% are primary brain neoplasms, and the remaining 10% corresponds to secondary tumors progressing from LGA to GBM (Urbańska et al. 2014). According to US data health institutes (2006–2010), the incidence of GBM rises with increasing age, being the most common range of age, patients from 74 up to 85 years old. GBM accounts for 45.2% of primary malignant brain and CNS tumors, 54% of all gliomas, and 16% of all primary and CNS tumors. Similar percentages are reported in other countries (Thakkar et al. 2014). GBM has the most reduced survival, with only 0.05–4.7% of diagnosed patients living 5 years after the diagnosis (Ostrom et al. 2014).

Exposure to ionizing radiation has been the only established risk factor for gliomas (Ostrom et al. 2014). However, other potentially hazardous chemicals, such as pesticides, polycyclic aromatics compounds, and solvents are considered risk potential factors (Ostrom et al. 2014; Spinelli et al.

2010). Despite GBM and other gliomas are recognized as nonheritable diseases, diagnosed patients could have relatives affected by gliomas. A multifactorial model could explain this fact: the patient may be exposed to environmental risk factors and has a genetically determined susceptibility (McKinney 2004). Patients with tuberous sclerosis, Turcot syndrome, multiple endocrine neoplasia IIA, neurofibromatosis type I or brain head injuries could be diagnosed with GBM (Urbańska et al. 2014).

Furthermore, the genomic profile of GBM displays several molecular subtypes, which are defined by essential alterations in gene expression, and they are associated with specific mutations and copy number alterations. For instance, the epidermal growth factor receptor (EGFR) gen associated with cell growth, migration, and cell survival. EGFR found in 40% of GBM, and 50% of EGFR amplification cases contain a truncated variant of the gene that encodes a protein that lacks an extracellular domain and remains constitutively active (EGFRvIII). Both of them are frequent alterations in GBM cells (Liu et al. 2014; Ohgaki and Kleihues 2007; Parsons et al. 2008).

On the other hand, the TP53 gene encodes the protein p53 that acts as a transcription factor and binds to promoters of genes involved in the DNA repair. Around 65% of GBM type 2 have TP53 mutations (50% at codons 248 and 273) (Ohgaki and Kleihues 2007). Other alterations are the mutation in the substrate-binding domain of the enzyme NADPH-dependent isocitrate dehydrogenase (IDH) type 1 and 2, which participates in converting isocitrate to alpha-ketoglutarate in the NADPH production process. These mutations promote epigenetic alterations and increase the expression of genes that control cell differentiation and proliferation. IDH mutations are frequent in grade II and III gliomas. Still, they are absent in pilocytic astrocytomas (Dunn et al. 2013; Ohno et al. 2013; Yang et al. 2012).

The current medical treatment is a combination of resection, to the extent feasible, followed by radiotherapy and chemotherapy. This treatment is aggressive and unspecific due to the following factors: (a) the complete resection is impossible since these tumor cells easily infiltrate surrounding tissues, (b) the drug delivery to the tumor site is difficult since the blood–brain barrier (BBB)—a physiological barrier—blocks the entrance and (c) the tumor cells are resistant to radiotherapy (Davis 2016). A 2015 report mentioned that the most common cancer drugs for GBM are temozolomide (TZM), carmustine (BCNU), and lomustine, being TZM the first cancer drug option due to its fast absorption and capability to cross the BBB (Wait et al. 2015). However, in the best scenario, patients treated with radiotherapy combined with TZM expect a median survival of 15 months (Barker et al. 2014). Therefore, it is precise to seek more efficient medical treatments that achieve specificity, safety, and reduce current therapies' adverse effects. Reports on experimental therapies are vast, particularly nano-formulations, such as liposomal and other lipid-based nanoparticles, showing promising CNS drug delivery results.

This review focuses on the state-of-the-art on lipid-based nanoparticles developed as potential vehicles for delivering antitumoral drugs in GBM. The authors describe different lipid-based nanoparticles' mechanisms for crossing the BBB and studies conducted within the last 5 years.

Lipid-Based Nanoparticles: Concepts and Preparation Methods

Liposomes

Liposomes were first described by the English hematologist Alec Bangham in 1961 (Bangham et al. 1965). The name liposome is derived from two Greek words: "Lipos" meaning fat and "Soma" meaning body. Structurally are defined as small artificial vesicles of spherical shape consisting of a central aqueous compartment surrounded by one or more concentric phospholipid layers (Laouini et al. 2012). The liposome size can vary from small (0.025 µm) to large (2.5 µm) vesicles. Based on their size and number of bilayers, liposomes are classified in multilamellar vesicles (MUV) and unilamellar vesicles (Akbarzadeh et al. 2013). Unilamellar vesicles (UV) are classified into two categories: (1) large unilamellar vesicles (LUV) with particles size from 100 nm up to 1000 nm, and (2) small unilamellar vesicles (SUV) with particle sizes from 20 nm up to 100 nm (Xu et al. 2017), (Fig. 1). Due to its hydrophobic and hydrophilic character, liposomes are promising systems for drug delivery, thus hydrophilic (in the aqueous cavity), hydrophobic (within the lipidic membrane), and amphiphilic substances incorporated within these vesicles for a large variety of potential biomedical applications. These structures can deliver both hydrophilic and hydrophobic drugs for cancer, antibacterial, antifungal, immunomodulation, diagnostics, ophthalmic, vaccines, enzymes, and genetic elements (Alavi et al. 2017). Their main advantages are nontoxic, nonimmunogenic, biodegradable, biocompatible, protect the encapsulated drug from the external environment, and reduce the exposure of sensitive tissues to toxic drugs (Akbarzade et al. 2013). The main disadvantages of liposomes are production cost is high, short half-life, leakage, and fusion of encapsulated drugs/molecules (Shashi et al. 2012). Thus, the current main challenge for liposomes formulation is to obtain efficient drug entrapment, narrow particle size distribution, and long-term stability (Popovska et al. 2013). In general, liposomes preparation involves four primary stages: (1) drying down lipids from organic solvents, (2) dispersing the lipid into an aqueous media, (3) purifying the resultant liposome, and (4) analyzing the final product (Shashi et al.

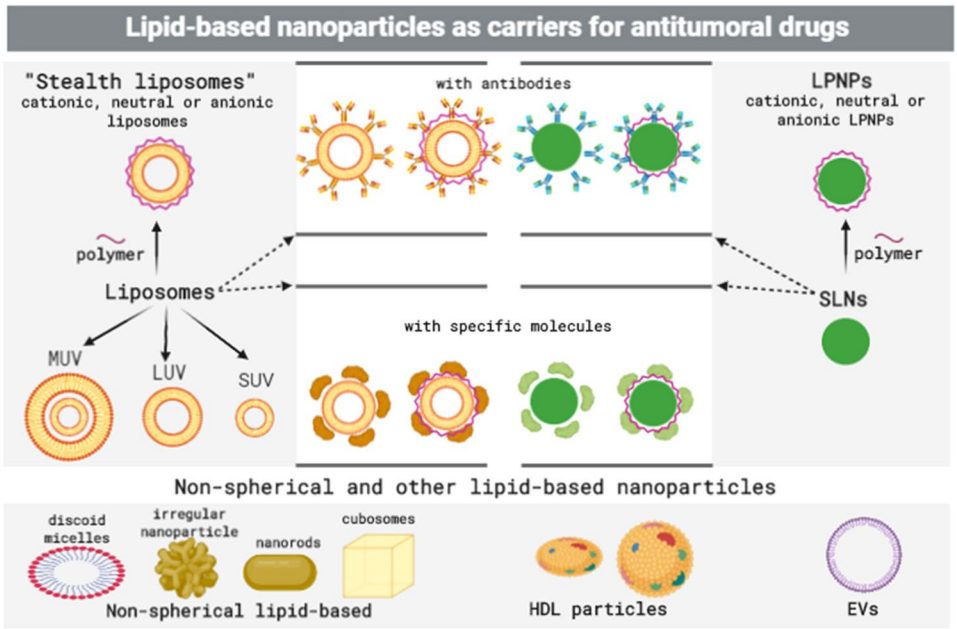

Fig. 1 Lipid-based nanoparticles. MUV: multilamellar vesicles; LUV: large unilamellar vesicle; SUV: small unilamellar; SLNs: solid lipid nanoparticles; LPLPs: lipid–polymer hybrid nanoparticles; HDL: high density lipoproteins. Created in BioRender.com

2012). Passive or active drug loading methods are applied to encapsulate molecules into liposomes. The passive loading involves the encapsulation before or during the manufacturing procedure (in situ entrapping). In contrast, in the active loading method, drugs can be loaded by creating diffusion gradients for ions or drugs across the external and internal aqueous phases (Shashi et al. 2012).

The assembly of conventional liposome structures occurs in aqueous solutions with natural and/or synthetic phospholipids: phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, or phosphatidylinositol. Cholesterol and its derivatives are often included in liposomes preparation as they can improve membrane fluidity and stability in the presence of biological fluids (e.g., plasma) and reduce the permeability of water-soluble molecules through the membrane (Vemuri and Rhodes 1995). In addition, liposomes can contain other molecules, such as hydrophilic polymers conjugated with lipids as an outer layer (known as "Stealth liposomes") (Laouini et al. 2012). For instance, unilamellar liposomes (102 ± 3.3 nm) formulation prepared with polyethylene glycol (PEG) was used to encapsulate donepezil, a drug against Alzheimer's disease, and administered to rats via intranasal route, finding higher donepezil concentrations in the plasma and brain of animals treated with loaded liposomes, in comparison with the free donepezil administration; and histopathological examination showed that PEGylated liposomal donepezil was safe and nontoxic (Al-Asmari et al. 2016).

