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REVIEW

Getting into the brain: liposome-based strategies for effective drug delivery across the blood–brain barrier

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Abstract: This review summarizes articles that have been reported in literature on liposome-based strategies for effective drug delivery across the blood–brain barrier. Due to their unique physicochemical characteristics, liposomes have been widely investigated for their application in drug delivery and in vivo bioimaging for the treatment and/or diagnosis of neurological diseases, such as Alzheimer's, Parkinson's, stroke, and glioma. Several strategies have been used to deliver drug and/or imaging agents to the brain. Covalent ligation of such macromolecules as peptides, antibodies, and RNA aptamers is an effective method for receptor-targeting liposomes, which allows their blood–brain barrier penetration and/or the delivery of their therapeutic molecule specifically to the disease site. Additionally, methods have been employed for the development of liposomes that can respond to external stimuli. It can be concluded that the development of liposomes for brain delivery is still in its infancy, although these systems have the potential to revolutionize the ways in which medicine is administered.

Keywords: Alzheimer, Parkinson, stroke, cerebral ischemia, glioma, liposomes, blood–brain barrier

Introduction

In the 1880s, Paul Ehrlich intravenously injected dyes (eg, trypan) into animals, and observed that the dyes were able to stain all organs except for the brain. He concluded that the brain had a lower affinity to the dye when compared to other organs.[1] In 1913, Edwin Goldmann, a student of Ehrlich, did the opposite, and injected the very same dyes directly to the cerebrospinal fluid of animal brains. He found that in this case, the dyes readily stained the brain and not the other organs.[2] These experiments clearly demonstrated the existence of a separation between the blood and the brain. However, in 1898, Max Lewandowsky was the first to postulate the existence of a specialized barrier at the level of cerebral vessels: the blood–brain barrier (BBB), after he and his colleagues had carried out some experiments to demonstrate that some drugs were neurotoxic when injected directly into the brain and not into the vascular system.[3] It was just in the late 1960s that Reese and Karnovsky visualized the fact that the barrier was localized to the endothelium by electron-microscopy studies.[4]

The BBB is composed of polarized endothelial cells connected by tight junctions of the cerebral capillary endothelium and a variety of transporters (Figure 1), which are responsible for its extremely low permeability, limiting the delivery of drugs to the central nervous system (CNS).[5,6] BBB functionality is dynamically regulated by an ensemble of different cell types, such as astrocytes, pericytes, and neurons (Figure 1A).[7–9]

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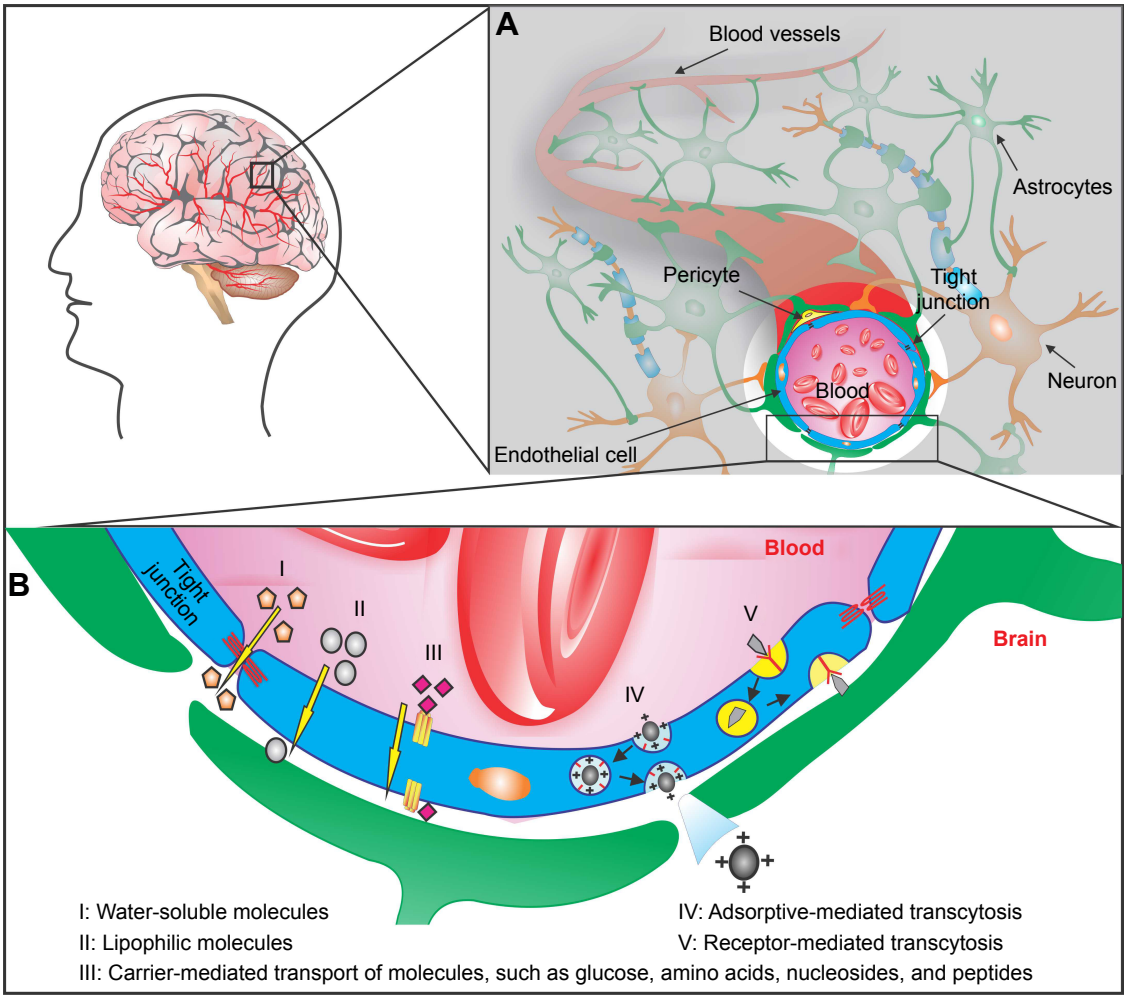


Figure 1 Pathways for crossing the blood–brain barrier (BBB).
Notes: The BBB is located at the walls of the blood vessels that supply the central nervous system, including the brain. (**A**) Cross-section of a cerebral capillary, showing the structure of the BBB. The barrier is composed of a network of astrocytes, pericytes, neurons, and endothelial cells that form the tight junctions. (**B**) Different mechanisms for drug delivery across the BBB: water-soluble molecules penetrate the BBB through the tight junctions (I); lipid-soluble molecules are able to diffuse across the endothelial cells passively (II); carrier-mediated transport machineries are responsible for transporting peptides and small molecules (III); cationic drug increases its uptake by adsorptive-mediated transcytosis or endocytosis (IV); larger molecules are transported through receptor-mediated transcytosis (V).

Endothelial cells are surrounded by a basal lamina, which is generally rich in laminin, fibronectin, type IV collagen, and heparin sulfate,[5,7–9] which may represent an interesting targeting for drug transport and provides a negatively charged interface.[10,11]

Aimed at the development of more efficient therapies for neurological disorders, extensive research is being done into the molecular and cell biology of many of these disorders. To date, human genetic mutations and defective cell-signaling pathways linked to a disease have been identified, and may contribute to the development of mechanism-based therapies and biomarkers for affected patients at early stages in the disease.[12,13] Moreover, pharmaceutical companies have spent billions of dollars in the hope that their scientists could develop drugs to defeat the brain disorders, eg, a drug that helps brain-cell growth, repairs damage, or slows down tumor progress, something that is not available now. However, obstacles to effective therapy delivery remain, and one of the most notable obstacles for drugs to penetrate the brain effectively is the BBB.[14,15]

How to circumvent the blood–brain barrier?

Based on better knowledge of BBB biology, several different strategies for delivering molecules across the barrier have been developed for treating CNS diseases, and can be broadly classified as invasive, pharmacological, and physiological approaches.[15–19] The invasive method is based on direct delivery of drugs into the brain tissue through varying techniques, such as the use of polymers or microchip systems,

stereotactically guided drug insertion through a catheter, and transient disruption of the BBB. However, these approaches are invasive, leading to risks of infection, damage to brain tissue, and toxicity. Furthermore, invasive approaches are costly and require hospitalization.[20–22]

The pharmacological method for crossing the BBB is based on modifying, through medicinal chemistry, a drug molecule to enable BBB permeability and making it insusceptible to drug-efflux pumps, such as P-glycoprotein (PgP).[17] One early strategy was based on the development of highly lipophilic and small drugs, allowing them to diffuse successfully through the brain's endothelial cells (Figure 1B). Unfortunately, synthesizing drugs that fulfill this condition eliminate a vast number of potentially useful polar molecules that could be used to treat CNS disorders. A second possibility is to use small water-soluble drugs to facilitate traversal of the BBB by the paracellular hydrophilic diffusion pathway (Figure 1B), though the majority of these molecules are just able to penetrate the interendothelial space of the cerebral vasculature up to the tight junctions, and not beyond. Moreover, modifications to drug structure often result in loss of the drug's biological activity.[23]

Among all the approaches employed in drug delivery to the brain tissues, the physiological method is the most advantageous, as it takes advantage of the transcytosis capacity of specific transporting receptors expressed at the BBB surface in order to penetrate the barrier (Figure 1B). For example, the occurrence of low-density lipoprotein receptor-related protein on the BBB is of critical importance for therapeutic proteins or peptides to glial cells or neurons across the whole CNS.[24–26] Another method consists in the use of receptor-mediated endocytosis by conjugation of drug molecules to ligands, such as antibodies and peptides, against receptors that are expressed on the surface of endothelial cells of the barrier,[6] allowing the drug to be transported into the brain (Figure 1B). In addition, cationic compounds are able to bind to the negatively charged plasma membrane of the endothelial cells by electrostatic interactions.[10,11] Therefore, the cationic substance crosses the BBB by adsorption-mediated transcytosis or endocytosis (Figure 1B). However, a low rate of drug dissociation from the ligands, nonspecific drug–receptor interactions, and the limited concentration of cationic substances in the brain are disadvantages for this kind of approach.

Undoubtedly, all three of these approaches have strong disadvantages that limit the successful treatment of neurological diseases. In response to this insufficiency in methods to transport therapeutic drugs across the BBB, aggressive research efforts into the use of nanotechnology to deliver drugs effectively across the BBB without altering their effect is being done. For this purpose, a broad range of nanoparticles with different sizes, architectures, and surface properties have been engineered for brain drug delivery.[27,28] These include liposomes,[29,30] polymeric nanoparticles,[31,32] carbon nanotubes,[33,34] nanofibers,[35,36] dendrimers,[37,38] micelles,[39] inorganic nanoparticles made of iron oxide,[40] and gold nanoparticles.[41] Unfortunately, it is beyond the scope of this article to review potential advantages – or disadvantages – of each of these nanocarriers in the imaging and/or therapy of the brain. For a more detailed overview of nanotechnology-based systems on drug delivery to the CNS, we refer the reader to Vlieghe and Khrestchatisky.[27] Here we focus on the one of most promising approaches aimed at improving brain drug targeting and delivery: liposomes and molecules that can selectively target brain tissues. In fact, liposomes are at present the nanoparticle type with the most studies that have been published for delivery to the brain, representing in this way the most advanced material and thus with the highest potential for clinical applications.

Why use liposomes for treating neurological disorders?

Common diseases of the CNS, such as neurodegeneration, multiple sclerosis, stroke, and brain tumors, represent a huge medical need. According to a World Health Organization report, about 1.5 billion people globally are suffering from neurological diseases.[42] The prevalence of neurological disorders is expected to have a significant increase in the next decade, as the aging population is highly increasing and living longer. Drug therapies to the brain have been particularly inefficient, especially due to the BBB, as discussed earlier. It would be thus be desirable to gain a better understanding of the molecular mechanism of the disease and the development of improved diagnostic devices and treatments. In this way, liposomes have emerged as promising carriers for CNS delivery.

