ELSEVIER

Strategies for brain-targeting liposomal delivery of small hydrophobic molecules in the treatment of neurodegenerative diseases

Zi-Ying Wang[1,‡], Sravan Gopalkrishnashetty Sreenivasmurthy[1,‡], Ju-Xian Song[1,2], Jing-Yi Liu[1,3,4] and Min Li[1]

[1] Mr & Mrs Ko Chi-Ming Centre for Parkinson's Disease Research, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China
[2] Medical College of Acupuncture-Moxibustion and Rehabilitation, Guangzhou University of Chinese Medicine, Guangzhou, China
[3] State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics & Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen, 361102, China
[4] College of Medicine, Xiamen University, Xiamen 361005 Fujian, China

Neurodegenerative diseases (NDs), including Alzheimer's disease (AD) and Parkinson's disease (PD), threaten the health of an ever-growing number of older people worldwide; so far, there are no effective cures. Significant efforts have been devoted to developing new drugs for NDs in recent years, and some small molecules have been shown to be promising in preclinical studies. However, the major challenge for brain-targeting drugs is how to efficiently deliver the drugs across the blood–brain barrier (BBB) to desired targets. To address this issue, liposomal delivery systems have proved to be ideal carriers for neuroprotective small molecules. Here, we summarize recent advances in the brain-targeting liposomal delivery of small hydrophobic molecules (SHMs) and propose strategies for developing liposomal SHMs as disease-modifying neurotherapeutics for NDs.

Introduction

NDs, including AD, PD, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), share similar pathological features manifested as abnormal protein aggregations in specific degenerative neuronal cells. The ever-growing number of patients with NDs globally has become the biggest challenge for the healthcare system. For example, approximately 27 million people globally have dementia, of which half are related to AD. The prevalence of NDs is expected to triple by 2050 [1]. In the case of PD, the number of patients was reported to be over 6.2 million in 2015, and this is still increasing [2].

Given our limited understanding of the complicated pathogenesis of NDs, current therapies for NDs can only treat symptoms, and there are no disease-modifying drugs that can halt the progression of these diseases. The major challenges for development of new drugs for NDs include what to target and how to design and specifically deliver the drugs to the desired targets. If you have the wrong target, even a drug that hits that target will not treat the disease. However, if you have the right target and the right drug, but are unable to deliver the drug specifically to the target, then the treatment also fails. For instance, the failure of the amyloid-beta (Aβ)-lowering agent tarenflurbil in the largest clinical trial for patients with AD ever conducted raised the question of whether Aβ is the appropriate target for treatment [3]. However, the poor pharmacokinetic profile of tarenflurbil (with a cerebrospinal fluid: plasma ratio of 1.3% in rodents) and its poor brain penetration were also considered to be significant factors leading to the negative results in this Phase III clinical study. In the case of levodopa (L-DOPA), a direct precursor of dopamine (DA) and the most widely used symptomatic therapy for PD, only 5–10% of L-DOPA enters the central nervous system (CNS) from the usual oral dose. The remaining drug is metabolized by the liver and eventually causes serious peripheral adverse effects [North Maria, PhD Thesis, Linköping University, 2017]. To overcome this problem, researchers have been investigating new targeting delivery systems since

Corresponding authors: Liu, J.-Y. (ljy5165943@126.com), Li, M. (limin@hkbu.edu.hk)
‡ These authors contributed equally to this paper.

https://doi.org/10.1016/j.drudis.2018.11.001

the 1990s, reporting that one-tenth L-DOPA (5 mg/kg) incorporated into liposomes was equivalent to free drug (50 mg/kg) for increasing DA content in murine striatum, indicating the high efficiency of liposomal delivery for brain targeting [4]. Nevertheless, >40% of all drug failures in development can be attributed to inadequate drug delivery [5].

Liposomes are currently the most widely exploited system for brain-targeting drug delivery [6]. Several drugs, including doxorubicin (DOX) and daunorubicin, have already been approved in liposome-based formulations on the market or for clinical trials with NDs. Liposomes are also being used to deliver SHMs, such as the clinically approved drug DA and its derivatives, rivastigmine, tacrine and donepezil, to treat NDs [7–12]. In addition, natural hydrophobic compounds, such as curcumin, quercetin, and resveratrol, have been increasingly reported to be neuroprotective in animal models of NDs [13–15]. However, the main limitation to the further development of these and SHMs is their poor bioavailability because of insolubility and/or susceptibility to degradation. However, liposomal delivery systems in many cases appear capable of overcoming the problems of delivering SHMs to disease targets, and promising preclinical results confirm their potential for application in clinical practice, particularly in the treatment of NDs [16] (Table 1).

Here, we summarize recent advances in the brain-targeting liposomal delivery of SHMs. We illustrate the advantages and strategies of liposomal systems for brain-targeting delivery of SHMs using curcumin as an example. By critically analyzing the data, we highlight those strategies that appear to have the most promise for developing effective disease-modifying neurotherapeutics for NDs.

Application of liposomal delivery systems in the treatment of NDs

Liposomal brain delivery of poorly permeable hydrophobic molecules

Most drugs designed to treat NDs fail to enter the CNS to generate their therapeutic effects because of the BBB. After oral administration or injection, therapeutic agents circulating in the blood meet the BBB, which separates the blood from the brain and extracellular fluids in the CNS [17]. The BBB comprises endothelial cells with tight junctions, a basement membrane, and astrocyte end-feet. To cross the BBB, drugs must first cross the endothelial cells. However, the limited permeability of endothelial cells hinders them from entering the brain.

The mass distribution of P-glycoprotein (P-gp) in endothelial cells inhibits the transport of drugs into the brain. P-gp is an ATP-dependent efflux pump that resists the influx of a variety of lipophilic compounds as well as large (>400 Da) hydrophilic molecules [18]. Consequently, only small lipophilic molecules, such as oxygen, carbon dioxide, and nicotine, can permeate the BBB into the CNS. Although it is reported that heterogeneous BBB disruption is observed in NDs [19], it has proven impossible to deliver drugs by taking advantage of pathological BBB leakage, owing to the distribution of P-gp and the downregulation of pinocytosis.

The limited transportation across BBB leads to the poor brain targeting of most therapeutic agents. Thus, for the drug to reach therapeutic levels in the CNS, a larger dosage might be required. However, because NDs are chronic diseases and patients are mostly older, larger doses can bring a high risk of peripheral adverse effects, especially after long-term administration. Thus, strategies to overcome these problems are vital [6]. Several nanoparticles (NPs) have been engineered to effectively deliver drugs across the BBB without affecting its function. There are many types of NP, including liposomes, micelles, dendrimers, carbon nanotubes, and metal particles, that are able to do this. Among these, liposomes have received widespread attention because they are nontoxic, biodegradable, and biocompatible, and, therefore, show potential for clinical application [20].

Conventional liposomes are natural or synthetic lipid spheres that comprise single or multiple lipid bilayers surrounding an aqueous core. The lipid components are mostly phosphatidylcholine, derived from egg or soybean lecithin. Given their unique self-enclosed structure, liposomes can entrap hydrophilic agents in their aqueous core and hydrophobic molecules into their lipid membranes. Given their unique structure, liposomes overcome the shortcomings of traditional formulations, such as poor aqueous solubility, low bioavailability, and nonspecific distribution in the body [21,22].