Another interesting approach is incorporating biomolecules, such as antibodies and other proteins onto the liposome surface to target specific sites in the organism. Asahi et al. (2003), using a rat model of embolic focal stroke, showed that tissue plasminogen activator loaded in an anti-actin-targeted immunoliposome significantly reduces hemorrhage. In addition, doxorubicin-loaded liposomes functionalized with transferrin and folate (dual-targeting), can cross the BBB and distribute in the brain glioma, also exhibited the least tumor area and the highest apoptotic activity in the glioma cells among all the treated group (Gao et al. 2013).

Preparation of Liposomes

Preparation of liposomes involves physic techniques where lipids and other components (cholesterol, modified lipids, drugs) are placed in organic solvents in a homogeneous solution at defined temperatures and concentrations. Then, the removal of solvents and the incorporation of aqueous media followed by a physic method favors the lipid assembly, and finally, the resulting liposomes are purified and characterized. Drug loading can be achieved by passive (while liposomes are assembled, the drug is incorporated) or active (the drug loading takes place in assembled liposomes) steps defined by the drug's polarity. Thus, the nonpolar organic solvents are appropriate for passive loading of hydrophobic drugs, while hydrophilic drugs in aqueous solutions are ideal for active incorporation. The percentage of the total drug incorporated into the liposomes is known as entrapment efficiency, trapping effectiveness, or encapsulation efficiency (%EE) and is limited by the trapped volume in the liposomes, drug polarity, and evidently, by the selected way to incorporate the drug.

Three methods are commonly reported for assembling liposomes: (1) thin-film hydration, (2) by solvent injection (ether or ethanol), and (3) by reverse-phase evaporation.

Briefly, the thin-film hydration method requires well-defined molar proportions of phospholipids and cholesterol dispersed in an organic solvent and reduced pressure to evaporate the solvent. The dried film deposited in a flask is re-hydrated by adding an aqueous buffer solution under agitation at a temperature above the lipid transition temperature (the temperature required to induce a change in the lipid physical state from the ordered gel phase to the disordered liquid crystalline phase) (Laouini et al. 2012). The thin-film hydration method produces heterogeneous MUV in size and shape (1–5 μm diameter). However, techniques, such as sonication or extrusion can later downsize the particle size (Mui et al. 2003). On the other hand, the solvent injection method aims to encapsulate lipids dissolved in diethyl ether or ether–methanol mixture by gradually injecting the lipidic solution to an aqueous solution at 55–65 °C, under reduced pressure. This reduced pressure allows removing the organic solvent but not the aqueous medium, which leads to forming MUV. The size distribution is not homogeneous (from 70 up to 200 nm), and high-temperature conditions could affect the passive encapsulation (Vemuri and Rhodes 1995). Finally, the reverse-phase evaporation method requires a water-in-oil emulsion obtained by the sonication of two phases system: (1) phospholipids in an organic solvent (ether, diethyl ether or isopropyl ether or a mixture of isopropyl ether and chloroform) and (2) an aqueous buffer. The residual solvent removed by continuous rotary evaporation under reduced pressure allows the formation of liposomes in the aqueous phase remained (Akbarzadeh et al. 2013). This methodology achieves liposomes with a high aqueous space-to-lipid ratio and entrapment of a large percentage of the aqueous material (Laouini et al. 2012). The vesicles obtained SUV with diameters of 0.1–1.2 μm (Otake et al. 2001).

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are lipid-based nanostructures with an average diameter ranging 50–1000 nm. SLNs reported in the early 1990s decade represented an alternative to previous colloidal liquids, such as liposomes, emulsions, and polymeric micro and nanoparticles. Lipids in the SLNs structure remain at solid-state at physiological temperature (Kumar et al. 2014; Rosmati et al. 2014). Typically, the design of SLNs is a lipidic matrix core in solid-state coated with a phospholipid core constituted by the hydrophobic aliphatic tails of the phospholipid molecules (Fig. 1). Thus, the center of the SLNs is only hydrophobic and could enhance the entrapment efficiency of hydrophobic drugs in the core when compared with conventional liposomes (Ganesan and Narayanasamy 2017).

Besides solid lipids, SLNs contain surfactant (emulsifier and co-emulsifiers) and water. The solid lipids to be incorporated in SLNs include: (i) triglycerides, such tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, (ii) partial glycerides: glyceryl monostearate, glyceryl behenate, glyceryl palmitostearate, (iii) fatty acids (saturated, monounsaturated and polyunsaturated fatty acids), (iv) steroids, manly cholesterol, and (v) waxes, e.g., cetyl palmitate (Kammari et al. 2017).

The use of SLNs as drug delivery vehicles could enhance the physical stability of the formulation, conferring protection to the active compound, improving its controlled release, increasing the biocompatibility and selective targeting, decreasing the use of organic solvents and facilitating the large scale production (Rostami et al. 2014).

Preparation of SLNs

SLNs are obtained by three fundamental physical methods. The first method is the preparation by high-pressure homogenization or HPH, which pushes a fluid (with 5–10% of lipid content) with a pressure of about 100 and 2000 bar through a narrow window (few microns length). The lipidic fluid accelerates on a short distance to high velocity (over 1000 km/h). The SLNs formation mechanism is explained by both very high shear stress and cavitation forces, which disrupt the particles down to the submicron level (Mehnert et al. 2001). The incorporation of the active compound or drug proceeds in the preparatory stage, where the liquefied lipid mixture contains the active compound (Lasoń and Ogonowski 2011). A first variation of the method occurs at temperatures higher than the lipid melting point and is called hot HPH. The use of higher temperatures allows smaller particle sizes in nanoemulsions in a liquid state due to the decreased viscosity of the inner phase (Ferreira et al. 2016). The hot HPH is easily scalable and commercially available. However, some factors could limit the application of the hot HPH, such as the degradation of thermolabile drugs and the complexity of the crystallization step of the nanoemulsion (Ganesan and Narayanasamy 2017) A second variation, the cold HPH method, is performed with solid lipids and is similar to milling a suspension at elevated pressure. Once the drug and the melted lipid form a mixture, the temperature cools down using dry ice or liquid nitrogen. The product is pulverized to microparticles (50–100 μm) by ball/mortar milling (Mukherjee et al. 2009) and allows the incorporation of thermolabile drugs or active compounds (Ganesan and Narayanasamy 2017).

A second method to obtain SLNs is microemulsion. The application of the microemulsion technique needs first to melt the fatty acids. Then, a mixture of water and surfactants (emulsifiers and co-emulsifiers) is heated to the lipids temperature and blended under mild conditions. The final product under adequate conditions is a transparent microemulsion, thermodynamically stable. The hot microemulsion is then dispersed in water at 2–3 °C by smooth mechanical

stirring, ensuring that the small particle size results from precipitation and not from the mechanical stirring. The microemulsion technique allows obtaining stable products with low energetic cost, overcoming the consuming time procedure and the low SLNs concentration (Ganesan and Narayanasamy 2017; Lasoń et al. 2013).

Furthermore, the solvent emulsification/evaporation method considers, as a first step, to place the mixture of fatty acids in organic solvents. Later, the addition of a water-based phase forms the emulsion. The SLNs (about 25 nm) formation requires the evaporation of the organic solvent, which allows the precipitation of the SLNs in the aqueous phase (Lasoń and Ogonowski 2011). In addition to the low energetic cost, this method generates a low viscosity emulsion and is adequate for thermolabile drugs. However, the emulsion containing SLNs maybe not stable, and the residual organic solvent might induce toxic effects on biological systems (Ganesan and Narayanasamy 2017).

Lipid Polymer Hybrid Nanoparticles

Lipid-polymer hybrid nanoparticles (LPNPs) consist of (i) a hydrophobic polymeric core, which facilitates the incorporation of poorly water-soluble drugs; (ii) a lipid layer surrounding the polymeric core to increase the biocompatibility and drug retention inside the core; and (iii) a hydrophilic polymer stealth layer outside the lipid shell to confer stability and a long systemic circulation lifetime (Zhang and Zhang 2010) (Fig. 1). The use of LPNPs favors the drug control release since the structures have both liquid state and solid-state and due to their high structural integrity (Chan et al. 2009; Wu 2016).

Variations in the components of the LPNPs structures provide different types of hybrids: (a) polymer core with lipid shell, which contains a partial polymer core coated with one or two layers (e.g., lipid polyethylene glycol); (b) core–shell type lipid polymer–lipid hybrid nanoparticles that consist of a polymer core surrounding by a single or multiple lipid layers; (c) erythrocyte membrane-camouflaged polymeric nanoparticles, also known as biomimetic nanoparticles, which are constituted by a polymer core, lipid shell and an outer layer (erythrocyte membrane decorated with polymers); and (d) monolithic lipid polymer hybrid nanoparticles assembling a nanoparticle lipid or lipid polyethylene glycol in a polymeric core, containing drug molecules (Zhang and Zhang 2010).

Preparation of LPNPs

A first approach to obtain LPNPs is the two-step method, where the polymer core and the lipid shell are obtained separately and finally merged to provide LPNPs as a lipid bilayer or multilayer shell. In the second step of the process, both components, the polymeric cores, and lipid layers are carefully combined in selected molar ratios by vortexing, needle extrusion, and HPH (Zhang and Granick 2006; Zhang and Zhang 2010).

Contrary, the one-step method approach reduces the time and effort to prepare LPNPs by producing both polymer core and lipid shell in a single solution. The solution contains the selected polymer and active compound in a polar organic solvent, and then an aqueous solution containing lipids is incorporated while under sonication. The polarity of the polymeric solution allows diffusion into the aqueous phase with coprecipitation of both the active compound and the polymer into nanoparticles. The lipids quickly cover the polymeric nanoparticles in the same solution. This method is limited to hydrophobic active compounds or drugs, partially soluble in polar organic solvents (e.g., acetone) (Cheow and Hadinoto 2011).