Liposomes are roughly nano- or microsize vesicles consisting of one or more lipid bilayers surrounding an aqueous compartment. The potential use of these vesicles as a carrier system for therapeutically active compounds was recognized soon after its discovery in the early 1960s. In recent years, liposomes have been explored as carriers of therapeutic drugs, imaging agents, and genes, in particular for treatment and/or diagnosis of neurological diseases.[29,43–45] Due to their unique physicochemical characteristics, liposomes are able to incorporate hydrophilic, lipophilic, and

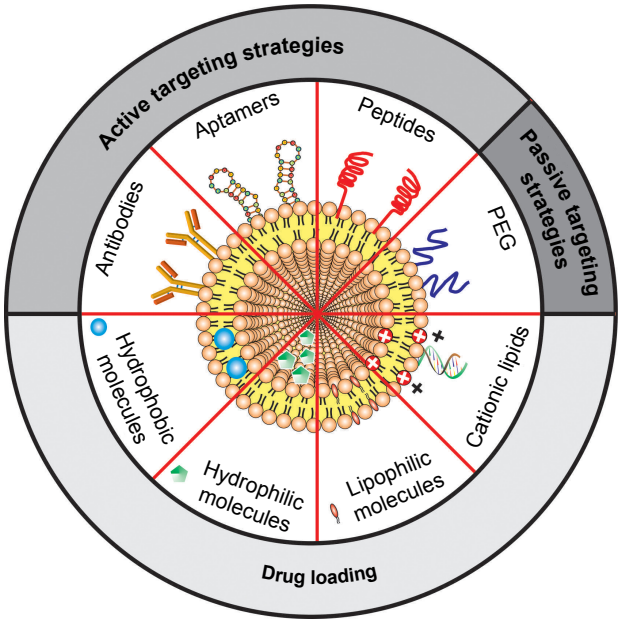


Figure 2 Schematic representation of the main liposomal drugs and targeting agents that improve liposome affinity and selectivity for brain delivery.
Abbreviation: PEG, polyethylene glycol.

hydrophobic therapeutic agents. Hydrophilic compounds may either be entrapped into the aqueous core of the liposomes or be located at the interface between the lipid bilayer and the external water phase. Lipophilic or hydrophobic drugs are generally entrapped almost completely in the hydrophobic core of the lipid bilayers of the liposomes. In addition, the use of cationic lipids allows the adsorption of polyanions, such as DNA and RNA (Figure 2). They also have the advantage of presenting good biocompatibility and biodegradability, low toxicity, drug-targeted delivery, and controlled drug release.[46,47] In order to improve blood circulation and brain-specific delivery, the liposome surface can be further modified by the inclusion of macromolecules, such as polymers, polysaccharides, peptides, antibodies, or aptamers (Figure 2). Unfortunately, efficient brain-specific drug delivery by liposomes is not in clinical practice. However, several liposomal drugs are either approved for clinical use or in clinical trial studies (Table 1).[48–59]

Optimizing the ideal liposome for crossing the BBB has important implications for the treatment of neurological diseases. Different liposomal formulations and strategies have been developed for enhancing drug delivery across the BBB. The following examples illustrate current strategies using liposomes as brain vectors (Table 2).[60–69] Cationic liposomes are successfully used as carriers for the delivery of therapeutic drugs and genes.[70–72] Several studies have shown that these cationic nanocarriers are more efficient vehicles for drug delivery to the brain than conventional, neutral, or anionic liposomes, possibly due to the electrostatic interactions between the cationic liposomes and the negatively charged cell membranes, enhancing nanoparticle uptake by adsorptive-mediated endocytosis.[60–62] But there is a major drawback to the use of cationic nanocarriers for brain delivery: due to nonspecific uptake by peripheral tissues and their binding to serum proteins that attenuates their surface charge, large amounts of these nanocarriers will be required to reach therapeutic efficacy, and those carriers are potentially cytotoxic. Therefore, there is a need for the development of liposomes that efficiently target diseased areas in the brain.

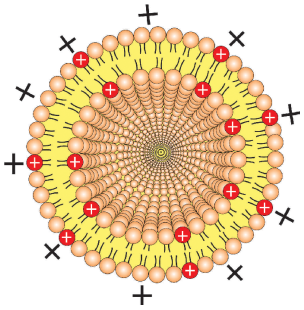
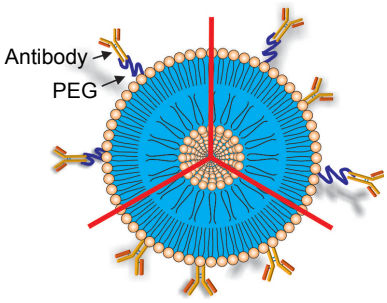
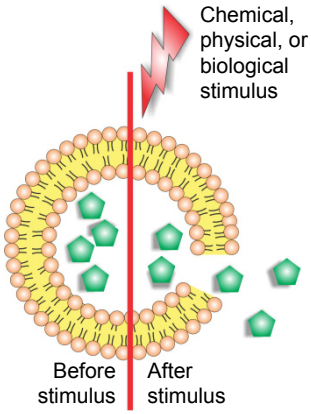
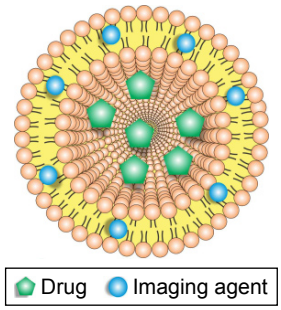
Surface-functionalization methodologies improve, at least in part, the pharmacokinetics and biodistribution of liposomes into the brain. For example, the addition of polyethylene glycol (PEG) or polysaccharides forms a protective layer over the surface of liposomes and protects the vehicle from the binding of plasma proteins, preventing the opsonization process and subsequent clearance of liposomes. Even though the PEGylation of liposomes prolongs their circulation time in the body, it does allow liposomes to cross the BBB. Therefore, their functionalization with biologically active ligands, such as peptides, antibodies, aptamers, and others, which specifically bind to receptors that are expressed

Table 1 Liposome-based drugs on market or in clinical trials for brain-targeted drug delivery

Commercial name	Compound	Lipid composition	Indications	Trial phase	References
AmBisome	Amphotericin B	HSPC, DSPG, and cholesterol	Cryptococcal meningitis	NA	48, 49
Abelcet®	Amphotericin B	DMPC and DMPG	Cryptococcal meningitis	NA	48, 49
DaunoXome®	Daunorubicin	DSPC and cholesterol	Pediatric brain tumors	I	50
Depocyt®	Cytarabine	Cholesterol, triolein, DOPC, and DPPG	Lymphomatous meningitis	NA	51
Doxil®/Caelyx®[a]	Doxorubicin	HSPC, cholesterol, and DSPE-$PEG_{2,000}$	Glioblastoma multiforme	II	52–55
			Pediatric brain tumors	II	56, 57
Myocet®	Doxorubicin	EPC and cholesterol	Glioblastoma multiforme	II	58

Note: [a]PEGylated liposomal doxorubicin is known as Doxil® in the US and Caelyx® in Europe.
Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DOPC, dioleoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; DSPC, distearoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; DSPG, distearoylphosphatidylglycerol; EPC, egg phosphatidylcholine; HSPC, hydrogenated soy phosphatidylcholine; NA, not applicable; PEG, polyethylene glycol.

Table 2 Means by which liposomes can penetrate the BBB

Strategies to permeate the BBB	Short description	References
Cationization of the vector 	The use of cationic liposomes is an interesting strategy, due to electrostatic interaction between their positive charges and the polyanions present at the BBB, resulting in adsorptive-mediated endocytosis.	60–62
Targeting ligand 	To increase liposomal drug accumulation into the brain, the use of ligand-targeted liposomes toward the receptors expressed on brain endothelial cells has been suggested, resulting in receptor-mediated transcytosis. One or more targeting ligands, such as antibodies and aptamers, can be covalently bound over the liposome surface or to the ends of the PEG chains.	44, 63–65
Triggered drug release 	Strategies developed for trigged drug release of liposome contents in response to specific external stimuli, such as variations in magnetic field, temperature, ultrasound intensity, light or electric pulses, and others.	66–68
Theranostic 	Liposomes are a very well-known carrier for drugs, but they can also incorporate a noninvasive contrast agent. This multifunctional theranostic liposomal drug-delivery system has advantages in diagnosis, real-time monitoring of disease treatment, and pharmacokinetics of liposomes.	30, 68, 69

Abbreviations: BBB, blood–brain barrier; PEG, polyethylene glycol.

on the surface of the brain endothelial cells, facilitates their binding and transport across the BBB.[73,74]

Although actively targeted brain drug delivery has improved the crossing of nanoparticles into the brain, additional properties can be included in liposomal systems to enhance the delivery of drugs at the targeted site in response to specific stimuli, such as variations in temperature, magnetic field, ultrasound intensity, or changes in pH. For example, recent reports introduced the concept of magnetic liposomes as a targeting moiety for delivering of therapeutic molecules

across the BBB. In one example, one or more drug molecules could be reversibly bound to the surface of iron oxide nanoparticles, and when encapsulated within the core of liposomes, bypassed an established in vitro model of the BBB by action of an external magnetic field.[67] Furthermore, it has been shown that magnetic liposomes can also be taken up into human monocytes, followed by the entry of nonmagnetic monocytes into the brain.[67] Although this approach has not been largely explored for brain delivery, this may become a good strategy for effective drug delivery by stimuli-responsive liposomes.

Furthermore, multifunctional liposomes can be engineered into a single structure, providing a powerful approach to improve disease-specific detection, treatment, and follow-up monitoring.[30] The term "theranostic" is used for nanoparticles that incorporate both therapeutic and diagnostic agents onto the same system.[75] One example of theranostic agent for brain delivery was described by Wen et al,[76] using quantum dots and apomorphine liposomally encapsulated for both brain therapy and imaging. The results showed that theranostic liposomes were transported across the BBB, providing a new and exciting strategy for brain-cancer imaging and therapy.[76]

It is worth mentioning that various routes of administration have been tested to access the brain for therapeutic purposes. For the delivery of liposomes to the CNS, intravenous injection seems to be the preferred route. The possibility of choosing between alternative routes of administration (oral, ocular, or mucosal) has been largely explored for bypassing the BBB, but it is beyond the scope of this article. For example, intranasal administration provides a practical and noninvasive approach to deliver drugs to the brain, allowing in this way an increase in the amount of drugs delivered across the barrier.[77–79] It was shown that a liposomal formulation of rivastigmine was able to prevent degradation of the drug in the nasal cavity and to carry it through the mucosal barriers.[80] Furthermore, the ability of cationic liposomes to delivering proteins to the brain via the intranasal route has also been demonstrated.[81]

In this review, a search of the literature was undertaken to investigate whether the use of liposomes offered any additional benefit than the therapeutic drug alone to treat most significant neurological diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, stroke, and brain cancer, and discuss its advantages and limitations. As a vast majority of CNS drugs have limited brain uptake, they may benefit from the use of liposomes as a drug-delivery vehicle into the brain. Moreover, liposomes have been widely explored as drug-delivery carriers to increase uptake of such drugs into the CNS. Therefore, there appears to be an obvious need for establishing CNS-penetrant and specific therapeutics to overcome the BBB and to do this in a controlled manner.

Materials and methods

Search strategy

A PubMed and Web of Science search was conducted to identify all known published articles on liposomes in drug development focused on the treatment of neurological disorders up to May 2016.

Study selection

Initially, articles were identified using a combination of the following keywords: 1) "liposomes" and "Alzheimer"; 2) "liposomes" and "Parkinson"; 3) "liposomes" and "Huntington"; 4) "liposomes" and "stroke" or "cerebral ischemia"; and 5) "liposomes" and "glioma". Reviews, patents, editorial materials, book chapters, conference publications, and articles not published in English were excluded from the literature search. Based on titles/abstracts, only studies that described in vivo experiments were selected for review. The final decision to include/exclude studies was based on full copies of articles.

Data extraction

In vivo studies with liposomes have been performed in most species, including mice, rats, dogs, monkeys, and humans. As in vivo study interpretation of results deserves attention, especially because of the biological differences between species, this was the parameter used to group the studies. Also, the following parameters of the liposome formulation were compared: 1) route of administration, 2) time points, 3) liposome composition, 4) ligands, 5) drug or imaging agent, and 6) particle size. Lately, biological outcome into the CNS has also been reported.

Results and discussion

Neurodegenerative disorders

AD, PD, and Huntington's disease were grouped together in this topic, because a growing number of studies indicates that these disorders share in common some features, such as the accumulation of intracellular or extracellular protein aggregates, selective degeneration of neurons, inclusion-body formation, and inflammation in particular brain regions.[82] However, the search for reports on the use of liposomes for delivery of active or imaging compounds against neurological diseases was done individually. A flowchart of the literature search is shown in Figure 3. An initial search yielded a total