There are several routes by which liposomes enable drug transportation across the BBB, including passive transcellular diffusion, carrier-mediated transport, receptor-mediated transcytosis (transferrin receptor, insulin receptor, etc.), absorptive-mediated transcytosis, cell-mediated transcytosis, and efflux pumps [23]. Receptor-mediated transcytosis is the most studied transport route for the liposome delivery system. With the help of transferrin (TfR) or insulin receptors (IR), which are highly expressed on BBB endothelial cells as surface conjugations, liposomes easily pass through the BBB and carry therapeutic drugs into the CNS [22]. Similarly, liposomes can also be conjugated to monoclonal antibodies of glial fibrillary acidic protein (GFAP) to cross the BBB. A recent study reported a liposomal nanohybrid cerasome fabricated from polysorbate 80 as a P-gp inhibitor to improve the BBB permeability of curcumin in the treatment of PD, with remarkable effect [24] (Fig. 1). Details of strategies to enhance the brain targeting of liposomal delivery systems are described below.

Application of liposomal delivery systems in preclinical drug development for NDs

In recent years, SHMs have showed significant protective effects on different NDs in *in vivo* and *in vitro* models [25–27]. Among them, the most popular and promising candidates are L-DOPA, DA derivatives, rivastigmine, and natural compounds, including curcumin, quercetin, and resveratrol. However, these SHMs still have some shortcomings, such as low bioavailability, systemic adverse effects, and water insolubility. Thus, prepared liposome formulations for SHMs could provide an efficient and reliable drug delivery system for the treatment of NDs.

L-DOPA, a bioprecursor of DA, is currently the most common clinical drug for the treatment of PD. It converts to DA after crossing the BBB and its activation of central DA receptors (D2) can ameliorate the symptoms of PD. However, the main issue associated with L-DOPA is its low bioavailability; 90% of a dose is metabolized by the liver, eventually causing several adverse effects, such as dyskinesias [28]. To address this problem, Cao *et al*. used chitosan-coated L-DOPA nanoliposomes (NL) to prevent

TABLE 1

Application of liposomal delivery systems in clinically approved drugs and preclinical drugs for NDs

Conventional drugs and/or natural products	Bioactivity	Problems	Liposomal composition (including targeting ligand)	Improvement	Refs
L-DOPA (Levodopa)	Precursor to DA, norepinephrine, and epinephrine	L-DOPA-induced dyskinesia	Chitosan-coated L-DOPA nanoliposomes	Scores of abnormal involuntary movement (AIM) decreased significantly in liposome group	[7]
DA HCL	Neurotransmitters	DA does not penetrate BBB	Transferrin-functionalized liposomes	Provided higher permeability across BBB	[8]
DA derivative *N*-3,4-bis(pivaloyloxy)-dopamine (BPD)	DA analog	Short half-life; not all BPD crossed BBB	29 amino-acid peptide (RVG29)-functionalized liposomes	Crossed BBB and reached striatum and nigra efficiently; improved therapeutic outcome of BPD in murine PD model	[9]
Rivastigmine	Acetylcholinesterase inhibitor	N/A	Electrosteric stealth (ESS) liposomes	Fourfold increase in both plasma and brain drug levels *in vitro*, *ex vivo*, and *in vivo*	[10]
Tacrine	Acetylcholinesterase inhibitor	Increased liver function tests	Multifunctional liposomes: EPC and CHO (Lipo A) with Toc (Lipo B) or V3 (Lipo C) or both (Lipo D)	Marked increase in tacrine permeability	[11]
Donepezil	Binds and reversibly inactivates cholinesterases	Low bioavailability in brain	Traditional liposomes	Bioavailability of DNP in plasma and brain increased significantly	[12]
Quercetin	Shows antioxidative ability to eliminate free radicals and could ameliorate cognitive and memory dysfunction	Low solubility in blood, rapid metabolism in intestine and liver, and low uptake in brain	RMP-7- and lactoferrin-grafted liposomes	Decreased transendothelial electrical resistance and increased ability to cross BBB; could reduce neurotoxicity and improve neuronal survival	[42]
Curcumin	Interferes with amyloid plaques	Low solubility, instability low bioaccessibility; high degradability in basic medium; poor systemic bioavailability; unable to enter cells via plasmatic membrane on its own	Pluronics-modified liposomes	Improved stability and *in vitro* bioaccessibility of curcumin	[32]
			Wheat germ agglutinin- and cardiolipin-conjugated liposomes	Significantly improved permeation of CRM and NGF across BBB; reduced Aβ plaque deposition and malondialdehyde level; increased percentage of normal neurons and cholinergic activity in hippocampus of AD rats	[34]
			Xanthan gum-coated mucoadhesive liposomes	Rapid transport of curcumin in brain tissues	[16]
			Conventional liposome	Reduction in angiotensin-converting enzyme activity and anticytokine effect in target regions of brain, which helped memory recovery	[37]
Resveratrol	Activates sirtuin 1 and PGC-1α	Low aqueous solubility and relatively low bioavailability	Fusogenic liposomes	Resveratrol in fusogenic liposomes significantly enhanced fusion to cell membranes; activated Nrf2 and exerted antioxidative and antiapoptotic effects in aged cells	[45]

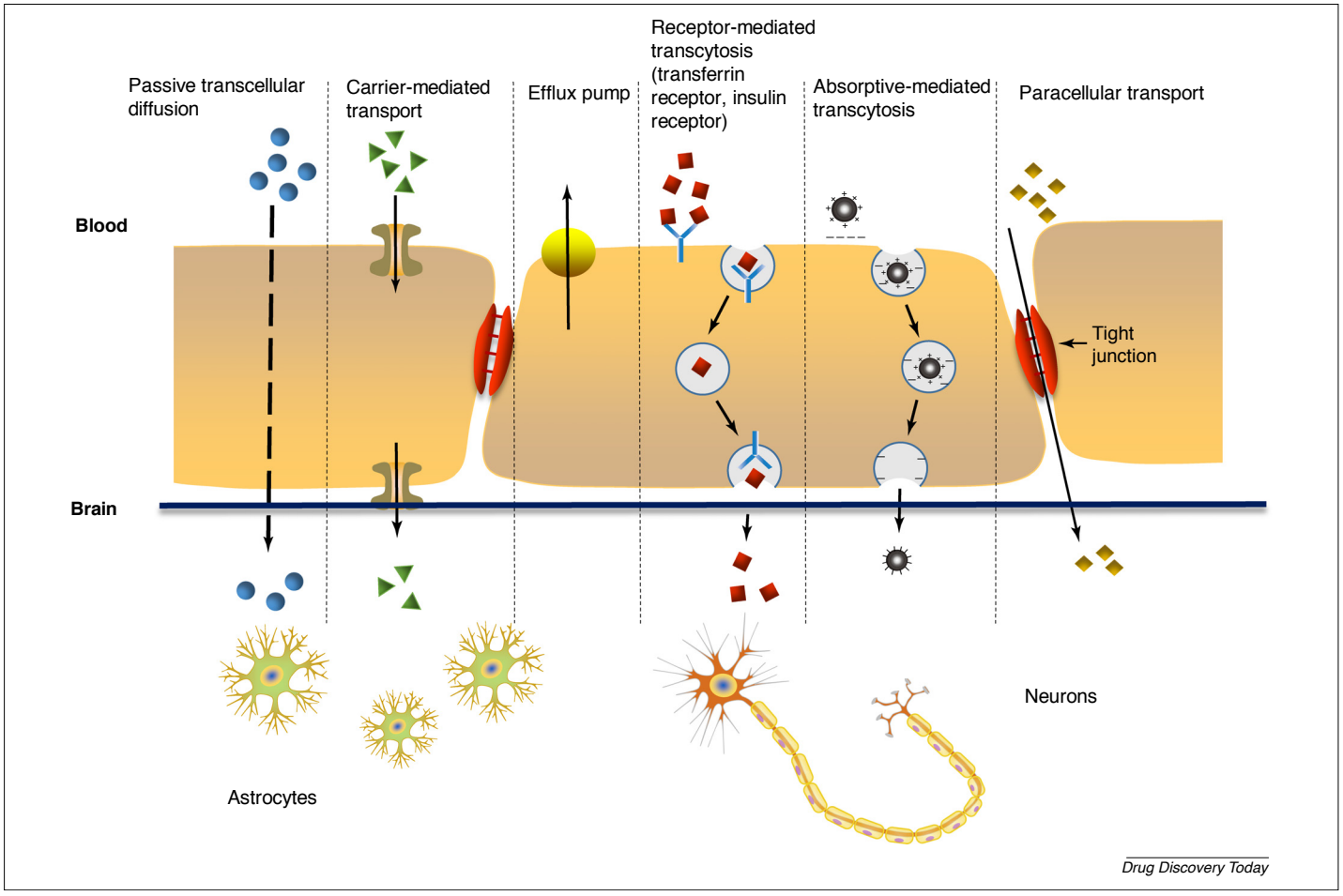

FIGURE 1

Different absorption pathways for the liposomal delivery of small hydrophobic molecules (SHMs) across the blood–brain barrier (BBB). Absorption pathways include passive transcellular diffusion, carrier-mediated transport, efflux pumps, receptor-mediated transcytosis (transferrin and insulin receptors), absorptive-mediated transcytosis, and cell-mediated transcytosis.