Immunoliposomes

Immunoliposomes consist of antibody or antibody fragments coupled onto the liposome surface (e.g., anti-transferrin receptor). There are two general strategies for immunoliposomes preparation: (a) the antibodies can be coupled to previously assembled liposomes, or (b) an antibody anchor can be incorporated after liposome preparation. The first method is more practical and results in an antibody attachment orientated exclusively outside the liposomes (Eloy et al. 2017). The conjugation of antibodies to the distal ends of PEG chains can coat the surface of the liposome to achieve enhanced permeability and retention effect (see section "Nanomaterials to Target the CNS: the Challenge of Crossing the BBB") (Mercadal et al. 2000).

Nonspherical Lipid-Containing Nanoparticles and Other Lipidic Nanostructures

The nonspherical nanoparticles or anisotropic nanoparticles correspond to nanostructures with morphologies different to spherical, such as 2D polygonal (triangle, square, disc), 3D polyhedral (tetrahedron, cubes, hollow nanocage), rod-like (nanobar, nanorod, nanowires), branched (monopod, bipod, tripod, star) complex (snowflakes, cones, filamentous, hemispheres) (Toy et al. 2014).

Since the chemical nature and shape of nonspherical nanoparticles are vast, several production methods are reported. Mathaes et al. (2015) describe in particular non-wetting templates for lipid–polymer complex or hybrids, where fluorinated materials are used to induce raw materials into a designable mold, and the shape takes places by a photochemical crosslinked reaction. The nonspherical nanoparticles with less than 100 nm of size are fabricated from PEG and polylactic-*co*-glycolic acid (PLGA)

and covered by lipids. Furthermore, nonspherical lipid platelets have been obtained by the HPH technique, Chen et al. (2020) presented a hybrid nanodisc synthesized from 1,2-distearoyl-sn-glycerol-3-phosphatidylethanolamine modified with PEG-NHS (DSPE-PEG-NHS) and other lipid–polymer molecules by thin-film hydration method. Recently, the influence of the nanoparticle shape has been highlighted as a crucial factor in biological structures, pharmacokinetic, and pharmacodynamic parameters. Zhu et al. (2019) and Mathaes et al. (2020) have compiled findings on nonspherical nanoparticles in nanomedicine. Nonspherical nanoparticles could exhibit a stronger targeting avidity than spherical shapes due to their ability to form a higher number of multivalent interactions between the ligand onto the nanoparticle surface and the target molecule on the tumor cell surface, including those in the CNS, (Mathaes et al. 2015; Toy et al. 2014; Zhu et al. 2019). The geometry affects the cellular uptake mechanism, for example, elongated ellipsoid shapes in nanoparticles are associated with inhibition of phagocytosis, but oblate ellipsoids may enhance phagocytosis (Sharma et al. 2010).

Other lipid-based nanoparticles have demonstrated their ability to cross the BBB. In this respect, high-density lipoproteins (HDL) are a heterogeneous structure of lipoproteins (apolipoproteine A-1 is one of the major components), which carries cholesterol in the blood. HDL particles (synthetic or reconstituted) are obtained by cholate dialysis, sonication, thermal cycling, and microfluidics (Kuai et al. 2016). Thus, HDL-like particles have been reported as promising drug delivery vehicles since Mooberry et al. (2010) reported the delivery of paclitaxel (PTX) in an HDL structure. The HDL-like structures exhibit various shapes leading to changes in the cellular uptake. Interestingly, Dal Magro et al. (2019) presented the protective role of HDL in Alzheimer disease, describing that discoidal HDL (11 ± 2 nm) but not spherical HDL (8.8 ± 0.2 nm) purified from human blood could cross the BBB in vitro. However, more information regarding HDL vehicles and glioma needs to be found.

Moreover, extracellular vesicles (EVs), such as exosomes and microvesicles have gained attention as promising lipid-based nanoparticles to deliver antitumoral drugs and other biological applications. EVs are released by live cells and can be found in biological fluids, such as serum, saliva, and breast milk (Ramis 2020). Naturally, EVs carry biomolecules and metabolites cargoes, which can be incorporated into other sites by fusion or endosomal uptake (Maas et al. 2017). The use of EVs as nano-vehicles requires the purification of the biological structures from cell cultures or fluids with serial ultracentrifugation and characterization by density gradient ultracentrifugation, flow cytometry, immunoblotting, and some other techniques (Nooshabadi et al. 2020; Zhu et al. 2019).

Nanomaterials to Target the CNS: the Challenge of Crossing the BBB

A crucial aspect of the delivery of drugs to the CNS disease, such as brain tumors, is the therapeutic molecules' effectiveness for crossing the BBB. This physiological barrier consists of an organization of nonfenestrated endothelial cells and support cells such, pericytes, astrocytes, microglia, and circulant immune cells (Fig. 2a). Furthermore, BBB structure involves (1) tight junction proteins and adherent proteins, which avoid movement between nonfenestrated endothelial cells or paracellular diffusion (passive or simple diffusion), (2) transporters, (3) basal lamina, and (4) extracellular matrix. Drug molecules may cross the BBB by simple diffusion if the molecule has lipophilic characteristics and weight (< 400 kDa) (Ambruosi et al. 2006; Wohlfart et al. 2012). Moreover, altered vasculature irrigates tumors and permeability is comprised; thus, the nanoparticle's entrance and retention in tumor tissue are favored and in tumor regions in a passive process known as enhanced permeability and retention effect (EPR), in where molecules, such as liposomes, nanoparticles or drugs being to accumulate preferentially in a tumor tissue more than healthy tissues (Maeda 2012).

Some molecules, such as peroxynitrite, nitric oxide, tumor necrotic factor α, vascular endothelial growth factor (VEGF), and bradykinin have been related to promoting EPR (Kalyane et al. 2019). Significantly, bradykinin has been associated with EPR in glioma by acting on bradykinin 1 receptor (B1R) and B2R. B1R is involved in the induction of cyclooxygenase-2 production and glioma cell migration. Moreover, B2R increases the VEGF expression and enhances angiogenesis in prostate cancer (Yu et al. 2014). Kalyane et al. (2019) presented an extensive review describing EPR and poor lymphatic system in tumors, as crucial opportunities to deliver chemotherapeutic drugs in nanomaterial vehicles, including lipid-based nanoparticles, which have shown higher accumulation in tumor tissues than in healthy organs. Significantly, the drug delivery by EPR mechanism depends on the nanoparticle size and shape, tumor perfusion, vascular permeability, interstitial penetration, blood pressure, tissue lymphatic drainage function, cancer type, among others (Kalyane et al. 2019; Nakamura et al. 2016).

On the other hand, therapeutic substances could enter the CNS by facilitated diffusion, active transport, or even paracellular diffusion. In this respect, the physicochemical properties of a given drug rule the pathway of the entrance. Notably, high hydrophobicity, low molecular weight, and nonionic drugs are more easily delivered to CNS (Banks 2016). When the potential therapeutic molecule does not exhibit the mentioned physicochemical characteristics,