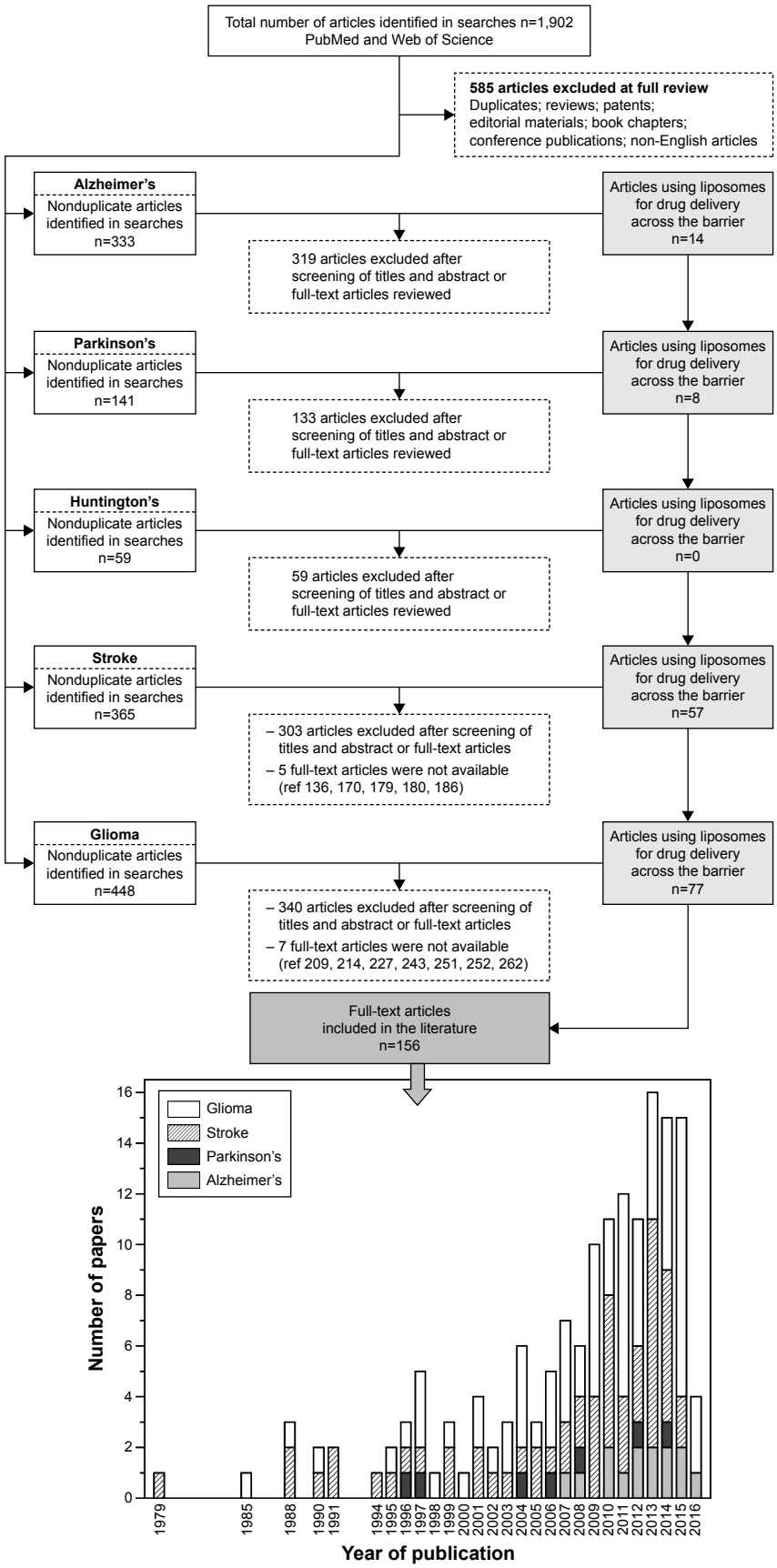


Figure 3 Flow diagram of studies that were identified based on the search terms described in the body of this article.
Abbreviation: ref, reference.

of 319 articles for AD, 141 articles for PD, and 59 articles for Huntington's, after excluding duplicate articles found in the PubMed and Web of Science databases. For AD, 26 full-text article reviews were performed, and 12 studies were included for their fulfillment of inclusion criteria (Figure 3, Table 3).[80,83–95] For PD, 19 full-text articles were reviewed, and eight articles had all the requisites to be considered here (Figure 3, Table 3).[96–103] Unfortunately, for Huntington's disease, just one full article was analyzed and this article did not show any outcome of interest for this disease, and for this reason was not included here (Figure 3).

Liposomes in the treatment of Alzheimer's disease

AD is a progressive and irreversible disease of the brain, affecting mainly people aged over 65 years. The neuropathogenesis of AD is a critical unsolved question. Progressive production and accumulation of insoluble protein aggregates, such as neurofibrillary tangles of hyperphosphorylated tau and amyloid-β (Aβ) plaques are thought to underlie the neuropathology of AD, leading to brain atrophy and neurodegeneration.[104] In addition, some studies have also suggested that deficits in cholinergic neurotransmitter systems and increased levels of free radicals or proinflammatory cytokines might be involved in AD neuropathogenesis.[105–108] More recently, a new potential cause for AD has been found in the behavior of certain immune cells that normally protect the brain instead beginning to consume a vital nutrient: the amino acid arginine.[109] This new discovery has implications not only in a new potential cause of the disease but also as a new strategy for targeting disease.

To date, the US Food and Drug Administration (FDA) has approved three acetylcholinesterase inhibitors – rivastigmine, galantamine, and donepezil – for the treatment of AD, which lead to an increase in central cholinergic action in the brain areas affected by the disease.[110] However, the administration of these inhibitors is associated with some severe side effects. It would thus be desirable to develop new formulations to avoid these side effects, and all studies proved that the use of liposomes was a good strategy in the treatment of AD.[80,86,90–92,111] Intranasal delivery of rivastigmine or galantamine liposomes has been shown to be a viable and effective route to improve drug bioavailability for brain drug targeting.[80,90,92] Intranasal delivery was also used as a successful approach for delivery of liposomes containing quercetin, which has antioxidant properties. As oxidative stress plays a very important role in the neuropathogenesis of AD, the use of quercetin liposomes has been shown to decrease neuronal oxidative stress.[93–95]

Moreover, there have been several studies exploring different strategies to block the effects of Aβ and tau proteins that constitute major hallmarks of AD.[84–87,91] Once encapsulated into liposomes, the H102 peptide, a β-sheet breaker, was able to block the early steps of aggregation and misfolding of the soluble Aβ, improving the spatial memory impairment of AD in rats.[112] α-Mangostin is a polyphenolic xanthone that exhibits pharmacological effects, such as anti-inflammation, antioxidant, and antitumor effects. When administered intravenously, α-mangostin liposomes have been shown to protect and improve the neurons against Aβ-oligomer toxicity in rats.[88]

Methoxy-XO4, a highly specific Aβ plaque ligand with the dual role of targeting moiety and fluorescent marker, has been conjugated to liposomes. When administered intravenously, these liposomes were able to cross the BBB in vivo and specifically bind to Aβ-plaque deposits, labeling vascular and parenchymal amyloid deposits in brain tissue.[87] For example, glutathione PEGylated liposomes demonstrated efficient encapsulation of an antiamyloid single-domain antibody fragment (V_HH-pa2H), increasing its transport from blood into the brain.[83] It has also been demonstrated that bifunctionalized liposomes decorated with phosphatidic acid and a modified ApoE-derived peptide are able to cross the BBB in vivo and destabilize Aβ aggregates, suggesting that this approach is a good option for AD treatment.[84,85]

Although the scope of this review is on liposome-strategies with the aim of facilitating BBB crossing, it is important to mention that other strategies have been developed for the use of liposomes for AD treatment.[89,113–116] Curcumin is a natural compound extract from the plant *Curcuma longa*, and has been reported to be a fluorescent molecule with high affinity for the Aβ peptide and able to reduce Aβ aggregation. In this way, intracranial injection of liposomes encapsulating curcumin efficiently labeled Aβ deposits in both human and mice tissues, proving to be an effective formulation for diagnosis and treatment of AD.[113] Also, intraperitoneal injection of liposomes containing phosphatidic acid or cardiolipin was able to reduce Aβ peptides in the plasma and shifted the equilibrium that exists between brain and blood Aβ peptides, slightly affecting the plaques in the brain.[89] Lastly, different liposome-based vaccines were developed and directed toward Aβ plaques[115,116] and tau.[114]

Liposomes in the treatment of Parkinson's disease

PD affects 4 million people worldwide.[117] The neuropathogenesis of PD is characterized by motor symptoms, such as tremor, rigidity, slowness of movement, difficulty with walking, and problems with gait. These motor symptoms result primarily

Table 3 Studies on liposome application in Alzheimer's and Parkinson's diseases

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Alzheimer's disease (AD)								
Mice	Intravenous injection	5–1,440 minutes	DMPC, cholesterol, and DSPE-$PEG_{2,000}$ EYPC, cholesterol, and DSPE-$PEG_{2,000}$	Glutathione	^{111}In-V_HH-pa2H antibody	110	Both ligand-targeted liposomes showed ability for specific brain delivery of single-domain antibody fragments beyond the BBB.	83
Mice	Intraperitoneal injection	3 weeks	SM and cholesterol	mApoE and phosphatidic acid	NI	121	Bifunctionalized liposomes decreased brain Aβ-plaque deposits, improving mouse impaired memory.	84
Mice	Intravenous injection	1, 4, or 24 hours	SM and cholesterol	mApoE and phosphatidic acid	^{14}C and ^{3}H	123	Bifunctionalized liposomes were also able to affect brain Aβ-oligomer aggregation/ disaggregation in vivo.	85
Mice	Intraperitoneal injection or oral administration	6, 8, and 24 hours	DPPC and cholesterol	NI	RIVA	3,400	Acetylcholinesterase inhibition was higher when RIVA liposomes were intraperitoneally injected in mice.	86
Mice	Intravenous injection	72 hours	DPPC, DSPE-$PEG_{2,000}$, and cholesterol	Methoxy-XO4	Methoxy-XO4	150	Liposomes might cross the BBB, since the nanostructures selectively bind to Aβ-plaque deposits to both parenchymal plates and cerebral amyloid angiopathy.	87
Rats	Intravenous injection	0.5, 2, 4, 8, 18, and 24 hours	DSPE-$PEG_{2,000}$	Tf	α-Mangostin	196	Targeted liposomes crossed the BBB and delivered α-mangostin into rat brain.	88
Rats	Intranasal administration	7 days	EPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	H102	112	Liposomes might have great potential for AD, since they ameliorate spatial memory impairment in rats.	112
Rats	Intranasal administration	15–240 minutes	EPC and cholesterol EPC, DSPE-$PEG_{2,000}$, and cholesterol	CPP	RIVA	166 179	The drug was efficiently delivered to the brain, especially by targeting liposomes.	90
Rats	Subcutaneous injection	3 months	PC, DC-Chol. and cholesterol	NI	RIVA	67 529	Despite liposome sizes, their therapeutic effect was evidenced by nearly preventing amyloid-plaque formation.	91
Rats	Intranasal administration	10 hours	PG	NI	GH	NI	Liposomes readily transported GH into brain tissues, suggesting some promise for this approach in brain drug targeting for AD treatment.	92
Rats	Intranasal administration	3 weeks	PC, EPC, and cholesterol	NI	QC	NI	The use of liposomes improved learning and memory deficits, possibly by reducing the levels of oxidative stress and acetylcholinesterase activity.	93
Rats	Intranasal administration	3 weeks	PC, EPC, and cholesterol	NI	QC	NI	Liposomes containing QC attenuated the death of neurons and cholinergic neuron cells in the hippocampus.	94

(Continued)

Table 3 (*Continued*)

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Rats	Intranasal administration	15–720 minutes	Soy lecithin and cholesterol	NI	RIVA	10,000	Liposome-encapsulated RIVA effectively delivered the drug into the brain.	80
Rats	Intranasal administration	4 weeks	PC, EPC, and cholesterol	NI	QC	NI	Liposomes increased anxiolytic activity and cognitive enhancement in the animals.	95
Parkinson's disease (PD)								
Mice	Intraperitoneal injection	5 days	HSPC, DSPE-$PEG_{2,000}$, and cholesterol	Chlorotoxin	Levodopa	107	Ligand-targeted liposomes increased the distribution of the drug in the brain, significantly attenuating serious behavioral disorders.	96
Mice	Intraperitoneal injection	7 days	EPC and cholesterol	NI	Levodopa	60	Liposome-encapsulated levodopa inhibited akinesia more effectively and increased muscle rigidity when compared to the free drug.	97
Mice	Intraperitoneal injection	14 days	EPC and cholesterol	NI	Levodopa	60	Treatment with levodopa incorporated into liposomes increased the quantity of dopamine in the mouse striatum.	98
Mice	Intraperitoneal injection	NI	EPC and cholesterol	NI	Levodopa	60	An extremely low dose of levodopa containing liposomes increased the rate of dopamine metabolism and altered the metabolism of signal phospholipids in the striatum.	99
Rats	Intranasal administration	3–4 weeks	DOPC, cholesterol, and stearylamine	NI	GDNF	149	Treatments with liposomes induced a neurotropic effect in the rat brain.	100
Rats	Intraperitoneal injection	8 weeks	DPPC, DODAB, and DSPE-$PEG_{2,000}$	OX26	GDNF plasmid	117	Sustained therapeutic effects are achieved in experimental PD with the formulation described here.	101
Rats	Intraperitoneal injection	430 minutes	DMPC and cholesterol	NI	Levodopa prodrugs	NI	Liposome formulations were demonstrated to be a good delivery and release system for the brain striatum of anti-PD agents.	102, 103

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; CPP, cell-penetrating peptide; DC-Chol, 3β-(*N*-[*N*′,*N*′-dimethylaminoethane]-carbamoyl)-cholesterol hydrochloride; DCP, dihexadecyl phosphate; DMPC, dimyristoylphosphatidylcholine; DODAB, dioctadecyldimethylammonium bromide; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; EPC, egg phosphatidylcholine; EYPC, egg-yolk phosphatidylcholine; GH, galantamine hydrobromide; HSPC, hydrogenated soy phosphatidylcholine; methoxy-XO4, 4,4′-[(2-methoxy-1,4-phenylene)di-(1*E*)-2,1-ethenediyl]bisphenol; mApoE, apolipoprotein E-derived peptide; NI, not informed; OX26, anti-transferrin receptor antibody; PC, phosphatidylcholine; PEG, polyethylene glycol; PG, phosphatidylglycerol; QC, quercetin; RIVA, rivastigmine; SM, *N*-palmitoyl-D-erythro-sphingosylphosphorylcholine; Tf, transferrin.

from the death of dopamine-generating neurons in an area of the brain called the substantia nigra, leading to the decreasing of dopamine levels.[118] Also, misfolding and intracellular aggregation of α-synuclein fibrils, also known as Lewy bodies, are pivotal to PD neuropathogenesis.[117,118] Mitochondrial dysfunction and oxidative stress may also be implicated in PD neurodegeneration.[118] However, the mechanisms underlying PD pathogenesis have not been fully elucidated.