the dyskinesias caused by L-DOPA overdoses. Chitosan is a cationic polyelectrolyte that has been used for the preparation of films, gels, and microcapsules. The authors found that the scores of abnormal involuntary movement (AIM) were reduced markedly in the liposome L-DOPA-treated cohort [7].

Other researchers have focused on increasing the permeability of DA to across the BBB. Lopalco *et al.* encapsulated DA hydrochloride (DA-HCl) into Tf functionalized liposomes (DA-HCl-LPs) to study its permeability in an *in vitro* model of the BBB [8]. Their results showed that liposomes decorated with the uptake-facilitating Tf receptors resulted in higher permeability across the monolayer. Qu *et al.* developed a brain-targeted drug delivery system to improve the therapeutic effects of a DA derivative *N*-3,4-bis(pivaloyloxy)-DA (BPD) [9]. They used a 29 amino-acid peptide (RVG 29) from rabies virus glycoprotein as the targeting ligand. BPD loaded in RVG29 functionalized liposomes (BPD-RVG29-lip) showed a high penetration efficiency across the BBB. Moreover, *in vivo* and *ex vivo* distribution experiments showed that RVG29-lip was selectively distributed to the brain.

Rivastigmine is an acetylcholinesterase inhibitor used for the treatment of early-stage AD and PD [25]. Nageeb El-Helaly *et al.* designed an electrosteric stealth (ESS) liposomes loaded with rivastigmine [10]. The ESS liposomes showed a higher percentage of drug permeating the BBB via a nasal administration compared with the drug solution. *In vivo* pharmacokinetic study also showed 486% relative bioavailability of the mean brain levels compared to rivastigmine solution [10].

Tacrine is another US Food and Drug Administration (FDA)-approved drug for the treatment of AD [29]. However, because of its rapid clearance from the systemic circulation, tacrine shows dose-dependent hepatotoxicity and peripherical cholinergic adverse effects. To increase the bioavailability of tacrine, Corace *et al.* utilized different liposomes to encapsulate tacrine [11]. The results indicated that multifunctional liposomes markedly increased the permeability of tacrine and showed neuroprotective effects against H_2O_2-induced oxidative injury.

Liposome formulations have also been studied for the bioavailability in the brain of the commonly used anti-AD drug donepezil. Donepezil binds and inactivates cholinesterases, therefore inhibiting hydrolysis of acetylcholine and increasing the acetylcholine concentrations. Adverse effects of donepezil included loss of appetite, vomiting, and difficulty sleeping [26]. Al Asmari *et al.* prepared liposome by using cholesterol, polyethylene glycol, and 1,2-distearyl-sn-glycero-3-phosphocholine (DSPC). The bio-

availability of donepezil in brain and plasma was significantly increased following liposomal donepezil administration via the intranasal route [12].

Curcumin is a natural compound isolated from turmeric *Curcuma longa*. It shows antioxidant, anti-inflammatory, and other pharmacological activities in many disease models. Several studies have demonstrated the neuroprotective effects of curcumin in experimental models of NDs [30,31]. A recent clinical study revealed that curcumin could lead to improved memory and attention in adults without dementia, and symptom benefits were associated with decreases in amyloid and tau accumulation in brain regions modulating mood and memory [32]. Similarly, in PD studies, curcumin was able to inhibit α-synuclein aggregation and fibril formation; in addition, orally administrated curcumin protected against neuronal loss induced by MPTP or 6-OHDA in rodent models [33].

Although curcumin demonstrates attractive neuroprotective efficacy, its poor aqueous solubility, low bioavailability, rapid metabolism, and rapid systemic elimination limit its clinical application [34]. After intravenous administration of curcumin, the maximum serum concentration achieved was 0.36 ± 0.05 μg/ml, whereas, upon oral administration, the maximum plasma concentration observed was 0.06 ± 0.01 μg/ml. Likewise, intraperitoneal administration of curcumin (0.1 g/kg) in mice showed a maximum plasma concentration of 2.25 μg/ml, whereas the plasma level was observed to be 0.22 μg/ml after oral treatment with curcumin (1.0 g/kg) [35].

Liposome systems can, at least partially, improve the above-mentioned unfavorable properties of curcumin. For example, the oral bioavailability of liposomal curcumin was enhanced more than twofold [36]. After intravenous administration of liposomal curcumin, its body circulation time also increased significantly [37]. Kuo *et al.* investigated the use of cardiolipin-conjugated liposomes with surface wheat germ agglutinin (WGA) carrying curcumin and nerve growth factor [38]. This modified liposome prevented neurodegeneration of SK-N-MC cells, reduced Aβ plaque deposition, and increased normal neuron percentages in the hippocampuses of AD rats. Moreover, the WGA-curcumin/liposome significantly promoted BBB permeability and ameliorated the low bioavailability of curcumin [39]. Sokolik *et al.* reported that liposomal curcumin could reduce angiotensin-converting enzyme activity in the target regions of the brain, which helped memory recovery in AD [40]. Specifically targeting the Aβ protein, Mourtas *et al.* synthesized a curcumin derivative encapsulated in multifunctional nanosized liposomes decorated with a BBB transport mediator (anti-Tr antibody). These anti-TrF liposomes showed a high binding affinity for amyloid deposits and considerably delayed the aggregation of $A\beta_{1\text{-}42}$ peptides [41]. Therefore, liposomal curcumin is a promising therapeutic drug for the treatment of AD.

Quercetin is a plant polyphenol found in many fruits and vegetables. It exhibits numerous biological and pharmacological activities. Many studies have demonstrated the antioxidative effect of quercetin in various disease models. For example, in a PD cell model, quercetin protected MN9D dopaminergic neurons against 6-OHDA-induced neurotoxicity [27]. Another study found that quercetin glycosides showed neuroprotection in a PD cell model by modulating *Nsf* and *Opa1* gene expression [42]. However, low solubility in the blood, rapid metabolism, and low uptake in the brain significantly restrain its clinical application [43]. Liposomal quercetin can considerately improve the solubility and bioavailability of quercetin, and this form of quercetin has been shown to be an effective antioxidant for protection against reactive oxygen species (ROS) [44]. Kuo *et al.* demonstrated that RMP-7- and lactoferrin-grafted liposomal quercetin protects SK-N-MC cells against apoptosis induced by Aβ [45]. Thus, liposomes loaded with quercetin show great promise as a system for delivering drugs to the brain in the treatment of NDs.