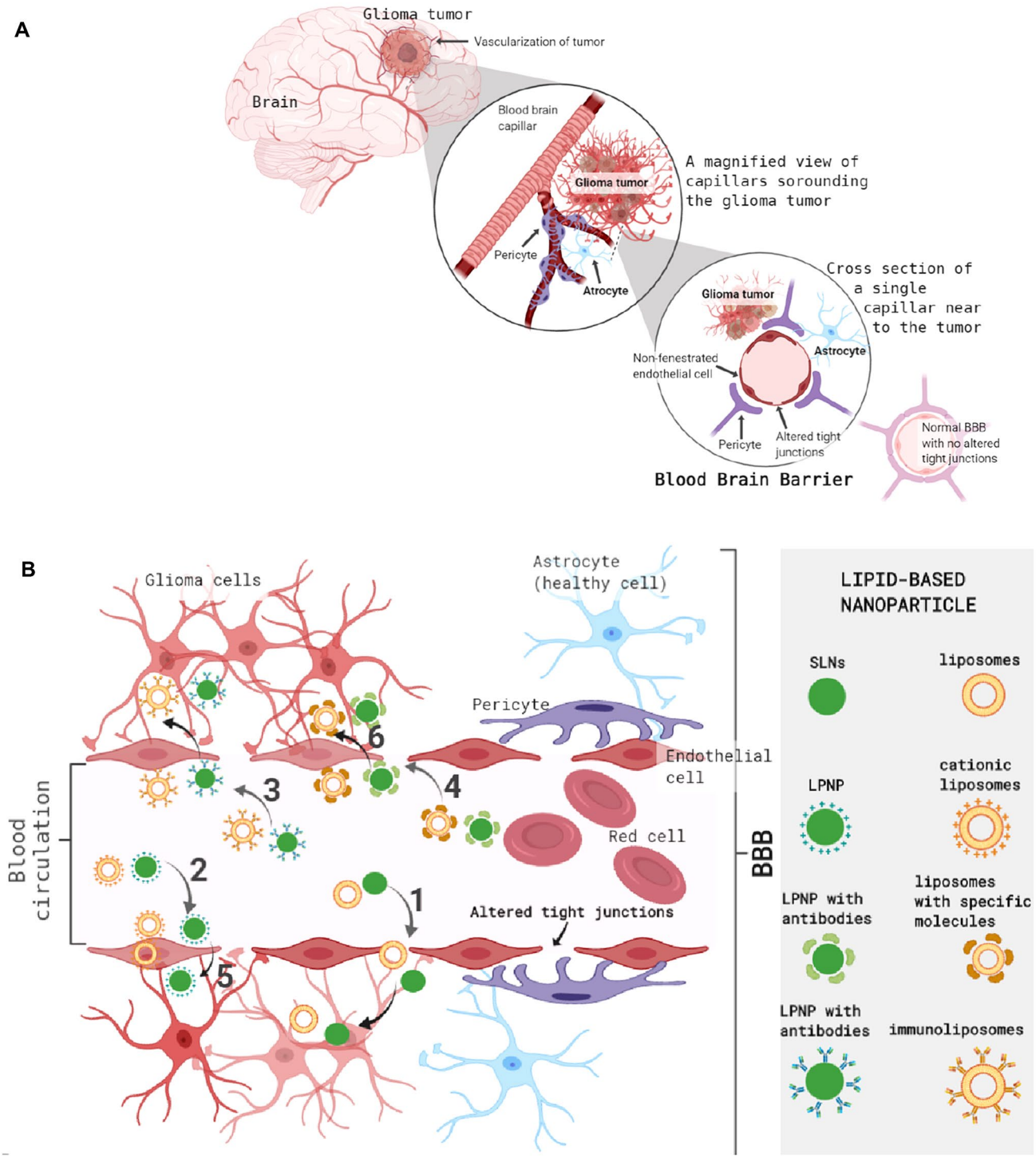

Fig. 2 Schematic representation of the BBB and location of a glioma tumor in brain (**a**). Possible entrance mechanisms of lipid-based nanoparticles to CNS (**b**). (1) Passive entrance by paracellular diffusion favored by altered permeability between endothelial cells in the BBB. (2) Cationic polymers covering lipid-based nanoparticles favor adsorption-mediated endocytosis, an active mechanism. (3) Incorporation of antibodies onto the lipid-based nanoparticles could favor the uptake by glioma cells by receptor-mediated endocytosis. (4) Molecules directed to specific targets (such as transferrin) favor active mechanisms by receptor-mediated endocytosis. (5) cationic polymers covering lipid-based nanoparticles favor transcytosis to the glioma tumor. (6) specific molecules directed to glioma cells enhance receptor-mediated transcytosis to the glioma tumor. Created with BioRender.com

modifications are required. Nanomaterials (< 100 nm) with lipophilic characteristics can enhance drug delivery (Pajouhesh and Lenz 2005). Several reports have highlighted the use of platforms based on the gold nanoparticles (Cheng et al. 2014; Khongkow et al. 2019), polymeric nanoparticles (Salvalaio et al. 2016), carbon nanotubes (Costa et al. 2018; Kafa et al. 2015), and silica nanoparticles (Bittner et al. 2019) as drug delivery vehicles.

Biological mechanisms of nanomaterials crossing the BBB can be passive or active mechanisms. The diffusion through the endothelial cells conforming to the BBB is a passive route generally reported for inorganic nanoparticles (such as gold nanoparticles) with minimal diameters < 10 nm and hydrophobic characteristics (Zhou et al. 2018) (Fig. 2b).

Furthermore, nanomaterials could temporarily disrupt the tight junctions between the endothelial cells forming the BBB, and finally, crossing to the CNS, as reported for inorganic structures with 100 nm or smaller (Chen et al. 2015; Trickler et al. 2010). Although, nanoparticles can enter through active mechanisms or energy-dependent routes: (1) carrier-mediated transport, as reported for self-targeting carbon dots smaller than 3 nm, which enter selectively to astrocytes through GLUT-1 glucose transporter (Zheng et al. 2015); (2) receptor-mediated endocytosis, when nanomaterials contain an adequate ligand or antibody; for instance, transferrin for transferrin receptor (TfR), or antibody anti-TfR (Ulbrich et al. 2009), (3) adsorption-mediated endocytosis, reported for cationic nanoparticles, which interact by electrostatic forces with the membrane cell components and activate endocytosis (Van Rooy et al. 2011; Zhou et al. 2018), (4) receptor-mediated transcytosis, nanoparticles targeting specific receptors (e.g., Tf) for specific molecules on the surface of endothelial cells (Akita et al. 2016; Lam et al. 2018; Sharma et al. 2019), and (5) absorptive-mediated transcytosis, for nanoparticles positively charged (Arcella et al. 2018; Sharma et al. 2019). Trascytosis involves endocytosis and exocytosis processes that facilitate extravascular transport of nanoparticles, and consequently, enhance delivery of nanoparticles within the tumor (Lu et al. 2012).

Mainly, reports on lipid-based nanoparticles crossing the BBB describe active mechanisms for delivering drugs in CNS. In the next section, we emphasize the biological studies for understanding the delivery of antitumoral drugs in the CNS using lipid-based nanoparticles as a promising treatment for GBM.

Advances on the Use of Lipid-Based Nanoparticle as Drug Delivery Vehicles for GBM Treatment

Some commercial antitumoral drug formulations approved by international health agencies as The Food and Drug Administration contain lipid-based nanoparticles in the USA. Examples of liposomal formulations are DaunoXome® a liposomal formulation of daunorubicin citrate; Depocyt® or cytarabine liposomal; Myocet ®, doxorubicin citrate encapsulated in liposomes; Doxil® and LipoDox®, both containing doxorubicin hydrochloride encapsulated in polymer-coated liposomes (Vieira and Gamarra 2018). Since lipids are hydrophobic and biocompatible, lipidic nanostructures are promising drug delivery vehicles for cancer treatment in the CNS. Understanding the mechanisms of action of liposomes and other lipidic nanostructures as drug delivery vehicles in CNS is highly relevant to modify the liposome characteristics and target specific sites, such as glioma tumors (Fig. 2b). Vieira and Gamarra (2016) summarized the mechanisms for liposomes, highlighting the need for modified liposomes to lead an entrance pathway through the BBB. For instance, the incorporation of cationic, anionic, or neutral polymers onto the liposome surface favor adsorption-mediated endocytosis mechanisms. Joshi et al. (2015) described the association between the surface charge of liposomes (10 mM) and the delivery efficiency in a glioma C6 rat model. Cationic liposomes tended to accumulate at higher rates than both neutral and anionic nanostructures (e.g., the total area under the curve data were 48 ± 34 μM/s, 343 ± 36 μM/s, 56 ± 43 μM/s for anionic, cationic, and neutral liposomes, respectively). Furthermore, cationic liposomes were redistributed within the brain, suggesting that drug delivery can target glioma tumors (Joshi et al. 2015).

The incorporation of peptides targeting glioma cells could increase drug delivery in tumors, as described by Liu et al. (2017). The authors designed a liposome with a transferrin-cell penetrating peptide (Tf-CPP), which allows selective delivery of the nano-vehicle. The CPP is a positively charged peptide due to an arginine-rich residue peptide; thus, CPP interacts with anionic material onto the cell surface and activates the cellular uptake. Liposomes containing CPP trapped in endosomes take hydrogen ions, promoting the lipid fusion between CPP-H^+-liposome and the endosomal membrane by electrostatic forces (Liu et al. 2017). Those physical forces are due to the highly negatively charged molecules, such as laminin, fibronectin, type IV collagen, and heparin sulfate onto the surface of the cells (Vieira and Gamarra 2016). A sterically stabilized liposome (SSL) liposomal system modified with Tf and CCP (Tf-CPP-SSL) reported by Liu et al. (2017) is a

receptor-mediated and adsorption-mediate liposome. That resulted in a high targeting efficacy for the microvascular endothelial cells and delivery in glioma C6 cells but not astrocyte cells (healthy cells) in in vitro and in vivo studies. Tf-CPP-SSL entered to the glioma cells with nondrug leakage or liposome degradation due to (1) their entrapment in endosomes of malignant cells, and (2) the successful escape from lysosomes to release doxorubicin (DOX) into the cytosol (Liu et al. 2017). In a similar context, a study reported by Helm and Fricker (2015), a liposome covered by bovine serum albumin (BSA), which crossed an in vitro BBB with possible adsorption-mediated endocytosis.

On the other hand, targeting a specific receptor in the brain could be designed with immunoliposomes as previously described for Tf receptor (TfR) in other nanostructures (Ulbrich et al. 2009). Two interesting studies propose immunoliposomes with OX-26, an anti-TfR as drug delivery vehicles in the CNS (Johnsen et al. 2017; Markoutsa et al. 2011). Markoutsa et al. (2011) pointed out in a BBB in vitro model and pH-sensitive fluorescence signal that OX-26 immunoliposomes (~ 80 nm) uptake occurs via receptor-mediated mechanism and lysosomal localization. Moreover, Johnsen et al. (2017) conducted both in vitro and in vivo studies to evaluate the uptake of an antitumoral drug, oxaliplatin, with OX-26 immunoliposomes. The drug uptake is higher in treatments with OX-26 immunoliposomes than soluble drug treatments. In vivo evaluations with LysoTracker dye indicated that oxaliplatin on OX-26 immunoliposomes uptake could occur close to tight junctions. However, contrary to other reports, the authors did not find evidence on receptor-mediated endocytosis (Johnsen et al. 2017).

Although active mechanisms are widely reported, some lipid-based nanoparticles could achieve passive pathways to cross the BBB. In this respect, Song et al. (2018), evaluated SLNs obtained by chemical modification of dioleoyl phosphoethanolamine (DOPE) with borneol (BO-SLNs). Interestingly, both BO-SLNs and BO-SLNs with a model drug curcumin (BO-SLNs-CM), altered the permeability of in vitro BBB. A transepithelial/transendothelial electrical resistance (TEER) measurements determined the integrity of the tight junctions in endothelial cell cultures. The TEER value on controls (no treatments) was 277.57 Ω/cm^2, while the TEER value after BO-SLNs and BO-SLNs-CM decreased to ~ 80 Ω/cm^2 in the first 20 min of exposure. An SLNs without BO did not alter TEER values, suggesting that the incorporation of BO monoterpene enhances cellular uptake by a temporal disruption of permeability (Song et al. 2018).

Simple liposomes or SLNs are not a relevant drug delivery vehicle in CNS since pharmacokinetics and other parameters indicate the need to decorate nanomaterials with polymers, antibodies, or other molecules. The recent studies on lipid-based nanoparticles consider different modification on the lipidic nanostructures to cross the BBB and enhance the drug delivery in CNS. In this review, we summarized the information reported in the last 5 years regarding the biological evaluation of lipid-based nano-vehicles as a potential drug delivery vehicle for GBM treatment (Table 1).