Currently, available therapies for PD are essentially symptom-directed, having no effect on the disease progression. To date, the natural precursor of dopamine, levodopa or L-dopa, has been used in the clinic for several years.[119] However, levodopa cannot be administered alone, since it is converted to dopamine via peripheral dopamine-decarboxylase enzyme and causes such side effects as sleepiness, nausea, and dyskinesia.[120] A recently reported study overcame this problem, developing liposomes for site-specific delivery of levodopa into the CNS.[96] Chlorotoxin-modified stealth liposomes encapsulating levodopa proved to be an efficient nanocarrier, increasing levodopa concentration into the substantia nigra and striatum.[96] In the same way, it was also observed that intraperitoneal injection of liposome formulations encapsulating anti-PD drugs could improve the release of dopamine in the striatum region.[96–99,102,103]

Also, there are ongoing studies showing that GDNF is able to promote growth, regeneration, and survival of substantia nigra dopamine neurons, preventing the progression of PD if administered in the early stages of the disease.[121–124] Recently, a very promising study showed neurotrophic and neuroprotective effects of GDNF protein into the rat brain.[100] Although the liposomal preparation of GDNF offered no significant advantage of GDNF alone after intranasal injection, the liposomal formulation might have a protective effect on the protein, preventing it from degradation.[100] Another example that demonstrated the improvement of the treatment of the disease with GDNF is reported in Xia et al.[101] In this study, intravenous administration of OX26-targeted PEGylated liposomes was used as a nonviral gene-delivery system to deliver GDNF plasmid into the CNS. The expression of *GDNF* genes, under the influence of a rat tyrosine hydroxylase promoter, was observed in organs where the *TH* gene is highly expressed, including the substantia nigra, adrenal gland, and liver. Sustained therapeutic delivery was achieved at the neurons of the nigrostriatal tract in experimental PD.[101] Lastly, novel liposomal formulations have been characterized and efficacy in PD rats reported after intracerebral injection.[125–130] As the injection was at the local site of the disease and did not show any evidence of transposing the BBB, they were not considered in this review article.

Stroke or cerebral ischemia

Unlike the other neurological disorders described so far, stroke has high incidence, disability, and mortality rates in a modern society.[131] An ischemic stroke is characterized by the sudden reduction of brain blood flow due to obstruction of cerebral vasculature, damaging the neural tissue (ischemic penumbra zone).[132] Unfortunately, the treatment for stroke has its limitations, due to the poor ability to deliver therapeutic agents across the BBB. Therefore, efforts have been made to identify and develop drug-delivery systems to the brain. Liposomes are described as a possible valuable system to achieve better therapeutic effects in the treatment of stroke. The search for reports on the use of liposomes as drug-delivery nanocarriers for the treatment and/or diagnosis of stroke is shown in Figure 3. An initial search yielded a total of 365 articles after excluding duplicate articles found in the PubMed and Web of Science databases. In total, 62 articles were eligible.[133–194] Although all articles described new nanocarriers for the delivery of therapeutic molecules into the brain, only 57 studies are included in Table 4, because the full text of five articles[139,173,182,183,189] was not available to access.

The initial treatment for acute ischemic stroke consists in the administration of the FDA-approved tissue plasminogen activator (tPA), which is effective within the first 3 hours after the event occurs. This drug works on quickly dissolving the blood clot to restore brain perfusion.[195] However, its use is limited, due to elevated risk of cerebral hemorrhage, most probably due to the generation of free radicals posttreatment.[196] Because oxidative damage is an important aspect of the pathology of stroke and involved in vascular cell-membrane damage, researchers considered the possibility of developing a novel system to deliver tPA efficiently to the ischemic penumbra area in the brain. Actin is already known to be able to bind to antigens present at the surface of cells with damaged membranes. Therefore, actin-targeted liposomes for tPA delivery were developed, and this new drug-delivery system was in fact very efficient in delivering tPA within the brain, reducing hemorrhagic transformation in rats after focal embolic stroke.[173] Furthermore, the enzyme SOD was demonstrated to be an excellent biological natural free radical scavenger, and its ability as a neuroprotectant agent was tested. As free enzymes possess no BBB-penetration

Table 4 Studies on liposome application in stroke or cerebral ischemia

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Mice	Intraperitoneal injection	24 hours	Lecithin and cholesterol	NI	Chr	NI	Chr liposomes offered protection against cerebral ischemia–reperfusion injury by reducing either oxidative stress species or apoptosis.	133
Mice	Intravenous injection	6 hours and 1, 3, and 7 days	DSPC, DSPE-$PEG_{2,000}$, DSPE-$PEG_{2,000}$-Mal, and cholesterol	Anti-ICAM-1 antibody	Gd and Rhb	210	Direct in vivo MRI-based detection after stroke was achieved only with ICAM-1-targeted MPIO.	134
Mice	Via gavage	7 days	DOPE, CHEMS, and DSPE-$PEG_{2,000}$	NI	ATP, PBT, and suramin	150	Treatment with the liposomal formulation increased the number of surviving hippocampal CA1 neurons, possibly due to the increased antioxidant capacity of the mouse brain.	135
Mice	Intracarotid injection	24 hours	PC, DSPE-$PEG_{2,000}$, DSPE-$PEG_{2,000}$-Mal, and cholesterol	Anti-NR1-receptor antibody	SOD enzyme	160	Ligand-targeted liposomes offered protection against ischemia–reperfusion injury, reduced inflammatory markers, and improved behavior in vivo.	136
Mice	Intramuscular injection	1 or 7 days	PS and PC	NI	ATP	100	ATP liposomes offered protection for the retina against ischemia–reperfusion injury.	137
Mice	Intraperitoneal injection	24 hours	PS and PC	NI	ATP	100	The liposomal formulation reduced CNS damaged due to the increased survival of retinal neurons after ischemic injury.	138
Rats	Intra-arterial injection	7 days	PC, PE, and cholesterol	NI	Angiogenic peptides and ^{99m}Tc	128	Liposomes as imaging agents to the delivery of angiogenic peptides hold promise as an indicator of the effectiveness of angiogenic therapy in cerebral ischemia.	140
Rats	Intravenous injection	24 hours	Soy lecithin, DSPE-$PEG_{2,000}$, and cholesterol	TF peptide	ZL006	74	Ligand-targeted liposomes for the delivery of the ZL006 significantly ameliorated neurological deficit and reduced infarct volume induced by ischemia reperfusion.	141
Rats	Intravenous injection	7 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	Anti-HSP72 antibody	RhB, Gd, and citicoline	100	Treatment with liposomes containing citicoline reduced lesion volumes.	142
Rats	Intravenous injection	2 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	^{18}F	100	Accumulation of PEG liposomes in and around the ischemic region was observed.	143
Rats	Intravenous injection	24 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	230	The delivery of Hb by liposomes reduced cerebral infarct volume.	144
Rats	Intravenous injection	22 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	250	Liposome-encapsulated hemoglobin was protective in amygdala SAT in transient whole-brain ischemia.	145
Rats	Intravenous injection	24 hours	EPC, DOPC, and cholesterol	NI	NO	800	Ultrasound-controlled delivery of NO had potential for improving stroke treatment.	146
Rats	Intra-arterial infusion	24 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	200–250	Liposomal treatment reduced injury by decreasing the effect of MMP9, due to higher production of neutrophils.	147
Rats	Intravenous injection	1 hour	DSPC and cholesterol	NI	DiI	114.5	Liposomal drug delivery to an ischemic zone was observed, even when cerebral blood circulation was reduced.	148

Rats	Intravenous injection	3 or 24 hours	DPPC and DSPE-$PEG_{2,000}$	Tacrolimus	DiI	109	Reduction of cerebral cell death and improvement in motor function was observed after liposome administration.	149
Rats	NI	2, 3, or 5 hours	PC, DSPE-$PEG_{2,000}$, DPPC, and cholesterol	NI	Xe	NI	For maximal neuroprotection, administration dose of liposome-encapsulated Xe must be 7–14 mg/kg.	150
Rats	Intravenous injection	8 hours	DSPC and cholesterol	NI	ISA	200	ISA liposomes increased distribution of the drug into the brain and consequently enhanced therapeutic efficacy.	151
Rats	Intravenous injection	0, 1, 3, 6, or 24 hours	DSPC and DSPE-$PEG_{2,000}$	AEPO	DiI or ^{125}I	NI	This liposomal formulation was able to accumulate in the brain ischemic zone and be retained there for at least 24 hours after injection. Also, liposome treatment significantly reduced cerebral injury and ameliorated motor functions.	152
Rats	Intravenous injection	1, 2, 3, 5, and 7 days	DSPC and DSPE-$PEG_{2,000}$	AEPO	NI	NI	Liposomes significantly improved the neurological deficit. This might have been due to their neuroprotective properties.	153
Rats	Intravenous injection	2 hours and 7 days	DPPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	DXP	100	Treatment with this liposomal formulation significantly improved behavioral outcome in animals after stroke.	154
Rats	Intravenous or intraperitoneal injection	1, 3, and 7 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Citicoline	NI	Intravenously injected liposome-encapsulated citicoline caused a reduction in infarct sizes.	155
Rats	Intraperitoneal injection	13 days	Lecithin and cholesterol	NI	Vitamin E and luteolin	150	Liposome-encapsulated luteolin reduced brain injuries after postischemic treatment.	156
Rats	Intravenous injection	2 and 21 days	POPC, DDAB, and DSPE-$PEG_{2,000}$	Tf	VEGF	100	Tf-VEGF liposomes demonstrated neuroprotective properties and consequently vascular regeneration in the chronic stage of cerebral infarction.	157
Rats	Intracarotid injection	1 and 3 days	DPPC and DOPC	NI	Xe	NI	Xe liposomes promoted improved performance in all behavioral tests of animals.	158
Rats	Intravenous injection	30 minutes	PE, cholesterol, and dicetylphosphate	*p*-Aminophenyl-α-D-mannoside	CDP choline	60–90	CDP liposomes exhibited neuroprotection in both young and aged rats by inhibition of mitochondrial injury in moderate cerebral ischemia reperfusion.	159
Rats	Intravenous injection	7 days	NI	NI	Hb	230	Liposome-encapsulated Hb demonstrated neuroprotective effects against transient cerebral ischemia.	160
Rats	Intravenous injection	4 days	PC, cholesterol, and stearic acid	NI	Hb	NI	The treatment with Hb-liposomes suggested that depending on the cellular type of Hb, it is possible to alleviate ischemia in rats.	161
Rats	Intravenous injection	24 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	230	Liposome-encapsulated Hb promoted reduction in size of cerebral infarction in rats.	162
Rats	Intravenous injection	1 day	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	NI	Liposome-encapsulated Hb treatment significantly reduced edema formation into a large area of the brain.	163

(*Continued*)

Table 4 (*Continued*)

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Rats	Intravenous injection	60 minutes	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb and ^{18}F	211	For liposome-encapsulated Hb, was shown that the delivery oxygen happens even into the ischemic brain from the periphery toward the core of ischemia.	164
Rats	Intraventricular injection	3 hours	PS, PC, and cholesterol	NI	Antisense ODNs	NI	Successful application of liposome-mediated antisense ODNs delivery in vivo demonstrated knockdown of synaptotagmin I, attenuating ischemic brain damage in neonatal rats.	165
Rats	Intraperitoneal injection	24 hours	Lecithin	NI	QC	NI	QC-liposome treatment demonstrated neuroprotective effects after ischemia. Also, this study suggested that endogenous brain glutathione is critical in defense mechanisms against stroke.	166
Rats	Intravenous injection	24 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	230	Liposome-encapsulated Hb treatment provided a reduction in the area of infarction in the cortex, but not in the basal ganglia after ischemia.	167
Rats	Intravenous injection	30 minutes	PE, dicetylphosphate, and cholesterol	NI	QC	234	Liposome-encapsulated QC demonstrated in neuronal cells of young and old rats showed preservation of antioxidant enzymes and inhibition of cellular edema formation.	168
Rats	Intravenous injection	24 hours	DPPC, DPPS, GM_1 ganglioside, and cholesterol	NI	CDP choline	50	CDP choline-liposome treatment reduced cerebral infarction in rats after ischemia.	169
Rats	Intraventricular injection		DOPC and cholesterol	NI	NGF	NI	NGF-liposome treatment significantly reduced infarct volume after ischemia.	170
Rats	Transfusion	45 minutes	NI	NI	Hb	220	Hemodilution with Hb liposomes did not attenuate ischemia in rats.	171
Rats	Intracarotid injection	24 hours	EYPC and cholesterol	Antiactin antibody	tPA	200–250	tPA-liposome therapy reduced vascular membrane damage and hemorrhagic transformation after ischemia.	172
Rats	Intrathecal injection	48 hours	DOTAP	NI	Plasmid	NI	Transfections in vivo resulted in reduction in number of apoptotic cells in the infarct and penumbra area.	174
Rats	Intraperitoneal injection	20 minutes	EYPC and cholesterol	NI	L-Ascorbic acid or α-tocopherol	NI	L-Ascorbic acid- or α-tocopherol-containing liposome treatment prevented the production of diene in excess of 2 hours prior to cerebral ischemic insult.	175
Rats	Intrathecal injection	24 and 72 hours	HSPC and cholesterol	NI	Fasudil	110	Liposome-encapsulated fasudil treatment presented an improvement on neurological outcomes after 24 hours in vivo, due to a reduction in infarct area.	176
Rats	Intravenous injection	1 week	DPPC, DPPS, and cholesterol	NI	CDP choline	49	Liposome-encapsulated CDP-choline treatment provided a rapid recovery of the damaged membranes of neuronal cells, allowing an enhancement of brain functionality.	177
Rats	Intravenous injection	4 hours and 7 days	PC and cholesterol	NI	ALLNal	NI	Liposome-encapsulated ALLNal was able to inhibit calpain on neurotoxic damage, which offers an optional treatment for transient forebrain cerebral ischemia.	178