Similarly, resveratrol is another polyphenol with antioxidant properties found in common foods, particularly high in grapes, red wine, and peanuts. Resveratrol displays a range of beneficial effects against human diseases. Accumulating evidence indicates that the neuroprotective effect of resveratrol occurs through its modulation of the activation of SIRT1 [46]. One preclinical study provided evidence that resveratrol is a potent molecule for the treatment of AD and PD, and various studies have verified its protective effect against several diseases; however, its rapid metabolism in the liver and intestinal epithelial cells means that it has extremely low bioavailability and, therefore, has not been considered for clinical studies [47]. In this instance, liposomes could overcome this major limitation. Csiszar *et al.* encapsulated resveratrol within fusogenic liposomes and found significant reductions in cellularly produced ROS and apoptosis in aged cells treated with fusogenic liposomal resveratrol [48]. In another study, treatment of PD rats with liposomal resveratrol significantly reduced aberrant motor behavior and the loss of nigral cells [49].

Current strategies for developing brain-targeting liposomal delivery systems of SHMs

Liposomes have distinct advantages that enable them to deliver SHMs through the brain endothelial barrier. The major drawback of conventional liposomes, which comprise a lipid bilayer of phospholipids and cholesterol, is their inherent instability and susceptibility to breakdown during drug delivery. One strategy to overcome these problems is coating the surface of liposomes with macromolecules, such as polymers, polysaccharides, peptides, antibodies, or aptamers, which can increase the circulation time and the efficiency of brain-specific delivery [50–53]. Here, we discuss current strategies for developing liposomal delivery systems of SHMs for the treatment of NDs. In Table 2, we summarize the nanosystems and their physicochemical properties for enhancing the brain delivery of various SHMs [54–65].

Strategies for enhancing drug-loading efficiency and stability

Although liposomes can greatly improve drug delivery, they have limitations, one of which affects pH-sensitive drugs. For example, curcumin is unstable in neutral pH conditions [66]; however, to improve its stability, researchers have used an evaporation method to prepare curcumin-filled liposomes (Cur-LP). A mixture of phospholipids, cholesterol, and ethanol containing curcumin (2 mg/ml) was made into an emulsion, which was then vacuum-evaporated to remove the ethanol from the solution. To maintain the acidic pH environment inside the Cur-LPs, three different pH conditions were used (2.5, 5, and 7.4) and a neutral pH 7.4 was used on the outside. The physical properties of aggregation and sedimentation, and the chemical properties in serum were studied

TABLE 2

Characteristics of representative liposome-like NPs for brain-targeted delivery of curcumin in the treatment of NDs

Sl no.	Nanovehicle	Drug and/or probe	Particle size (nm)	Zeta potential (mV)	EE%	LC%	Disease	*In vivo, in vitro, ex vivo*	Route of administration	Other major findings	Refs
1	Curcumin-loaded nanocapsules	Curcumin chitosan-Si	250		80.2 ± 2.2			♂Swiss mice	Intravenous	Curcumin bioavailability from NPs higher than from curcumin suspension	[54]
		Curcumin chitosan-Ab	300		75.7 ± 3.9		Brain targeting				
		Curcumin chitosan-Bi	400		92.8 ± 3.7						
2	Multifunctional liposomes	Curcumin	136 ± 18	−3.71 ± 0.72			AD	FVB mice; APP PS1 mice; APPswe PSEN1dE9 mice Brain tissue from patients with AD	Intravenous	Increased stealth properties of liposomes by reducing uptake by liver and spleen	[55]
3	Multifunctional nanoliposomes	Curcumin	116.1–3.7	−6.07 + 0.47			AD			Showed better brain penetration and reduced Aβ aggregation	[56]
4	Lipid-based NPs	Curcumin and piperine	93 ± 11	−30.9 ± 0.88	65		PD	♂ Balb/c mice and ♂ C57BL/6 mice	Oral	Enhanced bioavailability of curcumin and decreased aggregation and fibrils of alpha synuclein compared with individual drugs	[57]
5	Lipid nanocarriers	Curcumin	20.75 ± 0.89	−9.68 ± 0.61	>99		Brain targeting	Porcine nasal mucosa		Curcumin uniformly distributed in nasal mucosa; no histopathological changes in nasal mucosa layer	[58]
6	Curcumin-decorated liposomes	Curcumin	135.3 ± 0.7 to 207.2 ± 8	−10.5 ± 1.2 to −14.5 ± 2.4			AD	*In vitro* binding to $A\beta_{1-42}$ fibrils		Showed highest affinity for $A\beta_{1-42}$ fibrils (1–5 nM)	[59]
7	Liposomes	Curcumin	100.2–150	−20.7	20.45–48.75	11.1–14.1	Brain targeting	♂♀Wistar rat	Intranasal	Enhanced intranasal penetration, and also increased stability and bioavailability	[60]

TABLE 2 (*Continued*)

Sl no.	Nanovehicle	Drug and/ or probe	Particle size (nm)	Zeta potential (mV)	EE%	LC%	Disease	*In vivo, in vitro, ex vivo*	Route of administration	Other major findings	Refs
8	Polysorbate 80-modified cerasome	Curcumin	110	−16.5–34.5	46–87	2.98–5.48	PD	C57BL/6 mice	Intravenous	Exhibited superior stability towards PS 80 surfactant solubilization and longer circulation lifetime.	[61]
9	Lactoferrin-nanostructured lipid carrier	Curcumin	103.8 ± 0.6	–5.80 ± 0.73	96.51 ± 1.87	2.60 ± 0.17	AD	SD rat and ICR mice	Intravenous	Showed sustained release of payload and also high concentration in brain coronal section	[62]
10	Borneol-modified ginkgolides liposomes	Gingkolides	128.01 ± 5.91	–27.2 mV	89.73 ± 3.45		Cerebral Ischemia	♂Kunming strain mice	Intravenous	Increased brain tissue penetration with enhanced blood brain barrier permeability	[63]
11	Cationic bovine serun albumin conjugated Tanshinone IIA PEGylated Nanoparticles	Tanshinone IIA	122 16	17.8 1.6	85.6 3.2	5.86 0.8	Cerebral Ischemia	♂SD rat	Intravenous	Enhanced brain uptake efficiency than unmodified NPs	[64]
12	Solid lipid nanoparticles	Quercetin	159	21.05	85.73		AD	♂Wistar rat	Intravenous	Quercetin-loaded SLNs in aluminium chloride-treated rats was able to reverse the deleterious neu-rodegenerative effects of aluminium chloride	[65]

and the *in vitro* release kinetics of Cur-LP was also evaluated. The prepared LPs showed higher thermodynamic and kinetic stability and a constant drug release profile until 72 h post administration *in vitro*. Therefore, by modulating the internal pH environment of the liposomes, the stability of hydrophobic drugs on the lipid bilayer could be maintained for accurate delivery and release at the relevant target site *in vivo*. Polyethylene glycol (PEG) is commonly used to increase liposomal stability and ensure a longer circulation time in the body. In another study, chitosan was used to coat liposomes for the stable nasal delivery of fexofenadine. Results revealed that a chitosan coating increased liposomal stability even when stored at 4 °C for 6 months and also decreased drug leakage by nearly 10% [67] (Fig. 2A). The stability of the liposomes can also be enhanced by optimizing other factors, such as temperature and the addition of cholesterol to the lipid bilayer [68].

Although currently available liposome formulations can maintain the stability of curcumin in various buffers, they all face the issue of membrane leakage of the loaded drug molecules, thereby affecting the bioavailability needed for effective therapy [69]. In one study, the authors loaded curcumin into liposomes that were coated with *N*-trimethyl chitosan chloride (TMC coating) along with D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (Table 2), which enabled effective brain penetration of the liposomes. Various parameters, such as zeta potential, particle size, *in vitro* release, encapsulation efficiency (EE %), and drug-loading efficiency (LE %), were evaluated. Addition of chitosan and TPGS reduced the fluidity of the liposomal structure, which significantly reduced the leakage and increased EE% and LE%. Thus, this can be considered a desirable curcumin-loaded liposomal structure for BBB penetration [70]. In another study, researchers isolated exosomes from mouse blood and characterized the *in vitro* and *in vivo* brain-targeting abilities of the exosomes. Thereafter, exosomes were loaded with DA and checked for brain distribution in 6-OHDA-treated mouse model. Results showed that exosomes enhanced the brain distribution of DA especially in the striatum and even enhanced tyrosine hydroxylase (TH) levels as well as neurogenesis in the mouse brain [71].