Studies on the Potential Use of Liposomes

As previously mentioned, TZM is the first choice in chemotherapy treatment for GBM; however, the high toxic effects and poor tumor selectivity require attention. The aim is to reduce the drug dosage by improving the half life and brain targeting. In this respect, Gao et al. (2015) conducted a pharmacokinetic study of TZM encapsulated in liposomes (156.7 ± 11.40 nm) with an entrapment efficiency of 35%. Overall, in vivo results in New Zealand rabbits showed that the half time in hours t1/2β of temozolomide liposomes was 3.57 folds greater than TZM solution treatments (13.3 h for TZM liposomes and 3.7 h for TZM alone). Simultaneously, the maximum concentration in plasma (Cmax) reached 6.26 mg/L for TZM liposomes and 5.68 for TZM in solution. Moreover, the area under the curve was about 0.5 times higher in the liposomal formulation. The TZM analysis in mice showed that TZM liposomes accumulation in heart and lung decreased when compared with TZM in solution, suggesting that the liposomal formulation could improve the therapeutic effect in the brain and also, reducing toxicity in other sites (Gao et al. 2015). Satapathy et al. (2016) optimized the assembly of unilamellar liposomes with 1,2-distearoyl-sn-glycerol-3-phosphatidylethanolamine (DSPE) encapsulating docetaxel (DTX). The DSPE liposomes (100 nm) with an encapsulation efficiency of 78% achieved a sustained drug release profile in vitro dialysis membrane system (about 58% of DTX release in 24 h). Interestingly, in vivo study regarding the CNS delivery of liposomes showed that the technetium-99 m labeled liposomes effectively crossed the BBB and accumulated in Sprague–Dawley rats' brain tissue a time-dependent manner (3 and 5 h after intravenous administration). Although the authors did not describe the possible entrance mechanisms, they carefully describe the design parameters involved in the obtention of liposomes to deliver DTX in the CNS (Satapathy et al. 2016).

A modified liposome was studied by Tang et al. (2015), who developed a lipid–protein complex modified with BO as a carrier of PTX, an antitumoral drug. The nanocarrier (BO–PXT–LAN) had a spherical multilayer structure separated by a BSA–water interlayer and a BO–PXT–BSA conjugated (107.5 ± 3.2 nm). The BO–PXT–LAN complex showed a high entrapment efficiency for BO ($85.89 \pm 0.78\%$) and PTX ($90.40 \pm 1.2\%$) and exhibited sustained drug release profiles in vitro. The authors report an increase in the

Table 1 Relevant studies on the use of lipid-based nanoparticles as drug delivery vehicles for GBM treatment

Size average (nm)	Composition	Antitumoral drug/agent and encapsulation efficiency	In vitro/in vivo model	Target ligand/target	Mechanism BBB and/or glioma cell entry	References
Liposomes						
107.5 ± 3.2 (MLV)	Bovine serum albumin, lecithin	Borneol/85% and paclitaxel/90%	C6 glioma cells, Kunming mice	Not mentioned	Transcytosis/pinocytosis	Tang et al. (2015)
74.6–138.6 (SUV)	Lecithin, cholesterol, DSPE	Docetaxel/63%	Swiss albino mice, Sprague–Dawley rats	Not mentioned	Endocytosis	Satapathy et al. (2016)
156.7 ± 11.40 (SUV)	Lecithin, cholesterol, sorbitol	Temozolomide/35%	New Zealand rabbits, Kunming mice	Not mentioned	Pinocytosis	Gao et al. (2015)
~ 41.4 Stealth liposomes	DOTAP, DOPE, TfRscFv	Temozolomide/45%	U-87 MG and U-251 MG cells, BALB/c mice	TfRscFv/transferrin receptor (OX26)	Receptor-mediated transcytosis	Kim et al. (2016)
~ 137 liposomes	Tf-NP with the bromodomain inhibitor JQ1 DOPG and POPG	Temozolomide/48%	U87MG and GL261 cells	Transferrin/OX26	Receptor-mediated transcytosis	Lam et al. (2018)
~ 140 immunoliposomes	Immunoliposomes with OX26 receptor	Oxaliplatin/1.27 mg/mL	BCECs	Transferrin receptor/ OX26	Endocytosis	Johnsen et al. (2017)
~ 100 Stealth liposomes	Phosphatidylcholine, DSPE-PEG-NHS CPP (GGRRRRRRRRR-amide), Transferrin	Doxorubicin/90%	BBB model with BMVECs, C6 glioma cells BALB/c nude mice	Transferrin/OX26	Endocytosis	Liu et al. (2017)
100–175	Solid lipid NPs, tamoxifen, lactoferrin	Carmustine/40%	HBMEC and U-87 MG cells	Lactoferrin/LfR	Endocytosis	Kuo and Cheng (2016)
105–17	DPPC, phosphatidylcholine, aprotinin, melanotransferrin antibody (Anti-MTf)	Doxorubicin/	HBMEC and U87 MG cells	Aprotinin/LDLR Anti-Mtf/ p97	Apr–Dox–SLNs could traverse the BBB via the preferential affinity of Apr to LDLR	Kuo and Lee (2016)
~ 139	Lactoferrin- and arginine–glycine–aspartic acid dual-ligand-comodified	Temozolomide and vincristine/ ~ 80%	U87MG and T98G cells BALB/c nude mice	Lactoferrin/LfR	Receptor-mediated transcytosis	Zhang et al. (2018)
~ 43.82	Monomeric/di-chain (3 μM) or gemini (1.5 μM) surfactants	None	U87 MG and U373 MG cells	Not mentioned	Transcytosis/endocytosis	Mendes et al. (2018)
~ 87	DOPE-modified with borneol	Borneol/80.1%	HBMECs	Not mentioned	Improving the permeability through a physiological process	Song et al. (2018)
~ 180	SPIONs were encapsulated in SLNs	Nutlin-3a/2%	U87 MG cells	Not mentioned		Grillone et al. (2019)
Lipid polymer hybrid nanoparticles						
~ 178	Lecithin, stearic acid, poloxamer 188, TOC, PEG	Paclitaxel/ ~ 88%	C6 glioma and NIH 3T3 cells, Sprague–Dawley rats	TOC/SSTR2	Receptor-mediated transcytosis	Banerjee et al. (2016)

Table 1 (continued)

Size average (nm)	Composition	Antitumoral drug/agent and encapsulation efficiency	In vitro/in vivo model	Target ligand/target	Mechanism BBB and/or glioma cell entry	References
~ 70	Glycerol tricaprylate, lecithin, PEG, SDS, chitosan, CpG	Paclitaxel/ 98%	GL-261 cells, C57BL/6J mice	CpG/Toll-like receptor (TRL9)	Endocytosis	Lollo et al. (2015)
84.04–117.3	Conjugated linoleic acid, DSPE, PEG	Paclitaxel/90%	B16-F10, MDA-MB-231 and U87 MG cells, ICR mice	Not mentioned	Not mentioned	Zhong et al. (2016)
126.9–141.3	Solid lipid NPs of glyceryl monostearate, distearate and tristearate, phospholipon 90H, poloxamer 188	Asiatic acid/98%	SVG p12 and U-87MG cells	Not mentioned	Energy-dependent endocytosis	Garanti et al. (2016)
Nonspherical nanoparticles and other lipidic nanostructures						
10 – 12	sHDL nanodiscs	Docetaxel/~2% (w/w)	GBM cells/GL26 syngeneic mouse glioma model	CpG/TRL9	Not mentioned	Kadiyala et al. (2019)
30–150	Exosomes-derived from human endometrial stem cells	Atorvastatin/~20%	U87 cells	Not mentioned	Not mentioned	Nooshabadi et al. (2020)
16–17	Cubosomes from glyceryl mono oleate	AT101/97.7%	A172, LN229, SVGA and HMC3 cells	AT101/hydrophobic surface grooves	Endocytosis	Flak et al. (2020)
~ 100	Extracellular vehicles from NK-92MI cells	None	U87/MG, MDA-MB-231, and CAL-62/BALB/c nude mice	NK cells/ MHC class I	(1) receptor-mediated endocytosis, (2) lipid raft-mediated endocytosis, and (3) micropinocytosis	Zhu et al. (2019)

LDLR low-density lipoprotein receptor, *DOPE* dioleoyl phosphoethanolamine, *SPIONs* superparamagnetic iron oxide nanoparticles, *TOC* tyr-3-octreotide, *SSTR2* somatostatin receptor, *Tf-NP* transferrin-functionalized nanoparticle, *TfRscFv* anti-transferrin receptor single-chain antibody, *HBMECs* human brain microvascular endothelial cells, *BCECs* brain capillary endothelial cells, *BMVEC* brain microvascular endothelial cells, *LfR* lactoferrin receptor, *ICR mice* is a general-purpose model used for studies in a wide range of fields including toxicity, pharmacology, drug efficacy, and immunology, *sHDL* synthetic high-density lipoprotein, *SDS* sodium dodecyl sulfate, *PEG* polyethylene glycol

PXT uptake with PXT alone in glioma C6 cultures (about four times higher in BO–PXT–LAN). Confocal scanning microscopy images of glioma C6 cells demonstrated an increase in the cellular uptake (ratio of the concentration of nano-vehicle and drug/concentration of total protein). The use of PEG-veh zine before BO–PXT–LAN treatments significantly decreased the cellular uptake, suggesting the liposomal complex's internalization by clathrin-associated endocytosis. Supported by other evidence, the authors suggest that incorporation of an active molecule, such as BO could enhance the internalization through the BBB (brain microvascular endothelial cells or BMECs models) due to other mechanisms: (1) BO can temporally affect the tight junctions, (2) increase the membrane fluidity and (3) inhibit the Glucoprotein P1 (P-gp efflux) (see Fig. 3). This report also described an increase in the glioma C6 cytotoxicity when treated with BO–PXT–LAN as compared to PXT alone. However, the cytotoxicity in the healthy counterpart was not reported and omitted PXT alone (Tang et al. 2015).