Rats	Intravenous injection	8 days	DPPC, DPPS, GM_1 ganglioside, and cholesterol	NI	CDP choline	50	CDP-choline liposomes were active against ischemic injury, improving survival rates of rats.	179
Rats	Intravenous injection	11 days	DPPC, DPPS, GM_1 ganglioside, and cholesterol	NI	CDP choline	50	CDP-choline liposomes improved the survival rate of rats subjected to ischemia and reperfusion.	180
Rats	Intravenous injection	6 days	DPPC, DPPS, GM_1 ganglioside, and cholesterol	NI	CDP choline	50	CDP-choline liposomes were able to protect the brain against damage induced by ischemia.	181
Rats	Intracarotid injection	60 minutes	PC and cholesterol	NI	ATP	NI	The opening of the BBB under certain hypoxic conditions allowed the liposomes to reach the cerebral parenchyma.	184
Rats	Intravenous injection	30 minutes	DPPC, cholesterol, and stearylamine	NI	SOD enzyme	NI	Liposomes were able to deliver a higher amount of SOD into the brain.	185
Rats	Intravenous injection	1, 2, 8, and 24 hours	PC, stearylamine, and cholesterol	NI	CuZn-SOD enzyme	NI	Liposome-encapsulated SOD treatment reduced infarct sizes for the anterior artery area, middle artery area, and posterior artery area.	186
Rats	Intracarotid injection	NI	PC and cholesterol	NI	ATP	NI	Liposome-encapsulated ATP increased the number of ischemic episodes tolerated by rats.	187, 188
Rats	Intravenous injection	2 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	^{18}F	99	PEG liposomes radiolabeled with ^{18}F accumulated in and around the ischemic zone into the brain.	190
Rats	Intracarotid injection	24 hours	DPPC, DSPE-$PEG_{2,000}$, DHSG, and cholesterol	NI	Hb	262–269	Treatment with Hb liposomes significantly decreased the cerebral infarct volume of the cortex, improving motor-dysfunction score.	191
Gerbil	Intraperitoneal injection	2 hours	NI	NI	CuZn SOD	NI	Liposome-entrapped SOD increased endogenous SOD activity in normal brain tissue, and when given at the end of ischemia counteracted both the ischemic reduction in endogenous SOD and the increased peroxidation of mitochondrial membranes.	192
Monkey	Intravenous injection	8 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Hb	230	Liposome-encapsulated Hb treatment persistently reduced damage beyond the acute phase of ischemia.	193, 194

Abbreviations: ^{18}F, fluorine-18; ^{99m}Tc, technetium-99m; ^{125}I, 125 iodine; AEPO, asialo-erythropoietin; ALLNal, *N*-acetyl-leucyl-leucyl-norleucine amide; BBB, blood–brain barrier; CDP, cytidine diphosphate; CHEMS, cholesteryl hemisuccinate; Chr, chrysophanol; CNS, central nervous system; CuZn, copper-zinc couple; DDAB, dimethyldioctadecylammonium bromide; DHSG, 1,5-*O*-dihexadecyl-*N*-succinyl-L-glutamate; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DOPC, dioleoylphosphatidylcholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DPPC, dipalmitoylphosphatidylcholine; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine; DSPC, distearoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; DXP, dexamethasone phosphate; EPC, egg phosphatidylcholine; EYPC, egg-yolk phosphatidylcholine; Gd, gadolinium; Hb, hemoglobin; HSPC, hydrogenated soy phosphatidylcholine; ICAM-1, intercellular adhesion molecule 1; ISA, isopropylidene–shikimic acid; Mal, maleimide; MMP-9, matrix metalloproteinase-9; MPIO, micron-sized iron oxide particles; MRI, magnetic resonance imaging; NGF, nerve growth factor; NI, not informed; NO, nitric oxide; ODNs, oligodeoxynucleotides; PBT, pentobarbital; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEG, polyethylene glycol; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PS, phosphatidylserine; QC, quercetin; RhB, rhodamine B; SOD, superoxide dismutase enzyme; Tf, transferin; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; Xe, xenon; ZL006, 5-(3, 5-dichloro-2-hydroxybenzylamino)-2-hydroxybenzoic acid.

capacity and degrade rapidly in the serum, SOD encapsulation in liposomes was needed. In vivo experiments demonstrated the efficacy of SOD-loading liposomes to get into the brain, providing significant protection against free radicals.[136,139,185,186,189,192]

Moreover, a wide debate is ongoing in the literature about new strategies to treat this disease. Neuroprotective and neuroreparative drugs (for example, citicoline) are under development.[197] Citicoline, an exogenous form of cytidine-5′-diphosphocholine, is a key intermediate in the biosynthesis of phosphatidylcholine, the primary neuronal membrane phospholipid that is degraded during brain ischemia to free radicals and fatty acids. In addition, citicoline restored Na^+/K^+ ATPase, inhibited activation of phospholipase A_2, and accelerated cerebral edema reabsorption.[198] Therefore, citicoline was considered a good candidate for molecular therapy for stroke, since its acts at several points on the ischemic pathway. Unfortunately, due to the drug's polar nature, crossing the BBB was far lower than desired. It has been observed that liposome-encapsulated citicoline increases its bioavailability within the brain parenchyma and improves its therapeutic efficacy for the treatment of stroke in animals.[142,155,159,169,177,179–182]

Besides damaged blood vessels in cerebral ischemia, another important process that occurs in stroke is neovascularization or angiogenesis. This is the physiological process of forming new blood vessels from the existing vasculature in healthy brain tissue into areas of ischemic penumbra.[199] The outermost cells in the zone of ischemic penumbra slightly restore their metabolism activities, since they have more blood supply when compared to cells more centrally located in the ischemic area. At this site, where blood supply is limited, there is rapid consumption of ATP, due to low levels of oxygen. Therefore, the delivery of exogenous ATP by liposomes could restore the metabolism of ischemic cells and reduce the area of injury.[135,137,138,183,184,187,188]

As mentioned earlier, cells within the infarcted area of ischemic tissue do not receive enough oxygen or nutrients to generate ATP. For this purpose, liposome-encapsulated hemoglobin (Hb) was engineered as a pharmacological agent able to deliver oxygen for the treatment of ischemic diseases. Several studies reported in the literature suggest the efficacy of Hb liposomes in the treatment of stroke by enhancing the biodistribution of Hb liposomes within the ischemic area in the brain.[144,145,147,160–164,167,171,191,193,194] In the same way, liposomes for the delivery of angiogenic peptides[140] and VEGF[157] to promote angiogenesis in ischemic tissue were developed, and both formulations effectively promoted vascular regeneration.[140,157]

Over the years, many liposomal formulations have been developed for the treatment of stroke. Moreover, when liposomes were associated with contrast agents, researchers observed that they quickly accumulated in the ischemic zone.[134,143,148,152,190] Some formulations have demonstrated their ability to improve in vivo activity of drugs, such as chrysophanol,[133] dexamethasone phosphate,[154] nerve growth factor,[170] Xe,[150,158] FK506,[149] isopropylidene–shikimic acid,[151] asialo-erythropoietin,[153] antisense oligonucleotides,[165] plasmids,[174] quercetin,[166,168] fasudil,[176] nitric oxide,[146] *N*-acetyl-leucyl-leucyl-norleucine amide,[178] and a combination of synergistic drugs.[156,175] Very recently, a promising uncoupling new drug – ZL006 (5-(3, 5-dichloro-2-hydroxybenzylamino)-2-hydroxybenzoic acid) – was developed for stroke treatment. Its mechanism of action is based on the selective blocking of the coupling of nitric oxide synthase, and it was also recognized as a neuroprotective drug. As with many other drugs, ZL006 possesses low to BBB-permeability capacity. However, its encapsulation in immunoliposomes targeted the BBB and significantly enhanced the delivery of ZL006 within the brain. A remarkable neuroprotective effect was also observed.[141]

Brain cancer – glioma

There are more than 100 different types of brain and CNS tumors. In this article, we focused our search on the term "glioma", which encompasses all tumors that arise from glial cells, including astrocytomas, oligodendrogliomas, ependymomas, and glioblastomas multiforme.[200] Glioblastoma multiforme is by far the most common and aggressive cancer form of the glial tumors. The current standard of care for this type of cancer includes surgery, followed by treatment with radiation and/or chemotherapeutic drugs. The current median overall survival of patients with glioblastoma multiforme is less than 15 months after surgery, followed by synergistic combination of radiotherapy and chemotherapy with the anticancer drug temozolomide.[201] Treatment for this type of cancer has its limitations, due to the poor ability to deliver therapeutic agents across the two unique barriers present in the brain: the BBB and the blood–brain tumor barrier (BBTB). Moreover, the low accumulation of nanoparticles into brain tumors by the enhanced permeability and retention (EPR) effect should be also taken into account.[202] Therefore, efforts have been made to identify and develop drug-delivery systems for the brain. Liposomes are described as a possible valuable system to achieve better therapeutic effects in the treatment of gliomas, since several targeting strategies have been reported showing ability to reach the brain and to target the tumor. The search for reports on the use of liposomes as

drug-delivery nanocarriers for the treatment and/or diagnosis of gliomas is shown in Figure 3. An initial search yielded a total of 448 articles after exclusion of duplicate articles found in the PubMed and Web of Science databases. In total, 80 articles were eligible.[60,64,65,203–283] Although all described new nanocarriers for the delivery of therapeutic molecules into the brain, only 77 studies are included in Table 5, because the full text of seven articles[212,217,230,246,254,255,265] was not available to access.

Design of liposomal drug-delivery systems for glioma diagnosis

One of the most challenging problems in therapy of gliomas is their detection in the earliest stages of development. Like many tumor types, early detection correlates with successful therapy. Therefore, both new diagnostic and therapeutic approaches need to be developed for glioma-imaging oncology. For this purpose, a huge variety of contrast agents have been encapsulated into liposomes. These new nanomaterials may provide new opportunities for biomedical imaging, due to their unique magnetic, optical, and/or chemical properties, leading to the creation of better contrast-enhancement agents and increasing the sensitivity of techniques clinically available for diagnosis of brain tumors.[284]

Modern imaging techniques, such as magnetic resonance imaging (MRI), optical imaging, ultrasound, and single-photon-emission computed tomography (SPECT), are rapidly emerging as noninvasive modalities for detection and follow-up posttreatment of gliomas.[285,286] MRI is the preferred approach for glioma imaging, since it provides high-spatial-resolution anatomic images of this tumor type.[287] Optical imaging applied to glioma therapy has the potential to localize and identify intrinsic brain tumors for removal during surgery.[288] Ultrasound, unlike MRI, defines tumor volume and provides intraoperative localization of tumor tissue, although its use is limited by the presence of the skull.[285] SPECT yields growth rate and gives information about the heterogeneity of gliomas, but provides low-spatial-resolution images.[289] Positron-emission tomography (PET) provides functional information, since this technique is highly sensitive for measurements of biological processes, such as cell proliferation, angiogenesis, and glucose consumption.[290]

Paramagnetic contrast agents are the most widely used agents to enhance the visibility of gliomas in MRI images. Gadolinium (Gd)-based compounds, such as Gd–diethylenetriaminepentaacetic acid, gadodiamide, and gadoteridol, are effective contrast agents, owing to their seven unpaired electrons. Although Gd-based compounds are able to cross the BBB, a key advantage of using liposomes as Gd carriers is preferential localization at the tumor site through the EPR effect. In this way, it was shown that Gd liposomes with prolonged blood-circulation time tend to accumulate in the intratumoral extravascular space after moving across the tumor's leaky vasculature.[208,254] Moreover, a recent advance was reported in the design of a pH-responsive Gd liposome that was able to release the imaging agent into a cerebral glioma rodent model, detecting with 0.2 pH precision the mildly acid tumor microenvironment.[208]