Bioactive small molecules as targeting ligands on liposomal surfaces

Despite the low stability of hydrophobic molecules, they do bind to Aβ aggregates *in vitro* and *in vivo*. Thus, researchers conjugated curcumin covalently to the polar head groups of phosphatidylcholine, which enabled the direct binding of curcumin to Aβ deposits in *in vitro* hAPP-overexpressing cells lines. Furthermore, upon stereotaxic injection of Aβ in the brain of mice, curcumin-conjugated nanoliposomes (CnL) interacted with the Aβ deposits directly. Postmortem brain tissue sections from APPxPS1 AD mice as well as from patients with AD also showed that CnLs directly interacted with and stained the Aβ deposits, revealing the clinical relevance of CnL structures [72]. Similarly, in another study, a small unilamellar liposome conjugated with curcumin and its analogs was prepared using click chemistry ('click-curcumin liposomes'). The propensity of these liposomes to inhibit Aβ fibrils and Aβ oligomers were evaluated by a thioflavin T (ThT) assay and Aβ oligomer immunoassay, which revealed click-chemistry liposomes as a promising AD therapeutic strategy [73] (Fig. 2B). Other studies also showed that the small amphiphilic molecule monosialotetrahexosylganglioside (GM1) was incorporated into liposomal lipid membranes, the resulting GM1-modified rHDL (GM1-rHDL) significantly reduced $A\beta_{1\text{-}42}$ compared with GM1 alone. After intranasal administration of GM1-rHDL, observations showed enhanced BBB penetration and amelioration of memory deficits in AD mouse models [74].

Strategies for enhancing BBB permeability

For more efficient and effective therapeutic actions, drug-loaded liposomes need to readily cross the highly electrostatic BBB membrane. One of the parameters for enhanced brain permeability concerns the surface charge of the liposomes (positive, negative, and neutral) [75]. Usually, positively charged liposomes are considered to have lower brain-targeting efficiency compared with negative and neutral liposomes, owing to their ability to bind proteins and poor brain availability [76,77] (Fig. 2C). The brain consumes glucose as its major energy source and can be taken up by GLUT proteins on endothelial cells; thus, one approach to enhance the BBB permeability of drug-loaded liposomes is to glycosylate liposomes to enhance interactions with GLUT transporters, enabling them to cross the BBB. Researchers reported that *N*-acetyl-glucosamine (NAG) and RGD decoration on the surface of liposomes improved its BBB penetration [78].

In one study, ginkgolide B liposomes modified with borneol (GGB-LP) were prepared and optimized by resonance surface methodology (RSM). Researchers compared ginkgolide and GGB-LP and observed that borneol-modified ginkgolide liposomes improved BBB penetration and also enhanced the physicochemical properties of the modified liposome. The concentration of ginkgolide B (GGB-LP) in the brain tissue was higher than the injected forms of ginkgolide B (Table 2). Thus, this study provides a theoretical basis for studying the neuroprotective effects of *Ginkgo biloba* lactones in *in vivo* models of cerebral ischemia [79]. Studies have shown that modifying the liposomal surfaces with cell-penetrating peptides (CPPs) could further increase the efficiency of delivery to the brain (Fig. 2D). Accordingly, in one study, NL were covalently attached to a curcumin derivative (Curc) and modified HIV transactivating transcriptional activator (TAT) peptides. TAT is a CPP that enhances the permeability of liposomes through the BBB. *In vitro* human cerebral microvascular (hCMEC/D3) cultured cells, a known BBB cellular model, was used to evaluate the efficacy of NL to permeate artificial BBB and target Aβ. NL-Curc-TAT was observed in perinuclear regions. Interestingly, endosomal pathway activation was not observed, which confirmed the stability of curcumin, because it would have otherwise been degraded in the endosome/lysosome compartments [80]. Thus, NL-Curc-TAT can be considered as a promising strategy for AD treatment.

Delivery routes of liposomal SHMs in the treatment of NDs

The delivery route is another significant factor determining the efficiency of brain-targeted drug delivery. Strategies have been developed to enhance the brain targeting of SHMs. For local delivery, drugs are injected directly into brain parenchyma or ventricles via a catheter. Convection-enhanced delivery (CED) is a local delivery approach that has successfully been used to deliver liposomes to primate brains [81]. However, this approach requires accurate imaging guidance, and the short drug diffusive distances as well as high cost limit its wider application [82,83].