Furthermore, in vivo imaging using C6 xenograft bearing mice showed a maximum fluoresce signal for fluorescence-labeled LAN within 2 h post intravenous injection, and the signal decreased gradually after 24 h postinjection. Importantly, BO–PXT–LAN presented the strongest fluoresce signal until 24 h postinjection, suggesting the passive internalization of LAN with no BO modification (by EPR effect). Simultaneously, the proposed mechanism to deliver BO is the inhibition of P-glycoprotein-mediated efflux and further increases the internalization of the labeled complex in the tumor regions (Tang et al. 2015).

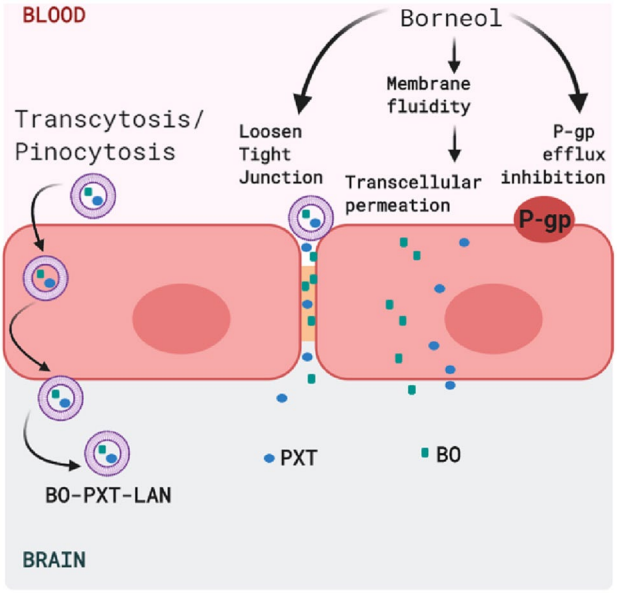

Fig. 3 Schematic illustration of PTX transport across the BBB (BMECs cells) (Adapted from Tang et al. (2015))

In the same context, Lam et al. (2018) developed a dual therapy, a transferrin-functionalized nanoparticle vehicle (Tf-veh) loaded with TMZ and the bromodomain inhibitor JQ1. The objective was to enhance the uptake by a receptor-mediated mechanism. Assays in NCR nude mice (Taconic) with multiphoton intravital live imaging (using Cys5.5 fluorescence) demonstrated the ability of Tf-veh-TZM-JQ1 to cross the BBB. Tf-veh-TZM-JQ1 achieve accumulation and retention on the surface of intracranial gliomas. In contrast, treatments with no functionalized NP showed no internalization into glioma tumors (see Fig. 4). Furthermore, the mice treated with Tf-veh-TZM-JQ1 had relative protection from adverse effects caused by TZM and percent survival of 100% at day 50 and 90% at day 60 (Lam et al. 2018).

Kim et al. (2016) described an evaluation conducted to a dual-targeting system to enhance the nanomaterial cellular uptake and target glioma cells. The nanosystem (~40 nm) consisted of cationic liposome encapsulating TMZ and decorated with anti-transferrin receptor single-chain antibody fragments (scL-TMZ) to facilitate the crossing of the BBB. The scL-TMX formulation was found stable with an encapsulation efficiency of 45.2%. The antitumoral effect of scL-TMX (15.3 μM) in U87 and U251 tumor cell lines was 2–3 times higher than the effect exerted by TMZ in solution. scL-TMX also increased the in vivo survival in mice bearing glioma tumors (37.5% of mice survive 45 days after the end of the treatment) (Kim et al. 2016).

Liu et al. (2017) reported a liposomal formulation designed to favor both receptor-mediated and adsorption-mediated entrance mechanisms by incorporating a Tf-cell penetrating peptide sterically stabilized (Tf-CPP-SSL). Measurements registered in flow cytometry (with coumarin-6 as fluorescence marker) indicated that Tf-CPP-SSL achieved the highest efficacy for BMECs and glioma C6 cells. Real-time confocal techniques (DOX-red fluorescence and lysosome-GFP marker) demonstrated that Tf-CPP-SSL favored the entrapment in endosomes of glioma C6 cells and DOX released in the cytosol by avoiding lysosomal degradation. Moreover, the in vivo studies in BALB/c nude mice bearing glioma C6 tumors showed that 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyannine Iodide labeled Tf-CPP-SSL increased brain accumulation after 8 h administration, while liposomes with no CPP where observed in the brain until 48 h after administration (Liu et al. 2017).

Bhunia et al. (2017a) reported a novel stable liposomal system (130–150 nm) prepared from a nipecotic acid-derived cationic amphiphile (NACA) co-loaded with CDC20siRNA (to silence the CDC20 oncogene) and PTX. The entrapment efficiency of siRNAs encapsulated within the liposomes of NACA was 90%. Findings in the cellular uptake experiments demonstrated that the liposomes of NACA containing encapsulated fluorescently labeled FAM-siRNA enter to IMR-32 human neuroblastoma cells via GABAA receptor, leading

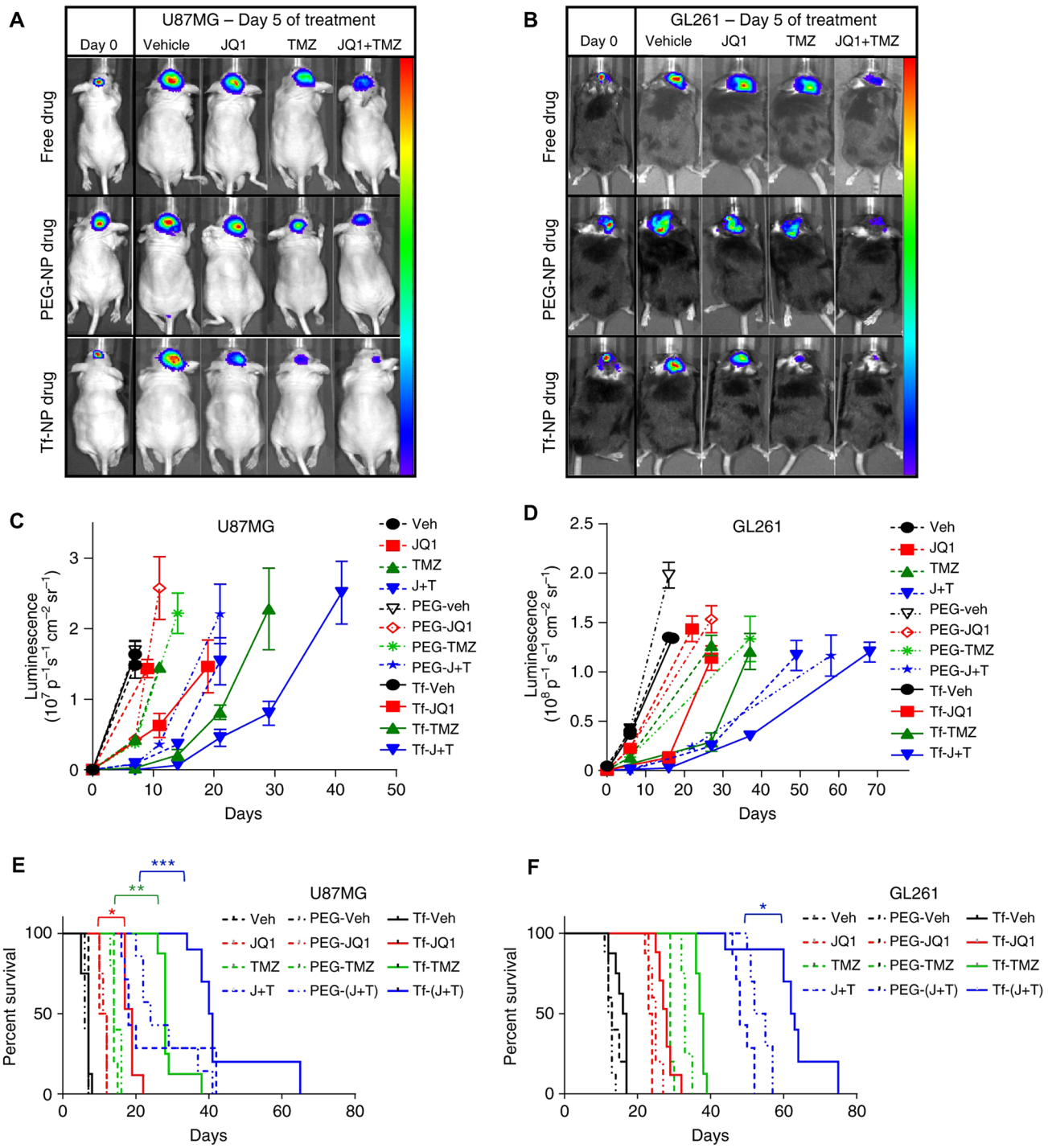

Fig. 4 Tf-NPs loaded with JQ1 and TMZ have superior pharmacodynamic effects in intracranial orthotopic models of glioblastoma. Representative bioluminescent images of (**a**) U87MG and (**b**) GL261 mice taken on day 0 and day 5 following initiation of treatment with free drug formulations (free drug), drugs loaded in PEG-Cy5.5 liposomes (PEG-NP drug), or transferrin-PEG-Cy5.5 liposomes (Tf-NP drug). Quantification of average tumor bioluminescence values in the different treatment arms throughout the course of treatment for (**c**) U87MG and (**d**) GL261 mice. Kaplan–Meier survival plots of **e** U87MG and **f** GL261 glioma mice in the different treatment arms. Study powered with eight mice per treatment arm for statistical significance. Log-rank (Mantel–Cox) test performed on survival plots. Student's *t* test used to quantify differences in treatment arms ($*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$) (Taken from Lam et al. (2018) under Creative Commons Attributions 4.0 International License (https://creativecommons.org/licenses/by/4.0/))

in a significantly higher CDC20 gene silencing efficiency in IMR-32 cell as compared to control (untreated cells). Interestingly, intravenous administration (dose 10 µg/mice) of the liposomal formulation induced a significant tumor growth inhibition in athymic nude mice bearing xenografted human neuroblastoma.