Methods for optical imaging of glioma are based on fluorescence. The lipid-binding fluorescent carbocyanine dyes DiD (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid, disodium salt), DiO (3,3′-dioctadecyloxacarbocyanine perchlorate), and DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylinocarbocyanine perchlorate) are widely used for imaging studies. The characteristics of aqueous-insolubility ease of aggregate formation and the fact that these dyes do not readily cross the BBB suggest that it would be desirable to develop a liposome-based system. In this way, the carbocyanine dyes have been encapsulated into liposomes with the ability to demarcate tumors.[60,256,269] The results of these studies suggest that those formulations, independent of the mode of administration, stained the tumor tissue and increased their bioavailability.[60,256,269] However, the use of these fluorescent probes has the disadvantage of requiring low-light conditions for the visualization of tumors in vivo, which is not useful in a surgical environment. It was recently reported that Evans blue liposomally encapsulated was able to demarcate visually the margins of invasive gliomas, which may not significantly change the surgical conditions for the resection of this type of tumor.[232]

Also, studies reported in Table 5 suggest that the use of MRI or optical imaging alone in the imaging of gliomas is not enough for their classification and grading, optimal treatment, and follow-up after treatment,[261,273] since each imaging technique is associated with individual advantages and limitations. Furthermore, it is generally observed that the presence of targeting ligands over the liposome surface improves the uptake of vesicles by target cancer cells and increases their retention time within tumors.[231,233,239,247,253] In gliomas, angiogenesis seems to be the preferable target area for diagnosis of this cancer. Angiogenesis, the formation of new vessels, is a key process for glioma survival and growth.[291] From the literature search, two molecules were identified for angiogenic cells: endoglin, also known as CD105, and the Ala-Pro-Arg-Pro-Gly peptide. MRI of endoglin-target liposomes was able to demonstrate tumor

Table 5 Studies on liposome application in glioma

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Mice	Intravenous injection	>50 days	PC, DSPE-$PEG_{2,000}$, and cholesterol	PTD_{HIVI} peptide	EPI plus celecoxib	108	Targeted liposomes were able to cross the BBB and accumulate in the tumor, leading to the destruction of glioma vasculogenic mimicry channels.	203
Mice	Intravenous injection	>50 days	SPC, DSPE-$PEG_{2,000}$, and cholesterol	Cell-penetrating peptides	PTX	100–120	Dual-targeted liposomes exhibited selective targeting and anticancer therapeutic effects.	204
Mice	Intravenous injection	>60 days	SPC, DSPE-$PEG_{2,000}$, and cholesterol	TR peptide	PTX	130	Ligand-targeted liposomes for the delivery of PTX showed an effective targeting ability to cancer stem cells, destroying the vasculogenic mimicry channels.	205
Mice	Intravenous injection	8 hours	DODAP, DSPC, C16 Cer-$PEG_{2,000}$, and cholesterol	CTX	miR-21	190	Ligand-targeted liposomes were able to efficiently deliver anti-miRNA oligonucleotides into the brain.	206
Mice	Intravenous injection	52 days	SPC, DSPE-$PEG_{2,000}$, and cholesterol	RGD peptide	PTX	107	Ligand-targeted liposomes for the delivery of PTX showed an effective targeting ability for brain-cancer stem cells.	207
Mice	Intravenous injection	60 minutes	DOPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Gd-DTPA	100	Liposome-encapsulated Gd-DTPA was able to release the contrast agent in response to a chance in the pH environment.	208
Mice	Intravenous injection	>77 days	NI	RGD peptide	DOX and iron oxide nanoparticles	90	Ligand-targeted liposomes were combined with iron oxide to produce a magnetic field-responsive liposome hybrid. These nanosystems presented enhanced targeting ability and facilitated site-specific drug delivery into the brain.	209
Mice	Intravenous injection	50 days	PC, DSPE-$PEG_{2,000}$, and cholesterol	WGA and TAM	DNR and quinacrine	104	Ligand-targeted liposomes for the delivery of DNR plus quinacrine exhibited evident capabilities in crossing the BBB, in killing glioma and glioblastoma stem cells, and in diminishing brain gliomas in mice.	210
Mice	Intravenous injection	7 days	SPC, DSPE-$PEG_{2,000}$, and cholesterol	RGD peptide and Tf	PTX	128	Ligand-targeted liposomes for the delivery of PTX were developed and presented high brain distribution.	211
Mice	Intravenous injection	4 hours	PC, DSPE-$PEG_{2,000}$, DSPE-$PEG_{1,000}$, and cholesterol	TAT-peptide and Tf	DOX	114	Ligand-targeted liposomes for the delivery of DOX possessed strong capability of synergistic targeted delivery of the chemotherapeutic drug for brain tumors.	213
Mice	Vascular and intratumoral injection by CED	>70 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Irinotecan	NI	Liposome-encapsulated irinotecan treatment demonstrated good antitumor activity on glioblastomas and a higher median survival time of tumor-bearing mice.	214
Mice	Intravenous injection	10, 14, and 20 days	DODAP, DSPC, C16 Cer-$PEG_{2,000}$, and cholesterol	CTX	asOs siRNA	178 144	Ligand-targeted liposomes for nucleic acids delivery demonstrated that CTX enhanced liposome internalization into glioma cells.	215
Mice	Intravenous injection	55 days	HSPC, DSPE-$PEG_{2,000}$, and cholesterol	RGERPPR peptide	DOX	90	Ligand-targeted liposomes for delivering of DOX showed an enhanced targeted therapeutic effect on glioblastomas.	216
Mice	Intravenous injection	30 minutes	DPPC, MSPC, and DSPE-$PEG_{2,000}$ EPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	DOX	121 123	Thermosensitive liposome-encapsulated DOX delivered DOX across the BBB and also improved median survival time of tumor-bearing mice.	218

Mice	Intravenous injection	>50 days	DPPC and DSPE-$PEG_{2,000}$	Angiopep-2 peptide	DOX	100	Ligand-targeted liposomes for the delivery of DOX demonstrated antitumor activity and prolonged median survival time of tumor-bearing mice.	219
Mice	Intravenous injection	70 days	SPC and DSPE-$PEG_{2,000}$	NI	Topotecan	100	Liposome-encapsulated topotecan delayed tumor growth and prolonged median survival time of tumor-bearing mice.	220
Mice	Intravenous injection	10 days	HSPC, DSPE-$PEG_{2,000}$, and cholesterol	CTX	DOX	100	Ligand-targeted liposomes demonstrated higher accumulation into tumors and antitumor activity.	221
Mice	Intravenous injection	4, 12, 24, and 48 hours	DOPC, DOPG, DOGS-NTA-Ni, DSPE-$PEG_{2,000}$, and cholesterol	Anti-EGFR antibody	BSH	100	Ligand-targeted liposomes were an effective tool for delivery of BSH to glioma cells into the brain.	222
Mice	Intraperitoneal injection	200 days	DPPC, DSPE-$PEG_{2,000}$, and cholesterol	IL-13 protein	DOX	100	Ligand-targeted liposomes for the delivery of DOX promoted a reduction in tumor size and prolonged median survival time on tumor-bearing mice.	223
Mice	Intravenous injection	6, 24, 48 and 72 hours	DPPC, DSPE-$PEG_{2,000}$, and cholesterol	Tf	^{10}B	100	Ligand-targeted liposomes delivered in a specific way a high concentration of BSH into the tumor tissue.	224
Mice	Intravenous injection	>60 days	NI	NI	U1snRNA/ ribozymes	NI	Delivery of U1snRNA/ribozymes by liposomes inhibited tumor growth and angiogenesis.	225
Rats	Intravenous injection + FUS	>80 days	HSPC, DOPE, CTAB, didodecyldimethylammonium bromide, and cholesterol	NI	DOX or QD	187 175	Combining the reversible opening of the by FUS and the ability of cationic liposomes to bind to glioma cells, it was possible to improve median survival of tumor-bearing rats.	226
Rats	Intravenous injection	9, 14, and 17 days	Liposomal doxorubicin	NI	Ultrasonic MB for delivery of lipo-DOX	100	FUS increased tumor drug delivery over time in glioma-tumor model.	228
Rats	Intravenous injection	24 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	^{188}Re	80	Liposome-encapsulated ^{188}Re prolonged median survival of tumor-bearing rats.	229
Rats	Intracarotid injection	2–5 minutes	DMPC, DOTAP, and cholesterol DMPC and cholesterol DMPG and cholesterol	NI NI NI	DiD DiD DiD	NI NI NI	The concentration of cationic liposomes was greater into the brain parenchyma compared to anionic and neutral liposomes. Cationic liposomes were also readily observed within glioma tissue after intra-arterial injection.	60
Rats	Intravenous injection	48 hours	DSPC, mPEG-DSPE, DSPE-$PEG_{2,000}$, and cholesterol	Anti-SAv and antiendoglin antibodies	Gd-DTPA	267	The two-step endoglin-targeted imaging using biotin–streptavidin interaction was demonstrated to induce intense enhancement of the tumor periphery, which implies that this advanced MR molecular contrast agent may be suitable for accurately delineating glioma tumor margins. Ligand-targeted imaging liposomes demonstrated enhancement of the tumor periphery, demarcating glioma tumor margins accurately.	231
Rats	Intravenous injection	4 weeks	DPPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Evans blue	173	Evans blue liposomes visually delineated invasive glioma margins.	232
Rats	Intravenous injection	48 hours	PC, DSPE-$PEG_{2,000}$, DDAB, and cholesterol	Anti-VEGF antibody	DiI	163	Ligand-targeted liposomes demonstrated specific accumulation of liposomes in glioma tumors.	233
Rats/ mice	Intravenous injection	46 days	PC, DSPE-$PEG_{2,000}$	MAN-$TPGS_{1,000}$, and DQA-$PEG_{2,000}$-DSPE	PTX and artemether	90	Ligand-targeted liposomes for the codelivery of PTX and artemether were able to deliver drugs across the BBB.	234

(Continued)

Table 5 (*Continued*)

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
Rats	Intravenous injection	>100 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Irinotecan	102–113	Liposome-encapsulated irinotecan prolonged median survival time in tumor-bearing mice.	235
Rats	Intravenous injection	18 days	PC, DSPE-PEG, Mal-$PEG_{2,000}$-DSPE, DC-Chol	Tf and CTX peptide	plasmid	120	Ligand-targeted liposomes for gene delivery were able to increase the transport of plasmid DNA and specifically delivery the gene for glioma cells into the brain.	236
Rats	Intracarotid injection	50 days	Lipoplatin or Lipoxal™	NI	Cis or oxaliplatin	NI	Tumor uptake was higher for Lipoxal than for the free drug oxaliplatin. Oppositely, lipoplatin led to lower tumor uptake compared with free cisplatin.	237
Rats	Intravenous injection	30 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	Tf and folate	DOX	180	Ligand-targeted liposomes for the delivery of DOX were able to deliver the drug across the BBB and were distributed mainly in brain gliomas.	238
Rats	Intravenous injection	24 hours	Lecithin, DSPE-$PEG_{2,000}$, and cholesterol	Anti-GFAP and anti-E2 extracellular loop of Cx43 antibodies	DiI or Gd-DTPA	140	Ligand-targeted liposomes demonstrated suitability as diagnostic agents to the peritumoral invasion zone of glioma.	239
Rats	CED administration	59 days	PC, CHEMS, and DSPE-$PEG_{2,000}$	NI	Cis	55	The formulation was highly neurotoxic after CED administration, and resulted in the death of animals after 24 hours.	240
Rats	Intravenous injection	>200 days	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	IB	160	Liposome-encapsulated IB treatment followed by liposomal DOX prolonged median survival of tumor-bearing rats.	241
Rats	CED administration	120	DSPC and cholesterol	NI	^{186}Re	NI	Prolonged survival of tumor-bearing rats was observed after brachytherapy with liposomally encapsulated ^{186}Re.	242
Rats	Intravenous injection	1, 2, and 3 weeks	Doxil®	NI	Ultrasonic MB for delivery of lipo-DOX	100	Posttreatment MRI revealed that the combination of FUS with liposomal DOX reduced tumor-growth rate.	243
Rats	Intravenous injection	>100 days	NI	Lactoferrin	DOX	208	Ligand-targeted liposomes for the delivery of DOX significantly prolonged median survival time of tumor-bearing rats.	244
Rats	Intravenous injection	72 hours	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	^{188}Re	80	This study showed that ^{188}Re-liposome was a good candidate for further development of a theranostic agent for treating glioma.	245
Rats	Intravenous injection	1 hour	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	Ala-Pro-Arg-Pro-Gly peptide	^{18}F and DiI	113	The smallest tumor among those tested, having a diameter of 1 mm, was successfully imaged by the liposomal ^{18}F.	247
Rats	Intravenous injection	>129 days	SPC and cholesterol	TAT peptide	DOX	105	Ligand-targeted liposomes for the delivery of DOX prolonged median survival of glioma-targeted rats.	248
Rats	Intravenous injection	16 days	EPC, DSPE-$PEG_{2,000}$, and cholesterol	Tf and TAM	EPI	110	Evident effect of targeting brain-tumor cells in vitro and extended median survival time in brain glioma-bearing rats.	249
Rats	Intravenous injection	14 days	EPC, DSPE-$PEG_{2,000}$, and cholesterol	MAN and Tf	DNR	122.8	Ligand-targeted liposomes for the delivery of DNR improved therapeutic efficacy for gliomas.	250
Rats	Intravenous injection	31 days	PC, DSPE-$PEG_{2,000}$, and cholesterol	TAM and WGA	Topetecan	100–110	Ligand-targeted liposomes for the delivery of topotecan prolonged median survival time of brain tumor-bearing rats.	251
Rats	CED administration	48 days	DSPC, DSPG, and cholesterol	NI	Topetecan and Gd	75–120	Liposomes for codelivery of topotecan and Gd were able to prolong median survival time of glioma-bearing rats.	252