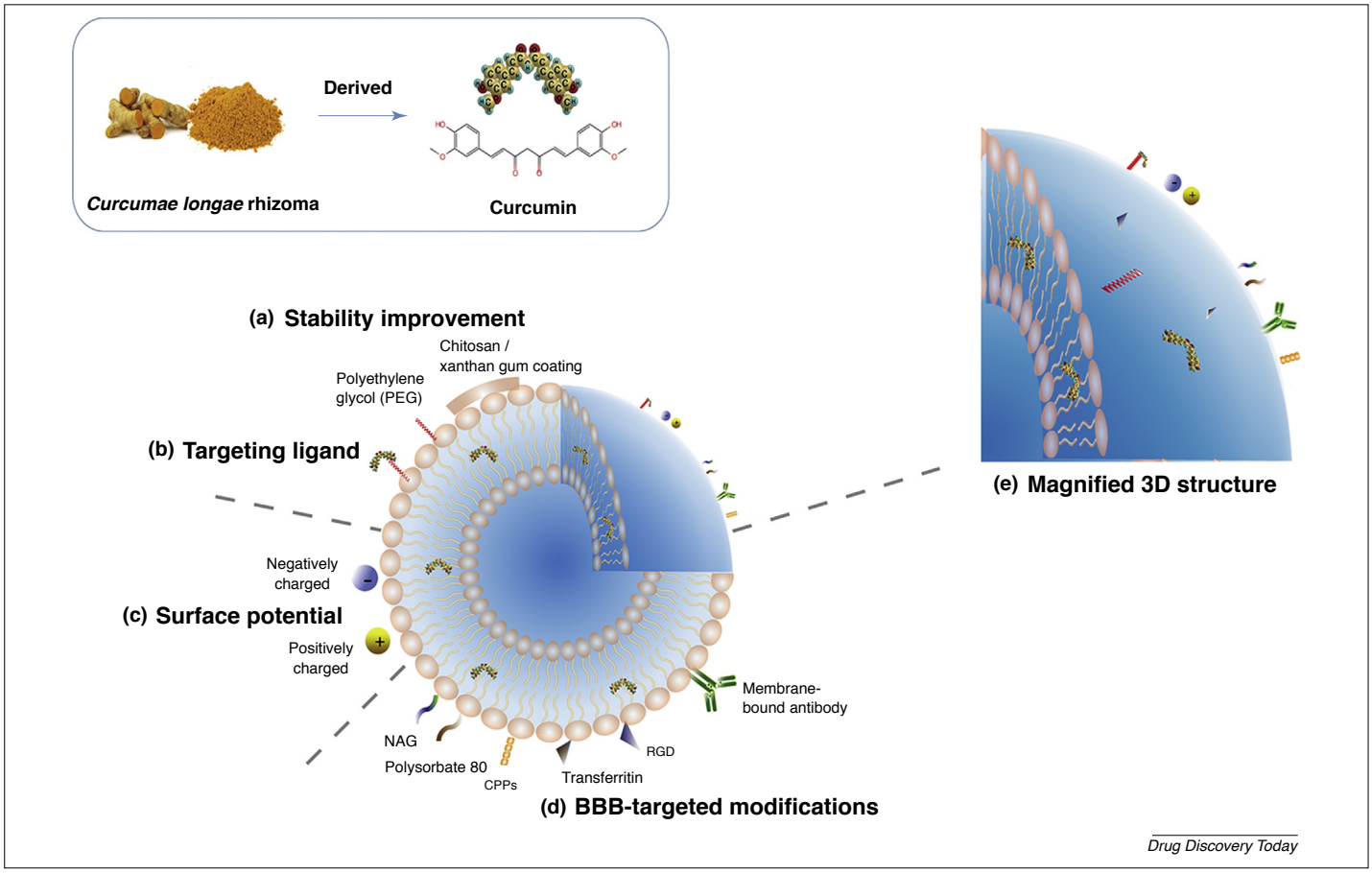

FIGURE 2

Liposomal surface engineering for drug delivery of curcumin as a representative small hydrophobic molecule (SHM). (a) Stability improvement: polyethylene glycosylation (PEGylation) improved the liposome stability. Biomolecules, such as xanthan gum and chitosan, are used to coat the outside of liposomes to increase their stability and mucoadhesiveness during nasal drug delivery. (b) Surface decoration by curcumin: hydrophobic curcumin directly bound to the liposomal surface via hydrophilic polymers, such as PEG, enhances direct binding to aggregated toxic proteins. (c) Surface potential: modification by cationic or anionic groups affects the brain penetration of liposomes to different levels. (d) Blood–brain barrier (BBB)-targeted modifications: liposomes can be engineered to specifically target the brain by attaching ligands, such as *N*-acetyl glucosamine (NAG), cell-penetrating peptides (CPPs), transferritin, arginine-glycine-aspartic acid (RGD), and membrane-bound antibodies on their outer surfaces. In particular, polysorbate 80 as a P-glycoprotein (P-gp) inhibitor enhances the BBB penetration of liposomal-delivered SHMs. Curcumin on the outer surface and within the hydrophobic liposomal lipid bilayer is represented by green stars.

Intranasal administration has shown advantages over conventional delivery systems. These advantages include: (i) bypassing the BBB, which is usually impermeable to SHMs; and (ii) avoiding passage of drugs into liver cells, where maximum metabolism, degradation, and elimination of major fraction of SHMs occur [84–86]. For example, intranasal administration of liposome-loaded curcumin has been shown to be more efficient in improving cognitive function and attenuating neuroinflammation in AD rat models compared with aqueous curcumin solutions [87].

Intranasally administered drugs can enter the brain either directly (via branches of the trigeminal nerve and olfactory bulbs) or indirectly (via systemic circulation) [88,89]. However, the exact mechanism and delivery efficiency (%) of intranasal delivery of such hydrophobic drugs have not been fully determined. Hence, estimating the amount of SHMs needed to be administered poses a significant problem. To circumvent this issue, two major parameters for nose-to-brain delivery have been established: (i) drug-targeting efficiency (DTE %); and (ii) direct transport percentage (DTP %). DTE describes the percentage of drug reaching the brain following intranasal administration relative to intravenous administration. DTP describes the percentage of intranasal drug delivery relative to other possible pathways of drug reaching the brain [90]. Other challenges for intranasal delivery of therapeutic agents include dealing with nasal metabolizing enzymes and mucociliary clearance. Moreover, the properties of small molecules, such as size, and lipophilicity, also affect their delivery to CNS following intranasal administration.

Challenges and future directions

Despite considerable applications of SHMs, such as DA and curcumin, there remains a bottleneck for the clinical translation of the liposome-assisted drug delivery of SHMs. Major reasons are attributed to their pharmaceutical manufacturing, government regulations, and intellectual property (IP) [91,92]. A significant challenge to their manufacturing is the quality consistency of SHM-encapsulated liposomes. As reported, the stability of liposomes can be affected by temperature, cholesterol content, and pH [68], and the surface charges of liposomes can also bring long-term stability problems, challenging the reliability and reproducibility of the manufacturing process. In addition, liposome production on an

industrial scale is another challenge. Optimization of the processing methods is required to reduce the manufacturing steps and the use of organic solvents [93]. Furthermore, although the surface coating of liposomes increases the brain delivery efficiency (e.g., [70]), it renders the system more complex and could bring challenges for large-scale good manufacturing (cGMP) production [74].

In addition, given that multiple patents might be associated with the biomedical applications of liposomal encapsulated SHMs, the pathway from invention to commercialization should be simplified to reduce the time and expense on IP issues [79]. Last but not least, a cost–benefit analysis is inevitable for the clinical translation of liposomal-based SHM therapies. Thus, communication and collaboration between experts in all stages, including manufacturing, laboratory research, and clinical evaluation, are strongly recommended.

Thus, liposomes carrying SHMs could provide solutions to overcome the limitations of SHMs *in vivo*. So far, results are encouraging, and our improved understanding of the challenges for clinical translation will guide the rational design of liposomal delivery systems with high-quality assurance and cost–benefit balances [91,92,94,95].

Acknowledgments

This work was supported by research grants HKBU12101417, HMRF 14150811, and HMRF 15163481 from the Hong Kong GovernmentNSFC 81773926, NSFC 81703487 from the Natual Science Foundation Council of China, and research grants FRG II 16-17/018, FRG I/17-18/0201, and FRG II/17-18/021 from Hong Kong Baptist University to M.L.

References

1 Disease, G.B.D. *et al.* (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388, 1545–1602

2 Kalia, L.V. and Lang, A.E. (2015) Parkinson's disease. *Lancet* 386, 896–912

3 Green, R.C. *et al.* (2008) Safety and efficacy of tarenflurbil in subjects with mild Alzheimer's disease: results from an 18 month multicenter phase 3 trial Late breaking news. *Eur. J. Neurol.* 15 412–412

4 Yurasov, V.V. *et al.* (1997) Effect of long-term parenteral administration of empty and L-Dopa-loaded liposomes on the turnover of dopamine and its metabolites in the striatum of mice with experimental Parkinson's syndrome. *Bull. Exp. Biol. Med.* 123, 126–129

5 Di Martino, P. *et al.* (2017) Nano-medicine improving the bioavailability of small molecules for the prevention of neurodegenerative diseases. *Curr. Pharm. Des.* 23, 1897–1908

6 Vieira, D.B. and Gamarra, L.F. (2016) Getting into the brain: liposome-based strategies for effective drug delivery across the blood-brain barrier. *Int. J. Nanomed.* 11, 5381–5414

7 Cao, X. *et al.* (2016) Effects and molecular mechanism of chitosan-coated levodopa nanoliposomes on behavior of dyskinesia rats. *Biol. Res.* 49, 32

8 Lopalco, A. *et al.* (2018) Transferrin functionalized liposomes loading dopamine HCl: development and permeability studies across an *in vitro* model of human blood–brain barrier. *Nanomaterials* 8 http://dx.doi.org/10.3390/nano8030178

9 Qu, M. *et al.* (2018) A brain targeting functionalized liposomes of the dopamine derivative N-3,4-bis(pivaloyloxy)-dopamine for treatment of Parkinson's disease. *J. Control. Release* 277, 173–182

10 Nageeb El-Helaly, S. *et al.* (2017) Electrosteric stealth Rivastigmine loaded liposomes for brain targeting: preparation, characterization, ex vivo, bio-distribution and *in vivo* pharmacokinetic studies. *Drug Deliv.* 24, 692–700

11 Corace, G. *et al.* (2014) Multifunctional liposomes for nasal delivery of the anti-Alzheimer drug tacrine hydrochloride. *J. Liposome Res.* 24, 323–335

12 Al Asmari, A.K. *et al.* (2016) Preparation, characterization, and *in vivo* evaluation of intranasally administered liposomal formulation of donepezil. *Drug Des. Dev. Ther.* 10, 205–215