The same research group developed a liposomal drug carrier (100–135 nm) prepared from a novel L-3,4-dihydroxyphenylalanine (L-DOPA) functionalized with amphiphile characteristics (Amphi-DOPA). The Amphi-DOPA functionalization aimed to target the large amino acid transporter-1 (LAT1) overexpressed in both brain capillary endothelial cells and glioma cells. The in vitro results in GL261 cells demonstrated that cellular uptake of this nanovehicle was LAT1-mediated (using a cell culture pretreated with anti-LAT1 antibodies as a control). The biodistribution study using labeled liposomes of Amphi-DOPA in GBM bearing mice showed a selective accumulation in brain tissue. Furthermore, the authors also described that an intravenous administration (dose 10 mg/kg) of the liposomes of Amphi-DOPA loaded with an activator of transcription 3 inhibitor WP1066, enhanced the overall survivability of C57BL/6J mice bearing orthotopically established mouse GBM by ~ 60% as compared to the untreated mice group, suggesting promising results in clinical evaluations (Bhunia et al. 2017b).

In the same context, Saha et al. (2017), elaborated two liposomal formulations (10–130 nm) based on the novel nicotinylated amphiphiles as nano-vehicles of anticancer drugs to orthotopic mouse glioblastoma. The authors evaluated in an orthotopic glioblastoma-bearing mice, the intravenous administration of WP-1066-loaded liposomes of nicotinylated amphiphiles in combination with in vivo dendritic cell-targeted subcutaneous genetic immunization (using tyrosinaserelated protein-2 encoded DNA vaccine). The treatment in the in vivo model enhances the overall survivability (OS) by > 500% as compared to the OS for the control group. Thus, the notable response could be directly associate to the incorporation of WP1066 in the nano-vehicles containing the nicotinyl-functionality molecules in the polar head-group region in the liposomal formulation of potent anticancer drug. Once the liposomes cross the BBB, the nicotinyl headgroup in the liposome exo-surface may further assist the delivery of the liposome-associated WP1066 to brain tumors, presumably via nicotinic acetyl choline receptors (Saha et al. 2017).

Furthermore, Saha et al. (2020), designed and synthesized three different β-amphetaminylated cationic lipid nanoparticles (150–195 nm) to exert the active transcytosis cellular uptake. Interestingly, the hexadecyl lipid nanoparticle (16-BACL) was able to simultaneously encapsulate PTX and programmed death receptor ligand-siRNA. These nanoparticles showed cytotoxicity against GL261 cell cultures and improved the overall survivability of orthotopic glioblastoma bearing mice as compared to its nontargeting counterpart (Saha et al. 2020).

Overall, preclinical evaluations of liposomes as nanovehicles for antitumoral drugs targeting the CNS show promising results. The incorporation of biomolecules, such as Tf, BO, L-DOPA derived, and siRNA have shown promising results in the research for more efficient vehicles targeting BBB and GBM.

Studies on the Potential Use of SLNs

A modification in the surface charge of nanomaterials from negative to positive could enhance the interaction and drug delivery in biological models. Thus, cationic and biocompatible polymers can decorate the lipid-based nanoparticles; however, controversy arises since cationic charges could also increase the cytotoxic effect in healthy tissues. An alternative suggested by Mendes et al. (2018) describes SLNs (~ 43 nm) with different surface modifications of positive charge by using monomeric and gemini surfactants either with conventional headgroups or serine-based molecules (with a zeta potential value of 26 mV). The authors propose dynamic molecular studies that cationic serine-derived surfactants can interact with cell membranes to achieve a higher cellular uptake. The theoretical studies were linked to the cellular uptake evaluations with confocal microscopy, where images of serine cationic surfactants were internalized by U-87MG glioma cells. The low toxicity presented in healthy astrocytes cell cultures (U-373 cells) exposed to the serine-derive surfactant SLNs suggests a potential alternative to deliver drugs in the CNS (Mendes et al. 2018).

Kuo and Lee (2016) reported SLNs conjugated system (150–180 nm) with grafted aprotinin (Apr) and melanotransferrin antibody (anti-MTf) to carry DOX (Apr–anti-MTf–DOX–SLNs) for GBM treatment. Apr and anti-MTf can join to proteins expressed by endothelial cells in the BBB (low-density lipoprotein receptor-related protein LRP1/LRP2 and MTf, respectively). The incorporation of Apr and anti-MTf enhanced the affinity for BBB and triggered endocytosis by targeting the endothelial MTf. A co-culture of human brain microvascular endothelial cells (HBMECs) with the regulation of human astrocyte and to a proliferated colony of U87MG cells was exposed to Apr–Anti-MTf–DOX–SLNs. The treatment demonstrated an enhancement on the ability of the antitumoral drug to cross the in vitro BBB and to inhibit the growth of tumor brain cells, with very low cytotoxicity on HBMECs (Kuo and Lee 2016).

In the same context, Kuo and Cheng (2016) conjugated SLNs with TXF and lactoferrin (Lf) as a vehicle for BCNU). The TXF–Lf–BCNU–SLNs system improved the characteristics of sustained release when compared with BCNU in

SLNs with no Lf. Moreover, the in vitro BBB model with HBMECs showed that TXF–Lf–BCNU–SLNs increased about ten times the BBB permeability coefficient for BCNU in SLNs. A fluoresce assay in the in vitro BBB indicates possible endocytosis via calcein in HBMECs. The biological assay in U87MG cells as a model for glioma tumor and HBMECs as a healthy counterpart showed the lowest cell viability in tumor cells (Kuo and Cheng 2016).

Zhang et al. (2018) incorporated an arginine-glycine-aspartic acid peptide (RGD) to SLNs to deliver TMZ into the SNC. The obtained nanoparticle lipid carrier (RGD-TMZ/NLCs) showed an inhibition of U87MG cells when in vitro assays were conducted. Furthermore, a U87MG tumor bearing BALB/c mouse model treated with RGD-TMZ/NLCs (containing 10 µg of TZM and/or peptide per mouse) also displayed the highest antitumor efficacy in vivo than all the other formulations (TMZ/NLCs, free TMZ solution, and blank NLCs) in the assays (about 80% of tumor inhibition rate compared with 20% when treated with free TMZ) (Zhang et al. 2018).

Other potential glioma treatments are based on the phytochemical compounds into lipid-based nanoparticles. For instance, asiatic acid is a pentacyclic triterpene isolated from *Centella asiatica* with neuroprotective and anticancer activity against glioma. Garanti et al. (2016) evaluated the incorporation of asiatic acid in SLNs (AA-MS-SLNs) with a size in a range of 126.9 and 141.3 nm, as a potential treatment against GBM. The design of SLNs considered the use of glyceryl monostearate (MS), glyceryl distearate, and glyceryl tristearate, with an entrapment efficiency of about 98% in all three systems. The system AA-MS-SLNs assessed a more favorable drug release profile and high cytotoxicity towards U87MG cells but not against SVG P12 cells (human fetal glial). However, SNLs-AA exerted a similar toxic effect than AA alone, suggesting that SNLs structures entrapped some AA molecules. SNLs marked with coumarin-6 dye for imaging and flow cell cytometry showed that cellular uptake of SNLs appeared to be an active mechanism via endocytosis (Garanti et al. 2016).

Furthermore, SLNs could be used in more complex nanosystems to enhance stability and biocompatibility. An interesting study in this respect was reported by Grillone et al. (2019), who proposed a nanocarrier based on the superparamagnetic nanoparticles encapsulated in SLNs as carriers of Nutlin-3a (Nut-Mag-SLNs). This drug is a cis-imidazoline molecule type with limited clinical use due to the high doses required to achieve a therapeutic concentration. Thus, Nut-Mag-SLNs (~180 nm) exhibited promising results compared with Nutlin 3a in solution: (1) excellent colloidal stability in different physiological mediums and up to nine months, (2) the ability to cross an in vitro BBB model with bEnd.3 cells, and (3) a superior pro-apoptotic activity toward GBM cells (U87MG) (Grillone et al. 2019).

Studies on the Potential Use of LPNPs

Reports on LPNPs currently focus on modifications with leading molecules for an active endocytosis mechanism. In this sense, Banerjee et al. (2016) designed and evaluated LPNPs with PEG and Tyr-3-octreotide (TOC), a known ligand for somatostatin receptors overexpressed in glioma cells. The resulting LPNPs-TOC with an average size of ~178 nm achieved an encapsulation efficiency of ~88%, as a vehicle of PTX. In vitro assays in glioma C6 cells, SLPs-PXT exhibited ~10% of early apoptosis and only ~1% of late apoptosis. In comparison, the modified formulation LPNPs–TOC–PTX showed about ~20% of early apoptosis and ~4% of late apoptosis, suggesting a process of active receptor-mediated endocytosis. Banerjee et al. (2016) determined the biodistribution of radiolabeled formulations, SLNs–PXT, and LPNPs–TOC–PTX. Notably, 2 h after intravenous administration, the SLNs–PXT biodistribution in brain and tumor brain was similar (0.86 and 0.78); however, LPNPs–TOC–PTX biodistribution in brain tumor was higher than its distribution in the brain (1.34 and 0.96), suggesting a selectivity for targeting glioma cells. These results consist of antiglioma efficacy in intracranial C6 glioma bearing rats with a median survival time of 36 days in rats treated with LPNPs–TOC–PTX, 19 days in the group treated with SLNs–PXT, and 15 days in a control group with Taxol (Banerjee et al. 2016).