Rats	Intravenous injection	30 hours	DSPE-S-S-PEG$_{5,000}$, DPPC, and cholesterol	Folate	NI	100	Ligand-targeted liposomes indicated that masking targeting ligands with cleavable phospholipid–PEG proved to be a good strategy for controlled exposure of targeting ligands to ensure that circulation times remained uncompromised.	65
Rats	Intravenous injection	5, 30, 60, and 120 minutes	EYPC, mPEG-DSPE, PDP-PEG-HEPE, and cholesterol	anti-CD105 antibody	Gd-DTPA	165.3	Ligand-targeted liposomes for tumor imaging detected early tumor angiogenesis on MR images.	253
Rats	CED administration	6 hours or 2 weeks	DSPC, DSPE-PEG$_{2,000}$, and cholesterol	NI	DiD	NI	Development of retroconvection-enhanced delivery method increased blood–brain transfer of macromolecules.	256
Rats	CED administration	>70 days	DSPC, DSPE-PEG$_{2,000}$, and cholesterol Doxil	NI	Irinotecan and DOX	95–110	Convection-enhanced delivery proved a good technique for delivery of liposomes to intracranial tumor-bearing rats.	257
Rats	Intravenous injection	42 days	DSPC, DSPE-PEG$_{2,000}$, and cholesterol	Folate	DOX	110–115	Ligand-targeted liposomes for the delivery of DOX prolonged the median survival time of tumor-bearing rats.	64
Rats	CED administration	>90 days	DSPC and cholesterol Doxil	NI	Topotecan and DOX	100–120	Convection-enhanced delivery seemed to be a good technique for delivery of liposomes to intracranial tumor-bearing rats.	258
Rats	CED administration	>100 days	DSPC, DSPE-PEG$_{2,000}$, and cholesterol	NI	Irinotecan	96–101	Convection-enhanced delivery was a good technique for the delivery of liposomes to intracranial tumor-bearing rats.	259
Rats	CED administration	NI	DSPC and cholesterol	NI	DiI and topotecan	112	Convection-enhanced delivery of liposomes enabled effective and continuous delivery of the chemotherapeutic drug in tumor-bearing rats.	260
Rats	CED administration	24 hours	DOPC and DSPE-PEG$_{2,000}$	NI	Gadodiamide and DiI	NI	Convection-enhanced delivery of liposomes allowed them to be spread into the tumor tissue. Greater penetration was observed for smaller liposomes and their coinfusion with mannitol.	261
Rats	CED administration	2 hours	DOPC and cholesterol	NI	DOX, Gd, and DiI	77	Theranostic liposomes allowed in vivo monitoring of therapeutic distribution of liposomes into brain tumor-bearing rats.	262
Rats	Intravenous injection	14 days	NI	Tf	siRNA	85	Cationic liposomes for siRNA transfection inhibited luciferase gene expression in brain gliomas up to 5 days after a single intravenous injection of nanoparticles.	263
Rats	Intravenous injection	24 hours	DPPC	NI	Photofrin	NI	Liposome-encapsulated Photofrin enhanced the PDT treatment of rat brain tumors.	264
Rats	Intraperitoneal injection	24 hours	DPPC	NI	Photofrin	NI	Liposome-encapsulated Photofrin enhanced the PDT treatment of rat brain tumors.	266
Rats	Intravenous injection	36 days	DSPC, DSPE-PEG$_{2,000}$, and cholesterol	NI	DOX	NI	Liposome-encapsulated DOX enhanced the therapeutic effect of the drug.	267
Rats	Intravenous injection and hyperthermia	60 days	DPPC and DSPC	NI	Cis	NI	The rats treated with CDDP liposomes + hyperthermia had the longest survival time, and the tumor CDDP level of this group was the highest when compared to the other groups. Histopathological examination showed that tumor cells were necrotized, but surrounding normal brain tissue remained	268

(Continued)

Table 5 (*Continued*)

Species	Administration	Time points	Liposome composition	Ligands	Drug/imaging agent	Particle size (nm)	CNS action	References
							undamaged. On the basis of these findings, we suggest that the combination of thermosensitive liposomes and localized hyperthermia may better focus antitumor drugs to the tumor, providing a significantly greater antitumor effect. Thermosensitive liposomes combined with localized hyperthermia provided higher antitumor activity, prolonging the median survival time of tumor-bearing rats.	
Rats	Intravenous injection	2 minutes	NI	NI	DiO	2,000	Lipid-coated microbubbles administered intravenously to rats bearing brain tumors specifically enhanced tumor visualization by ultrasound. Glioma tumors were able to internalize microbubbles.	269
Rats	Intracarotid injection	30 minutes	PC, DPPC, and cholesterol	NI	HRP	NI	These results indicate that liposomes can penetrate the blood–brain barrier. Liposome-encapsulated Cis were able to deliver drugs across the barrier.	270
Rats	Intravenous injection	43 days	DOTAP and DOPE	NI	Plasmid	NI	Liposomes for plasmid delivery inhibited rat glioma growth.	271
Dogs	CED administration	14 months	DSPC, DSPE-$PEG_{2,000}$, and cholesterol	NI	Irinotecan and Gd	93–108	This study provided a translational model system for convection-enhanced delivery in dogs.	272
Monkeys	CED administration	NI	DOPC, DSG-$PEG_{2,000}$, and cholesterol	NI	Gd or RhB	124	Theranostic liposomes in combination with CED allowed in vivo monitoring of therapeutic distribution of liposomes into brain tumor-bearing monkeys.	273
Humans	Intracranial injection	29 months	NI	NI	Plasmid	NI	Phase I clinical trial of *IFNB* gene therapy of glioma multiform via cationic liposomes demonstrated differentially expression of this and other genes of patients after gene delivery. A majority of the other genes were related to apoptosis, immune response, and angiogenesis.	274
Humans	CED administration	>25 months	Liposomal DNR	NI	DNR	NI	Progressive or recurrent high-grade gliomas are characterized by a very poor prognosis, and the relevance of second-line chemotherapy is still unassessed. Although it has been reported that liposomal anthracyclines and carboplatin show some activity in these patients, their association has never been investigated. We treated six children with recurrent high-grade glioma after surgery, radiotherapy, and chemotherapy, and one child with progressive teratoid/rhabdoid tumor with the combination of liposomal daunorubicin and carboplatin plus etoposide. Five of seven children showed a major response, and 29-month progression-free survival was 38%. The above-mentioned regimen was feasible, and children showed little and transient hematological toxicity.	275

							In our opinion, these results justify further investigation of this combination chemotherapy for recurrent or progressive malignant brain tumors in children. This study suggests the association of liposomal DNR, carboplatin, and etoposide as second-line chemotherapy in children with recurrent malignant brain tumors.	
Humans	Intracranial injection	6 months	NI	NI	IFNβ	NI	This study performed a pilot clinical trial by the transfer of *IFNB* gene therapy via cationic liposomes. The results demonstrated the feasibility and safety of this therapy for glioma treatment.	276
Humans	CED administration	42 days	NI	NI	SFVIL-12	90	A Phase I/II clinical trial of immunogenic therapy of recurrent glioblastoma multiforme with a liposome-encapsulated replication-incompetent Semliki Forest virus vector carrying the human *IL12*.	277
Humans	Intravenous injection	NI	Liposomal DNR	NI	DNR	NI	High concentration of DNR was detected in brain tumors after systemic administration of liposomal DNR.	278
Humans	Intravenous injection	165 weeks	Doxil	NI	DOX	NI	Long-term stabilization of glioblastoma multiforme observed in patients after treatment with liposomal DOX.	279
Humans	Intravenous injection	4 weeks	Doxil	NI	DOX and ^{99m}Tc-DTPA	NI	High concentration of DOX was detected in brain tumors in patients with glioblastoma multiforme and metastatic brain lesions after treatment with theranostic liposomes.	280
Humans	Intravenous administration	48 hours	DaunoXome® (liposomal DNR)	NI	DNR	NI	Liposome-encapsulated DNR presented good cytotoxicity toward human glioma tumors.	281
Humans	Intracranial injection	6 weeks	DPPC, DPPA, and cholesterol	NI	Bleomycin	NI	Although no clinically important side effects were observed, three of six patients showed progressive deterioration in their clinical condition.	282
Humans	Intracranial injection	24 hours	DPPC, DPPA, and cholesterol	NI	Bleomycin	NI	This study demonstrated the potential of liposomes in drug delivery in patients with brain cancer.	283

Abbreviations: ^{99m}Tc, technetium-99m; asOs, antisense oligonucleotides; BBB, blood–brain barrier; BSH, borocaptate sodium; CDPP, cis-diammino dichloroplatinum-II (cisplatin); CED, convection-enhanced delivery; Cer, ceramide; CHEMS, cholesteryl hemisuccinate; Cis, cisplatin; CNS, central nervous system; CTAB, Cetyltrimethylammonium bromide; CTX, chlorotoxin; DC-Chol, 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl]-cholesterol hydrochloride; DDAB, dimethyldioctadecylammonium bromide; DiD, 4,4′-diisothiocyanatostilbene- 2,2′-disulfonic acid; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DiO, 3,3′-dioctadecyloxacarbocyanine perchlorate; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DNR, daunorubicin; DODAP, 1,2-dioleoyl-3-dimethylammonium-propane; DOGS-NTA-Ni, 1,2-dioleoyl-*sn*-glycero-3-[(*N*-(5-amino-1-carboxypentyl)iminodiacetic acid) succinyl]; DOPC, dioleoylphosphatidylcholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOX, doxorubicin; DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate monosodium salt; DPPC, dipalmitoylphosphatidylcholine; DQA, dequalinium; DSPC, distearoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; DSPG, distearoylphosphatidylglycerol; DTPA, diethylenetriaminepentaacetic acid; EPC, egg phosphatidylcholine; EPI, epirubicin; EYPC, egg-yolk phosphatidylcholine; FUS, focused ultrasound; HEPE, hydrogenated egg phosphatidylethanolamine; HRP, horseradish peroxidase; HSPC, hydrogenated soy phosphatidylcholine; IB, imipramine blue; Mal, maleimide; MAN, *P*-aminophenyl-α-D-mannopyranoside; MB, methylene blue; miRNA, micro-RNA; mPEG, methoxy polyethylene glycol; MR, magnetic resonance; MRI, magnetic resonance imaging; MSPC, 1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; NI, not informed; PC, phosphatidylcholine; PEG, polyethylene glycol; PDP, pyridyldithiopropionate; PDT, photodynamic therapy; PTX, paclitaxel; QD, quantum dots; siRNA, small interfering RNA; SPC, sphingosylphosphorylcholine; TAM, tamoxifen; Tf, transferrin; TPGS, tocopheryl polyethylene glycol succinate; U1snRNA, small nuclear RNA component of U1 snRNP (small nuclear ribonucleoprotein); WGA, wheat-germ agglutinin.

angiogenesis[253] and delineate tumor margins, showing correlation between endoglin-associated neovasculature and tumor infiltration.[231] In the same way, PET of Ala-Pro-Arg-Pro-Gly peptide-targeted liposomes was able specifically to image the different structure of glioma vessels.[247] Also, liposome-targeted delivery of contrast agents containing antibodies to GFAP and the extracellular loop of Cx43 on its surface that selectively bound to brain-reactive astrocytes and faster-migrating glioma cells has been developed.[239] Also developed have been PEGylated liposomes containing VEGF antibody on the surface, increasing the distribution and efficacy of the delivery of liposomes to glioma.[233] However, it is still unclear if any of the strategies described could enhance detection of the earliest stage of tumors.