13 Di Legge, A. *et al.* (2011) Phase 2 trial of nonpegylated doxorubicin (Myocet) as second-line treatment in advanced or recurrent endometrial cancer. *Int. J. Gynecol. Cancer* 21, 1446–1451

14 Wagner, S. *et al.* (2008) Pegylated-liposomal doxorubicin and oral topotecan in eight children with relapsed high-grade malignant brain tumors. *J. Neurooncol.* 86, 175–181

15 Benesch, M. and Urban, C. (2008) Liposomal cytarabine for leukemic and lymphomatous meningitis: recent developments. *Expert Opin. Pharmacother.* 9, 301–309

16 Samudre, S.T. *et al.* (2015) Xanthan gum coated mucoadhesive liposomes for efficient nose to brain delivery of curcumin. *Drug Deliv. Lett.* 5, 201–207

17 Daneman, R. and Prat, A. (2015) The blood–brain barrier. *Cold Spring Harb. Perspect. Biol.* 7 a020412

18 Ballabh, P. *et al.* (2004) The blood–brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol. Dis.* 16, 1–13

19 Zlokovic, B.V. (2008) The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57, 178–201

20 Spuch, C. and Navarro, C. (2011) Liposomes for targeted delivery of active agents against neurodegenerative diseases (Alzheimer's disease and Parkinson's disease). *J. Drug Deliv.* 2011, 469679

21 Loureiro, J.A. *et al.* (2015) Dual ligand immunoliposomes for drug delivery to the brain. *Colloids Surf. B: Biointerfaces* 134, 213–219

22 Lai, F. *et al.* (2013) Liposomes for brain delivery. *Expert Opin. Drug Deliv.* 10, 1003–1022

23 Xu, S. *et al.* (2013) Targeting receptor-mediated endocytotic pathways with nanoparticles: rationale and advances. *Adv. Drug Deliv. Rev.* 65, 121–138

24 Nisi Zhang, F.Y. (2018) Localized delivery of curcumin into brain with polysorbate 80-modified cerasomes by ultrasound-targeted microbubble destruction for improved Parkinson's disease therapy. *Theranostics* 8, 2264–2276

25 Moretti, R. *et al.* (2007) Rivastigmine and Parkinson dementia complex. *Expert Opin. Pharmacother.* 8, 817–829

26 Birks, J. and Harvey, R.J. (2006) Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst. Rev.* 2006 CD001190

27 Ay, M. *et al.* (2017) Molecular mechanisms underlying protective effects of quercetin against mitochondrial dysfunction and progressive dopaminergic neurodegeneration in cell culture and MitoPark transgenic mouse models of Parkinson's Disease. *J. Neurochem.* 141, 766–782

28 Thanvi, B. *et al.* (2007) Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. *Postgrad. Med. J.* 83, 384–388

29 Summers, W.K. (2006) Tacrine, and Alzheimer's treatments. *J. Alzheimers Dis.* 9 (Suppl. 3), 439–445

30 Darvesh, A.S. *et al.* (2012) Curcumin and neurodegenerative diseases: a perspective. *Expert Opin. Investig. Drugs* 21, 1123–1140

31 Cole, G.M. *et al.* (2007) Neuroprotective effects of curcumin. *Adv. Exp. Med. Biol.* 595, 197–212

32 Small, G.W. *et al.* (2018) Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: a double-blind, placebo-controlled 18-month trial. *Am. J. Geriatr. Psychiatry* 26, 266–277

33 Yang, J. *et al.* (2014) Neuroprotective effect of curcumin on hippocampal injury in 6-OHDA-induced Parkinson's disease rat. *Pathol. Res. Pract.* 210, 357–362

34 Wang, Z.Y. *et al.* (2017) Neuroprotective natural products for the treatment of Parkinson's disease by targeting the autophagy-lysosome pathway: a systematic review. *Phytother. Res.* 31, 1119–1127

35 Yang, K.Y. *et al.* (2007) Oral bioavailability of curcumin in rat and the herbal analysis from Curcuma longa by LC-MS/MS. *J. Chromatogr. B* 853, 183–189

36 Ziling Li, S.P. *et al.* (2018) Pluronics modified liposomes for curcumin encapsulation: sustained release, stability and bioaccessibility. *Food Res. Int.* 108, 246–253

37 Feng, T. *et al.* (2017) Liposomal curcumin and its application in cancer. *Int. J. Nanomed.* 12, 6027–6044

38 Kuo, Y.C. *et al.* (2017) Wheat germ agglutinin-conjugated liposomes incorporated with cardiolipin to improve neuronal survival in Alzheimer's disease treatment. *Int. J. Nanomed.* 12, 1757–1774

39 Kuo, Y.C. and Lin, C.C. (2015) Rescuing apoptotic neurons in Alzheimer's disease using wheat germ agglutinin-conjugated and cardiolipin-conjugated liposomes with encapsulated nerve growth factor and curcumin. *Int. J. Nanomed.* 10, 2653–2672

40 Sokolik, V.V. and Maltsev, A.V. (2015) Effect of curcumin liposomal form on angiotensin converting activity, cytokines and cognitive characteristics of the rats with Alzheimer's disease model. *Biotechnol. Acta* 8, 48–56