The use of immunostimulant molecules onto lipid-based nanoparticles has also been explored. Lollo et al. (2015) designed LPNPs consisting of a lipid core and a chitosan shell to deliver PTX combining with an immunostimulant CpG molecule. CpG is a Toll-like receptor ligand (TLR9) expressed by murine immune cells and can trigger immune rejection and induce long-term immunity against glioma tumors. The nanosystem exhibited a diameter size of 70 nm, a positive surface charge of 25 mV, and high entrapment efficiency of PXT (98%). Both in vitro study in GL261 glioma cells and in vivo study in bearing mice with GL261 tumor demonstrated a higher cytotoxic effect when compared with commercial drug Taxol® and lipid nanoparticles loaded with PXT (Lollo et al. 2015).

Another approach to obtain liposomes is using bioactive lipid molecules with dual function: to assembly the nano vehicle and act as an adjuvant in anticancer therapy. In this respect, conjugated linoleic acid exhibits in vitro and in vivo antitumoral effects, as we have also reported in our research group (Silva-Ramirez et al. 2018). Zhong et al. (2016) developed a conjugated linoleic acid–PTX conjugate decorated with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine [methoxy (polyethylene glycol)2000] (CLA–PTX–PEG). The LPNP, with a size of ~117 nm, is reported as stable, well dispersed, and with an encapsulation efficiency of 90%. CLA–PTX–PEG nanomaterial evaluated in U87MG

human glioma cells showed that CLA–PTX–PEG cellular uptake after 6 h of exposure was above two times higher than a CLA–PXT control (as a mixture of free molecules). CLA–PTX–PEG treatments in U87MG cells resulted in a lower inhibitory concentration (IC50) than the CLA–PTX mixture. The result was not similar to the IC50 of Taxol treatment (IC50 in μM for Taxol, CLA–PTX mixture and CLA–PTX–PGE were 2.85 ± 0.60, 6.83 ± 0.16 and 4.62 ± 0.59, respectively), suggesting further studies to improve the lipid nanocarrier with bioactive CLA molecules (Zhong et al. 2016).

Nonspherical Nanoparticles and Other Lipidic Nanostructures

Although spherical nanoparticles are one of the most reported in nanomedicine, nonspherical nanoparticles (like discoidal particles, nanorods, and filamentous particles) have also been studied in the recent years to display better performances in tumor treatment and their ability to cross biological barriers (Taiarol et al. 2020). Evaluations on the potential use of nonspherical nanoparticles have been reported for polymeric materials. Shamul et al. (2019) encapsulated verteporfin (VP) in a micellar vehicle composed in poly(ethylene glycol)-poly(-amino ester)-poly(ethylene glycol) (PEG–PBAE–PEG), results show that these filamentous micelles containing VP (fVPs)-induced selective cytotoxicity against GBM cells (Shamul et al. 2019). As typically reported for nanoparticles targeting cancer, nonspherical nanoparticles can penetrate solid tumors through the EPR effect; however, elongated shapes are more efficiently trapped and retained in the tumor, consequently producing an adequate drug delivery (Sharma et al. 2010; Taiarol et al. 2020). Notably, nonspherical lipid-based nanoparticles in the search for GBM treatments have been recently reported. Flak et al. (2020) obtained cubosomes with an internal membrane bilayer and bicontinuous cubic phase. The cubosome structure can have a larger membrane surface area than spherical vesicles, hence, higher drug payload (97.7% of entrapment efficiency). The Nile Red labeling nano-vehicle loaded with AT101 drug (palforzia, an oral peanut-protein derived) (119 nm) showed their penetration into 3D tumor spheroids (780 μm and 535 μm, respectively) of LN229 and A172 glioma cells. Cubosomes were also internalized by glioma cells in a process associated with endocytic mechanisms. Furthermore, 2.5 and 3.75 μM concentrations of free AT101 treatments did not induce a significant cytotoxic effect on LN229 and A172 glioma cell cultures; however, the same concentrations encapsulated in cubosomes significantly improve the toxic effect on the same glioma cells, suggesting that cubosomes may reduce the dose of A172 in further in vivo tests.

Kadiyala et al. (2019) reported HDL-mimicking nanodiscs (8–12 nm) as vehicles of DTX, a chemotherapeutic drug combined with CpG oligodeoxynucleotide. CpG is a TRL9 ligand expressed by most immune cells, and its activation is related to immune rejection and induce long-term immunity against gliomas. Studies in GL26, HF2303, and U251 glioma cells showed that HDL-mimicking nanodiscs loaded with DTX (HDL-CpG-DTX) have higher toxicity than free DTX. Furthermore, imaging studies with fluorescence-labeled HDL nanodiscs showed intracellular localization after 2 h incubation in all three cell cultures. Interestingly, in vivo evaluation in GL26 tumor-bearing mouse model indicated that fluorescence signal due to labeled HDL-CpG-DTX was detected only in brain (about 4×10^3 fluorescent counts). In contrast, signals were not significant in other organs or control mice (naïve), suggesting the high selectivity of the nanomaterial as well as activate $CD8^+$T cell-mediated immunity and developing a long-term immunological response. The authors presented an intracranial treatment results in a mice model. Nissl staining of brain sections exhibited a significant tumor reduction in mice treated with HDL-CpG-DTX (control: saline solution). The work of Kadiyala et al. (2019) suggest promising results in future clinical trials.

Zhu et al. (2019) isolated EV from natural killer (NK) cell cultures (related with immune regulation of tumor cells) without (NK-EV) or with IL-15 (NK-$EV_{IL\text{-}15}$). The IL-15 cytokine promotes the survival, proliferation, and toxicity of NK cells. Thus, U87 MG glioma cells were treated with both NK-EV and NK-$EV_{IL\text{-}15}$ in a range of 5 to 15 μg, demonstrating higher cytotoxic activity (54, 35, 11%) in treatment with NK-$EV_{IL\text{-}15}$ (57% cell decrease) via apoptosis. In addition, the NK-$EV_{IL\text{-}15}$ did not affect Phoenix Ampho cells' viability derived from a human kidney and on normal thyroid cells. An in vivo model of tumor-bearing BALB/c mice showed a significant inhibition of GBM xenograft cells when animals were intravenously injected every other day for eight days with 50 μg of either NK-EV or NK-$EV_{IL\text{-}15}$. However, NK-$EV_{IL\text{-}15}$ decrease the tumor volume from about 1300 mm^3 to < 600 mm^3 while NK-EV reduced the tumor to 900 mm^3. Furthermore, EV treatments did not induce toxic effects in normal cells, demonstrating the potential use of EVs priming with IL-15 as an alternative treatment for further evaluations (Zhu et al. 2019).

More recently, Nooshabadi et al. (2020) isolated exosomes (EXO) (30–150 nm) from human endometrial stem cells and incorporated atorvastatin, a statin reported as anti-glioma, with a %EE of 28%. The authors conducted in vitro assays in U87 cells obtaining a decrease in the cell viability when cultures were treated with 10 μM free atorvastatin for initial 24 h. The results improved when atovastatina was in EXO as the cell viability decreased and continued until 72 h. Annexin and assays related to

activation of apoptosis mechanisms, such as TUNEL assay and cell cycle biomarkers, indicated that EXO with atorvastatin (10 μL) displays the highest level of apoptosis at 30.37%. Hence, EXO loaded with atorvastatin are potential lipid-based nano-vehicles for further in vivo assays (Nooshabadi et al. 2020).

The recent investigations regarding nonspherical, HLD and exocellular vesicles point out new opportunities to find alternative GBM treatments. It is expected more data in the following years.

Current Challenges on the Design and Evaluation of Lipid-Based Nanoparticles

Effective drug delivery in the CNS is a pending task for biomedicine. Although several approaches have been studied, nanotechnology is the most promising avenue in the drug control released. Current medical treatments for GBM offer limited drug effectiveness and various adverse effects due to the brain tumors' complex physiology.

In this review, we have described recent information in the search for lipid-based nanomaterials capable to deliver anti-glioma drugs. To date, the potential use of liposomes, solid lipid nanoparticles, immunoliposomes, lipid–polymer hybrid nanoparticles, high-density lipoproteins like structures, nonspherical lipid-based nanoparticles and extracellular vesicles in GBM novel treatments demonstrate promising results in preclinical evaluations. Overall, lipid-based nanoparticles are stable formulation, for which surface modification, such as cationic coverage and/or molecules to target active endocytosis, could enhance the cellular uptake by BBB cells. Moreover, the amphipathic property of lipid molecules assesses a high encapsulation efficiency of hydrophobic drugs, maintaining interaction, and biocompatibility with physiological environments. Interestingly, new approaches with lipidic nanostructures found naturally in living organisms and nonspherical nanoparticles have shown their potential to overcome nano-vehicles challenges.

The information reported in the literature contributes to the understanding of the GBM physiology and physicochemical properties of the lipid-based nanoparticles and to developing more complex nanoparticles to target GBM cells with no toxic effect on healthy tissues. In following years, clinical evaluations of lipid-based nanoparticles loaded with antitumoral drugs for the CNS will decide their application in GBM.

On the other hand, the commercial production of nanomaterials requires the solution of deficiencies in the large-scale production, including purification (relevant for exosomes), and storage of the novel formulations.

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Author contribution GNT had the main idea for this article, searched and compiled part of the information, and revised the complete work. BOB performed the literature search and summarized information. CG contributed to the critical revision of concepts and perspectives in this work.

Declarations

Conflict of interest The authors declare no conflict of interest.

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