Design of liposomal drug-delivery systems for glioma therapy

Glioma therapy consists of surgery followed by radiotherapy, chemotherapy, or photodynamic therapy (PDT). Moreover, the stage and type of glioma often determines whether monotherapy or combined therapies are needed. Radiation therapy is a very common option for the treatment of gliomas, and has a variety of modalities, including external beam and brachytherapy.[292] External beam radiotherapy, the most common approach in the clinic, uses ionizing radiation to kill cancer cells, but its application is limited by doses lower than 80 Gy due to toxicity.[293] Brachytherapy or internal therapy uses a radioactive source that is delivered into or near the tumor itself, making it possible to deliver high radiation to the tumor and harming as few normal cells as possible.[294] In this way, recent studies suggest that brachytherapy with liposomally encapsulated ^{186}Re or ^{188}Re isotopes holds significant promise for glioma therapy.[229,242,245] These studies demonstrated that animals treated with ^{186}Re or ^{188}Re liposomes had significantly prolonged survival, independent of the route of administration.[229,242] Also, ^{188}Re liposomes have been explored for diagnostic evaluation, revealing the potential of these liposomes as a future theranostic agent for brain gliomas.[245]

A wide variety of liposome-encapsulated anticancer drugs have also been developed for both experimental and clinical oncology. By virtue of their unique physicochemical characteristics, liposomes have mainly shown improvement in the therapeutic index of chemotherapeutic drugs by enhancing their efficacy against aggressive and chemoresistant glioma cells and/or lowering drug side effects in the body. Antitumor antibiotics include doxorubicin (DOX), daunorubicin (DNR), and bleomycin. DOX, an anthracycline antibiotic, damages DNA by intercalation, inhibiting DNA synthesis or poisoning of topoisomerase II, by alteration of membrane function, or by generation of free radicals.[295,296] DNR, also an anthracycline antibiotic similar in its chemical structure to DOX, acts through intercalation into DNA, metal ion chelation, and/or by free radical formation.[296] Bleomycin, a polypeptide antibiotic, exerts its action by breaking the DNA double helix.[297]

Furthermore, antitumor antibiotics are among the most widely used and studied chemotherapeutic drugs. They are currently available in the market as free drugs (Adriamycin®, Cerubidine®, and Blenoxane®, trade names for DOX, DNR, and bleomycin, respectively), encapsulated in PEGylated liposomes (Doxil® [PEGylated form of liposomal DOX]), and encapsulated in conventional liposomes (Myocet® and DaunoXome® [liposomal DOX and DNR, respectively]). Although the anticancer activity of free drugs has been reported to be effective against gliomas cells in vitro, they present very poor efficacy in vivo, because these antibiotics do not readily cross the BBB.[298] In a rat brain-glioma model, prolonged survival of the animals was observed when PEGylated liposomes were used to deliver DOX.[267] In contrast, in a cohort of patients with brain cancer, liposomal DOX, DNR, or bleomycin was found moderately effective against glioma.[275,278,279,281–283]

Based on the moderate efficacy of liposomal formulations against brain tumors, it is clear that more effective drug-delivery strategies are needed. One promising alternative strategy involves the combination of ultrasound and microbubbles to induce BBB opening for local and transient delivery of drugs into the brain, leading to improvement in chemotherapy treatment.[226,228,243,265] Moreover, as Doxil has been already clinically approved for the treatment of some types of cancer, these results suggest that the use of ultrasonic microbubbles for glioma chemotherapy is highly clinically relevant.[228,243] Other alternative strategies were found in this search. Researchers developed stimuli-responsive liposomes that were able to release DOX in a controlled manner in response to an external low-power radio frequency field[209] or local temperature rise.[218] Both strategies showed an improvement of DOX delivery across the BBB and prolonged survival time of animals.

In fact, over the years many liposomal formulations have been developed for the treatment of gliomas. Some formulations demonstrated their ability to improve activity of anticancer drugs in vivo, such as topotecan,[220] irinotecan,[214,230,235,259] arsenic trioxide,[255] cisplatin,[237,240,268] and oxaliplatin,[237] and codelivery of synergistic two-drug combinations[257,258] into brain tumor-bearing animal models. Other formulations

demonstrated increased bioavailability of the bioactive compound celastrol,[217] carriage of small molecules[241] and large payloads,[225,270] and enabled efficient gene therapy.[271,274,276,277] Additionally, the design of liposomes that simultaneously carry imaging and therapeutic agents is promising for glioma therapy, since it allows the opportunity for real-time visualization of drug localization, drug delivery, and monitoring the tumor-therapy response.[252,260,262,272,280] However, although passively targeting liposomes are the only ones used in clinical therapy, they suffer several limitations, such as low EPR effect within the brain, nonspecific uptake, and the crossing of both barriers. Therefore, methods for enhancing the targeting of liposomes to brain tumors were developed.

According to our search, liposomes actively targeting strategies for CNS delivery of anticancer drugs across the BBB are basically divided into adsorptive-mediated transcytosis (AMT) and receptor-mediated transcytosis (RMT). AMT is trigged by electrostatic interactions between the negatively charged surface of brain endothelial cells and the positively charged moieties of macromolecules. AMT-based drug delivery for glioma therapy was performed using the cationic cell-penetrating TAT peptide to functionalize the surface of liposomally encapsulated DOX.[248] The authors demonstrated that the TAT peptide could penetrate the BBB, since DOX was efficiently delivered to brain tumor-bearing rats.[248] However, this cationization strategy suffers from several limitations, such as instability of the system in the serum, nonspecific interactions, immunogenicity, and toxicity.[202,299]

RMT-based drug delivery has been widely explored for liposome targeting to the brain. This strategy relies on liposomal ligand interaction with the very specific receptor-mediated transport system in the BBB. In fact, there are several kinds of receptors that are expressed on the surface of endothelial cells of the barrier, such as transferrin and lactoferrin, that have been explored to facilitate the crossing of liposomes into the brain. Transferrin liposomes have been reported to be able to deliver borocaptate (BSH) and small interfering RNA (siRNA) into the CNS, which is highly significant, because these compounds do not readily cross the BBB.[224,246,263] In the same way, the covalent binding of lactoferrin to the liposome surface proved to be an effective strategy for the treatment of brain tumors.[244]

In glioma therapy, similarly to the BBB, the BBTB also represents a challenge for glioma-targeted delivery.[300] Fortunately, many kinds of receptors are highly expressed in the BBTB (tumor vessels and/or glioma cells), and these receptors have been explored for the design of actively targeting liposomes for brain delivery of anticancer drugs across the BBTB. For example, such ligands as chlorotoxin,[206,215,221] TR peptide,[205] RGERPPR peptides,[216] folate,[64,65] anti-EGFR antibody,[222] and IL-13,[223] have been successfully attached to the surface of liposomes. As a result, these decorated liposomes were able selectively to bind, target, and enhance uptake by glioma cells. In the same way, hemagglutinating virus of Japan liposomes have successfully delivered foreign genes into murine glioma cells, representing a good system for gene delivery.[227]

More recently, researchers have developed liposomes that can penetrate the BBB and targeting brain-cancer cells. This new system, known as dual-targeting liposomes, was produced to deliver DOX,[211,213,238] DNR,[250] epirubicin,[249] topotecan,[251] plasmids,[236] and siRNA,[212] and for codelivery of synergistic two-drug combinations.[203,210] All of these dual-targeting liposomes proved to be effective in crossing the BBB and targeting glioma cells. It was also demonstrated that just angiopep-2 peptide was able to target BBB and glioma cells at the same time,[219] and RGD peptide targeted both BBTB and tumor cells.[204,207,209]

PDT uses photosensitizing agents, such as Photofrin, for brain tumors, along with light of appropriated wavelength to kill glioma cells. The PDT cell-killing mechanism is directly related to the production of reactive oxygen species, which leads to cell apoptosis, with minimal side effects.[301] Unfortunately, the efficiency of this therapy for gliomas is limited by the BBB. Just like for chemotherapy, the efficacy of PDT for the treatment of brain tumors was greatly improved when Photofrin was encapsulated into liposomes, since the photosensitizing agent was efficiently delivered within brain tumors.[264,266] Finally, it is worth mentioning here that although the preferred route for delivery of liposomes seems to be intravenous injection, alternative routes of administration, such as convection-enhanced delivery and intracranial, intracarotid, and intraperitoneal injections, have been also considered.

Conclusion

The BBB is the most important obstacle to effective brain drug delivery. There has been great interest in this area, especially in the development of targeted liposomes to cross the BBB and to deliver therapeutic molecules only to the disease site within the brain. From the reported articles, we could see that liposomes can get into the brain via different mechanisms. Examples of these mechanisms are: 1) transport of liposomes via RMT, followed by their internalization by neurons or glial cells and release of therapeutic molecules within those cells; 2) adsorption of cationic liposomes in the endothelial cells, which enhanced the concentration of

therapeutic molecules within the brain cells; 3) antibody- or peptide-conjugated liposomes used to transport and target encapsulated drugs into the brain via transcytotic pathways; 4) inhibition of efflux transporters, such as PgP, by coating liposomes with transporter-inhibitory substances; and 5) disruption of the BBB. In fact, liposomes have the potential to revolutionize drug development for therapy and/or diagnosis of neurological diseases. By their unique physicochemical properties, liposomes have shown great ability to compartmentalize and solubilize hydrophilic and hydrophobic drugs (Figure 4). Furthermore, liposomes are biocompatible and biodegradable systems, which make them suitable for neuromedicine.

Also, the functionalization of liposomes surface modified with antibodies, peptides, aptamers, and other small molecules has shown promise in delivering a huge range of therapeutic molecules to targeted sites in the body (Figure 4). Liposomes usually improve the therapeutic index of new or established drugs by prolonging biological half-life and reducing their side effects. More importantly, liposomes may provide an excellent therapeutic tool for treatment or diagnosis of neurological disorders, due to their ability to cross the BBB and efficiently deliver drugs and/or contrast agents into the CNS, as discussed in this article. Additionally, theranostic liposomes have been developed, allowing real-time therapeutic efficacy. Also, high efficacy in using liposomes to deliver a drug in a spatial and temporal manner has been demonstrated (Figure 4), which we believe may be critical for the success of more effective therapy for neurological diseases.

Unfortunately, most advances and breakthroughs in liposome-based approaches have just happened for glioma therapy. The development of effective therapy for AD, PD, and stroke has been largely constrained. This might be due to our deficiency in understanding the neurological mechanisms and pathogenesis of these disorders. Increasing our comprehension about these diseases will contribute to the development of novel potential therapeutic strategies. This is essential, since we are living in a modern aging society and an effective spectrum of treatments is urgently needed.

By crossing the BBB, achieving efficient drug delivery into the brain is possible, which leads to an intensive search for alternative administration routes for liposomes. In this review, various studies used different administration routes to access the brain for the therapeutic delivery of liposomes (Figure 4). Intravenous injection of liposomes was the preferred route in the majority of the works cited here. Alternatively, intranasal injections offered a direct mode of drug delivery into the brain for AD and PD. Convection-enhanced delivery provided interesting results

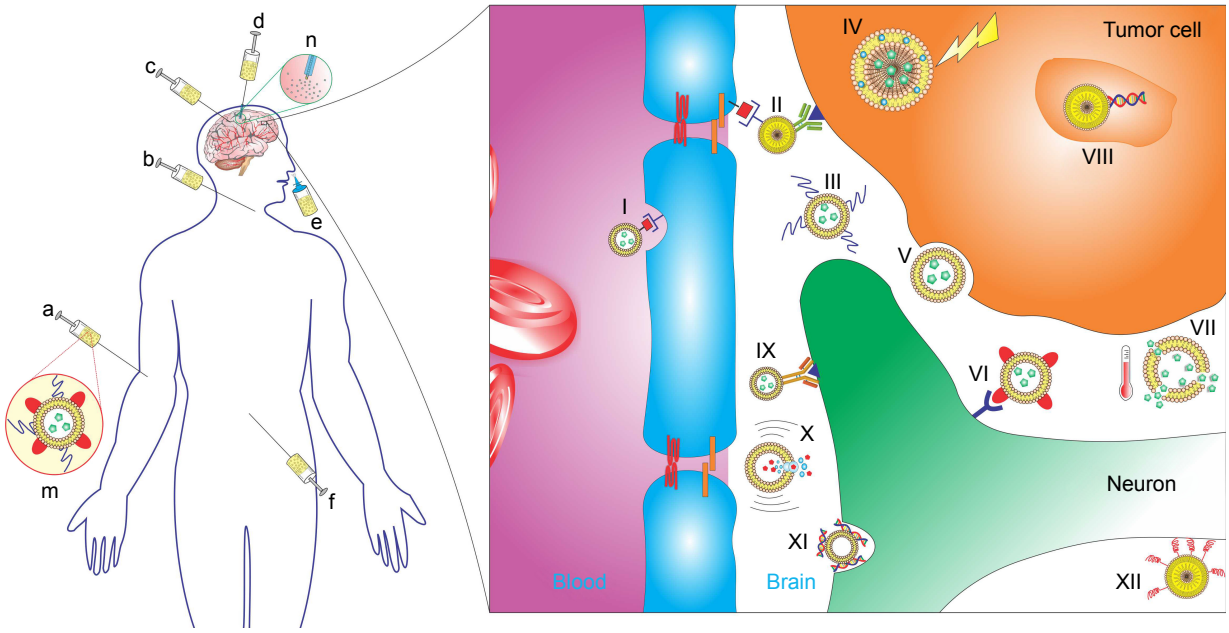


Figure 4 Delivery of therapeutic molecules or imaging agents to the brain by liposomes (m) is highly challenging.
Notes: Liposomes can be administered to the central nervous system via systemic delivery (a), intracarotid (b), intracranial (c), intranasal (e), and intraperitoneal (f) injections, or via convection-enhanced delivery (d/n). Liposome-based strategies consist in encapsulating the molecules of interest in liposomes (V). The ability to increase their blood-circulation time is created with the ligation of polyethylene glycol on the liposome surface (III). Liposomes can also be targeted to cross the blood–brain barrier (I), target the site of disease (IX), or both (II). Surface modification of liposomes can be achieved by covalent ligation of antibodies (IX), RNA aptamers (VI), or peptides (XII). Cationic lipids can be incorporated into the bilayer, facilitating their association with nucleic acids for gene therapy (VIII and XI). This figure also summarizes therapeutic mechanisms, such as hyperthermia (IV), temperature increase (VII), and ultrasound (X).

for efficient delivery of liposomes for drug delivery to tumor-bearing animal models (Tables 3–5). Administration of liposomal formulation via nonparenteral routes is highly desirable, since effective strategies for crossing the BBB are urgently needed.

Disclosure

The authors report no conflicts of interest in this work.

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