41 Mourtas, S. *et al.* (2014) Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. *Eur. J. Med. Chem.* 80, 175–183

42 Magalingam, K.B. *et al.* (2015) Quercetin glycosides induced neuroprotection by changes in the gene expression in a cellular model of Parkinson's disease. *J. Mol. Neurosci.* 55, 609–617
43 Dajas, F. *et al.* (2015) Quercetin in brain diseases: potential and limits. *Neurochem. Int.* 89, 140–148
44 Rezaei-Sadabady, R. *et al.* (2016) Intracellular ROS protection efficiency and free radical-scavenging activity of quercetin and quercetin-encapsulated liposomes. *Artif. Cells Nanomed. Biotechnol.* 44, 128–134
45 Kuo, Y.C. and Tsao, C.W. (2017) Neuroprotection against apoptosis of SK-N-MC cells using RMP-7- and lactoferrin-grafted liposomes carrying quercetin. *Int. J. Nanomed.* 12, 2857–2869
46 Kulkarni, S.S. and Canto, C. (2015) The molecular targets of resveratrol. *Biochim. Biophys. Acta* 1852, 1114–1123
47 Tellone, E. *et al.* (2015) Resveratrol: a focus on several neurodegenerative diseases. *Oxid. Med. Cell Longev.* 2015, 392169
48 Csiszar, A. *et al.* (2015) Resveratrol encapsulated in novel fusogenic liposomes activates Nrf2 and attenuates oxidative stress in cerebromicrovascular endothelial cells from aged rats. *J. Gerontol. A: Biol. Sci. Med. Sci.* 70, 303–313
49 Wang, Y. *et al.* (2011) Protective effect of resveratrol derived from *Polygonum cuspidatum* and its liposomal form on nigral cells in parkinsonian rats. *J. Neurol. Sci.* 304, 29–34
50 Eloy, J.O. *et al.* (2017) Immunoliposomes: a review on functionalization strategies and targets for drug delivery. *Colloids Surf. B: Biointerfaces* 159, 454–467
51 Kreuter, J. (2014) Drug delivery to the central nervous system by polymeric nanoparticles: what do we know? *Adv. Drug Deliv. Rev.* 71, 2–14
52 Raposo, G. and Stoorvogel, W. (2013) Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* 200, 373–383
53 Dua, J. *et al.* (2012) Liposome: methods of preparation and applications. *Int. J. Pharm. Stud. Res.* 3, 14–20
54 Marin, E. *et al.* (2017) New curcumin-loaded chitosan nanocapsules: in vivo evaluation. *Planta Med.* 83, 877–883
55 Papadia, K. *et al.* (2017) Multifunctional LUV liposomes decorated for BBB and amyloid targeting – B. *In vivo* brain targeting potential in wild-type and APP/PS1 mice. *Eur. J. Pharm. Sci.* 102, 180–187
56 Mourtas, S. *et al.* (2014) Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. *Eur. J. Med. Chem.* 80, 175–183
57 Kundu, P. *et al.* (2016) Delivery of dual drug loaded lipid based nanoparticles across the blood-brain barrier impart enhanced neuroprotection in a rotenone induced mouse model of Parkinson's disease. *ACS Chem. Neurosci.* 7, 1658–1670
58 Vaz, G.R. *et al.* (2017) Development of nasal lipid nanocarriers containing curcumin for brain targeting. *J. Alzheimers Dis.* 59, 961–974
59 Mourtas, S. *et al.* (2011) Curcumin-decorated nanoliposomes with very high affinity for amyloid-beta1-42 peptide. *Biomaterials* 32, 1635–1645
60 Subodhkant, S. *et al.* (2015) Xanthan gum coated mucoadhesive liposomes for efficient nose to brain delivery of curcumin. *Drug Delivery Lett.* 5, 201–207
61 Zhang, N. *et al.* (2018) Localized delivery of curcumin into brain with polysorbate 80-modified cerasomes by ultrasound-targeted microbubble destruction for improved Parkinson's disease therapy. *Theranostics* 8, 2264–2277
62 Meng, F. *et al.* (2015) A novel LDL-mimic nanocarrier for the targeted delivery of curcumin into the brain to treat Alzheimer's disease. *Colloids Surf. B Biointerfaces* 134, 88–97
63 Lv, Z. *et al.* (2018) Optimization of the preparation conditions of borneol-modified ginkgolide liposomes by response surface methodology and study of their blood brain barrier permeability. *Molecules* 23
64 Liu, X. *et al.* (2013) Protective effects of cationic bovine serum albumin-conjugated PEGylated tanshinone IIA nanoparticles on cerebral ischemia. *Biomaterials* 34, 817–830
65 Dhawan, S. *et al.* (2011) Formulation development and systematic optimization of solid lipid nanoparticles of quercetin for improved brain delivery. *J. Pharm. Pharmacol.* 63, 342–351
66 Shao, X.R. *et al.* (2017) Effects of micro-environmental pH of liposome on chemical stability of loaded drug. *Nanoscale Res. Lett.* 12 http://dx.doi.org/10.1186/s11671-017-2256-9
67 Qiang, F. *et al.* (2012) Enhanced systemic exposure of fexofenadine via the intranasal administration of chitosan-coated liposome. *Int. J. Pharm.* 430, 161–166
68 Sukowski, W.W. *et al.* (2005) The influence of temperature, cholesterol content and pH on liposome stability. *J. Mol. Struct.* 744–747, 737–747
69 Allijn, I.E. *et al.* (2016) Comparison of pharmaceutical nanoformulations for curcumin: enhancement of aqueous solubility and carrier retention. *Int. J. Pharm.* 506, 407–413
70 Chen, H. *et al.* (2012) N-trimethyl chitosan chloride-coated liposomes for the oral delivery of curcumin. *J. Liposome Res.* 22, 100–109
71 Choi, H.S. *et al.* (2010) Rapid translocation of nanoparticles from the lung airspaces to the body. *Nat. Biotechnol.* 28, 1300–1303
72 Lazar, A.N. *et al.* (2013) Curcumin-conjugated nanoliposomes with high affinity for Aβ deposits: possible applications to Alzheimer disease. *Nanomedicine* 9, 712–721
73 Taylor, M. *et al.* (2011) Effect of curcumin-associated and lipid ligand-functionalized nanoliposomes on aggregation of the Alzheimer's A β peptide. *Nanomedicine* 7, 541–550
74 Teli, M.K. *et al.* (2010) Nanotechnology and nanomedicine: going small means aiming big. *Curr. Pharm. Des.* 16, 1882–1892
75 Aharon, D. *et al.* (2011) The effect of liposomes' surface electric potential on the uptake of hematoporphyrin. *Biochim. Biophys. Acta Biomembr.* 1808, 2031–2035
76 Lockman, P.R. *et al.* (2004) Nanoparticle surface charges alter blood–brain barrier integrity and permeability. *J. Drug Target.* 12, 635–641
77 Campos-Martorell, M. *et al.* (2016) Charge effect of a liposomal delivery system encapsulating simvastatin to treat experimental ischemic stroke in rats. *Int. J. Nanomed.* 11, 3035–3048
78 Kamal, N. *et al.* (2015) QbD approach to investigate product and process variabilities for brain targeting liposomes. *J. Liposome Res.* 25, 175–190
79 Murday, J.S. *et al.* (2009) Translational nanomedicine: status assessment and opportunities. *Nanomedicine* 5, 251–273
80 Sancini, G. *et al.* (2013) Functionalization with TAT-peptide enhances blood-brain barrier crossing *in vitro* of nanoliposomes carrying a curcumin-derivative to bind amyloid-β peptide. *J. Nanomed. Nanotechnol.* 4, 1–8
81 Krauze, M.T. *et al.* (2009) Convection-enhanced delivery of liposomes to primate brain. *Methods Enzymol.* 465, 349–362
82 Gao, X. *et al.* (2017) Nanoagonist-mediated endothelial tight junction opening: A strategy for safely increasing brain drug delivery in mice. *J. Cereb. Blood Flow Metab.* 37, 1410–1424
83 Tosi, U. *et al.* (2017) Advances in molecular imaging of locally delivered targeted therapeutics for central nervous system tumors. *Int. J. Mol. Sci.* 18 http://dx.doi.org/10.3390/ijms18020351
84 Alam, M.I. *et al.* (2013) Intranasal infusion of nanostructured lipid carriers (NLC) containing CNS acting drug and estimation in brain and blood. *Drug Deliv.* 20, 247–251
85 Mittal, D. *et al.* (2014) Insights into direct nose to brain delivery: current status and future perspective. *Drug Deliv.* 21, 75–86
86 Merkus, F.W. and van den Berg, M.P. (2007) Can nasal drug delivery bypass the blood-brain barrier? questioning the direct transport theory. *Drugs R D* 8, 133–144
87 Vv, S. *et al.* (2017) Comparative analysis of nasal therapy with soluble and liposomal forms of curcumin on rats with Alzheimer's disease model. *J. Alzheimers Dis. Parkinson* 7 http://dx.doi.org/10.4172/2161-0460.1000357
88 Feng, Y. *et al.* (2018) An update on the role of nanovehicles in nose-to-brain drug delivery. *Drug Discov. Today* 23, 1079–1088
89 Quintana, D.S. *et al.* (2016) The promise and pitfalls of intranasally administering psychopharmacological agents for the treatment of psychiatric disorders. *Mol. Psychiatry* 21, 29–38
90 Kozlovskaya, L. *et al.* (2014) Quantitative analysis of drug delivery to the brain via nasal route. *J. Control. Release* 189, 133–140
91 Sercombe, L. *et al.* (2015) Advances and challenges of liposome assisted drug delivery. *Front. Pharmacol.* 6, 1–13
92 Krol, S. (2012) Challenges in drug delivery to the brain: nature is against us. *J. Control. Release* 164, 145–155
93 Jaafar-Maalej, C. *et al.* (2012) Lipid-based carriers: manufacturing and applications for pulmonary route. *Expert Opin. Drug Deliv.* 9, 1111–1127
94 Bozzuto, G. and Molinari, A. (2015) Liposomes as nanomedical devices. *Int. J. Nanomed.* 10, 975–999
95 Grimaldi, N. *et al.* (2016) Lipid-based nanovesicles for nanomedicine. *Chem. Soc. Rev.* 45, 6520–6545