Chapter 8

Liposomal Drug Delivery to the Central Nervous System

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70055

Abstract

Central nervous system diseases represent a huge world of burden of human suffering with negative economic results. Most therapeutic compounds cannot attain the brain because of the blood-brain barrier and its expression of efflux transporters. Among them, the P-glycoprotein plays a significant role leading to failure of various clinical treatments. A non-invasive strategy to circumvent the blood-brain barrier and P-glycoprotein emphasizes on the encapsulation and therefore masking of therapeutic compounds in drug delivery systems. Up to now, liposomes are the most widely studied drug delivery systems due to their biocompatibility, biodegradability, and less toxicity. The incorporation of polyethylene glycol-lipid derivatives within the bilayer of conventional liposomes significantly prolongs liposomal cargo half-life by steric stabilization. Interestingly, an increased brain accumulation of liposomal cargo is achieved by coupling targeting moieties on liposomes surface. These targeting moieties such as peptides or monoclonal antibodies recognize the biochemical transport systems at the blood-brain barrier and mediate the transport of liposomes and their cargo across this barrier. Moreover, stimuli-sensitive liposomes are programmed for cargo release when exposed to a particular microenvironment. Hence, this chapter highlights the potential liposomal applications for delivery of therapeutic compounds as well as diagnostic tools or both, in major central nervous system diseases.

Keywords: central nervous system diseases, blood-brain barrier, P-glycoprotein, liposomes, passive targeting, active targeting, stimuli strategies

1. Introduction

Most neurological disorders compromise the central nervous system (CNS) and its main organ, the brain. These disorders include stroke, brain cancer, Alzheimer's disease, Parkinson's

disease, epilepsy, multiple sclerosis, neuroinfections, and traumatic disorders of the nervous system, among others (http://www.who.int/en/). Because millions of people worldwide are affected by CNS disorders, they constitute 6.3% of the global burden of disease. In other words, CNS diseases are a huge world of burden of human suffering with negative economic results [1]. Most of the therapeutic molecules cannot attain the brain because of the presence of the blood-brain barrier (BBB), which separates the bloodstream from the cerebral parenchyma [2]. This barrier is mainly composed by endothelial cells, which are linked by tight junctions [3]. The BBB also contains a basal membrane, pericytes, and astrocytes [3]. More important is the presence of efflux transporters that perform active back-transport of the therapeutic molecules to the blood lumen. P-glycoprotein (P-gp) is the most important efflux transporter associated to the failure of various therapies to treat CNS diseases [4]. Advances in nanomedicine have created a non-invasive strategy for the management of CNS diseases [5]. This strategy emphasizes on the encapsulation of therapeutic compounds, which are mainly P-gp substrates, in drug delivery systems, also called nanocarriers, such as liposomes, lipid nanocapsules, polymeric nanoparticles, or polymersomes [6–10]. Encapsulation of therapeutic compounds in drug delivery systems improve their solubility and protect them from the biological environment and circumvent the P-gp at the BBB yielding higher concentrations of the therapeutic compounds in the brain parenchyma [5]. Among nanocarriers, liposomes have been the most studied due to their composition, which makes them biocompatible, biodegradable, and less toxic [11]. Liposomes not only hold potential as vehicles for therapeutic compounds (therapeutics) [7] but also for diagnostic tools (diagnostics) [12] directed to the CNS. Interestingly, recent efforts have combined therapeutics and diagnostics in the same unique nanocarrier, thus opening the way to theranostic liposomes, which represent an essential advancement for personalized nanomedicine [13]. To specifically target the therapeutic compound or the diagnostic tool to the pathological site, the CNS, two strategies are usually used [14]: (1) Passive targeting based on the longevity of the pharmaceutical carrier in the blood and its accumulation in pathological sites with compromised vasculature via the enhanced permeability and retention effect and (2) Active targeting based on the attachment of specific ligands to nanocarriers surface to recognize and bind specific biological receptors expressed at the BBB [14]. Later studies propose a new active targeting strategy in which liposomes take advantage of changes in the pathological microenvironment for localized and timely release of their cargo [15]. According to their formulation, stimuli-sensitive liposomes release may obey to internal stimuli such as pH, temperature, redox condition, and enzymatic activity or external stimuli such as magnetic fields, ultrasound, or irradiation [15]. Since innovative strategies are urgently needed to counteract CNS diseases, this manuscript summarizes the most relevant examples of passively and actively targeted liposomes, smaller than 200 nm, for therapeutics, diagnostics, or theranostics of major CNS diseases.

2. The blood-brain barrier

The blood-brain barrier (BBB) is an innate and selective barrier formed by endothelial cells lining ~650 km of microvessels, which constitute by far the largest interface for the blood-brain

exchange (**Figure 1**) [3]. The BBB endothelial cells differ from endothelial cells in the rest of the body by the absence of fenestrations and sparse pinocytic vesicular transport. The BBB endothelial cells display wider tight junctions known as zonulae occludens, and adherens junctions (AJ), which cover the vessels walls as a continuous sheath, leaving no space between cells [16]. Moreover, the BBB is also composed by an extracellular matrix (basal membrane), pericytes, and astrocyte foot processes [2, 4]. Because of this configuration, most molecular traffic takes a transcellular route across the BBB, rather than moving paracellularly as in most endothelia. The presence of specific transport systems on the luminal and abluminal membranes regulates the influx and efflux of various essential endogenous and exogenous substrates [17, 18]. Small gases such as oxygen and carbon dioxide but also small lipophilic agents, such as ethanol, caffeine, nicotine, and drugs like anesthetics and barbiturates, can diffuse freely through the lipid membranes [19]. Small polar molecules, such as glucose, amino acids, organic anions and cations, and nucleosides cross the BBB by carrier-mediated transport. Large solutes, such as proteins and peptides, are transported across the BBB by receptor-mediated or adsorption-mediated endocytic transport [17, 18]. In parallel, it was originally stated that therapeutic compounds transporting across the BBB were dependent on their physicochemical properties such as lipophilicity, molecular weight, and ionic state. However, it is the presence of efflux

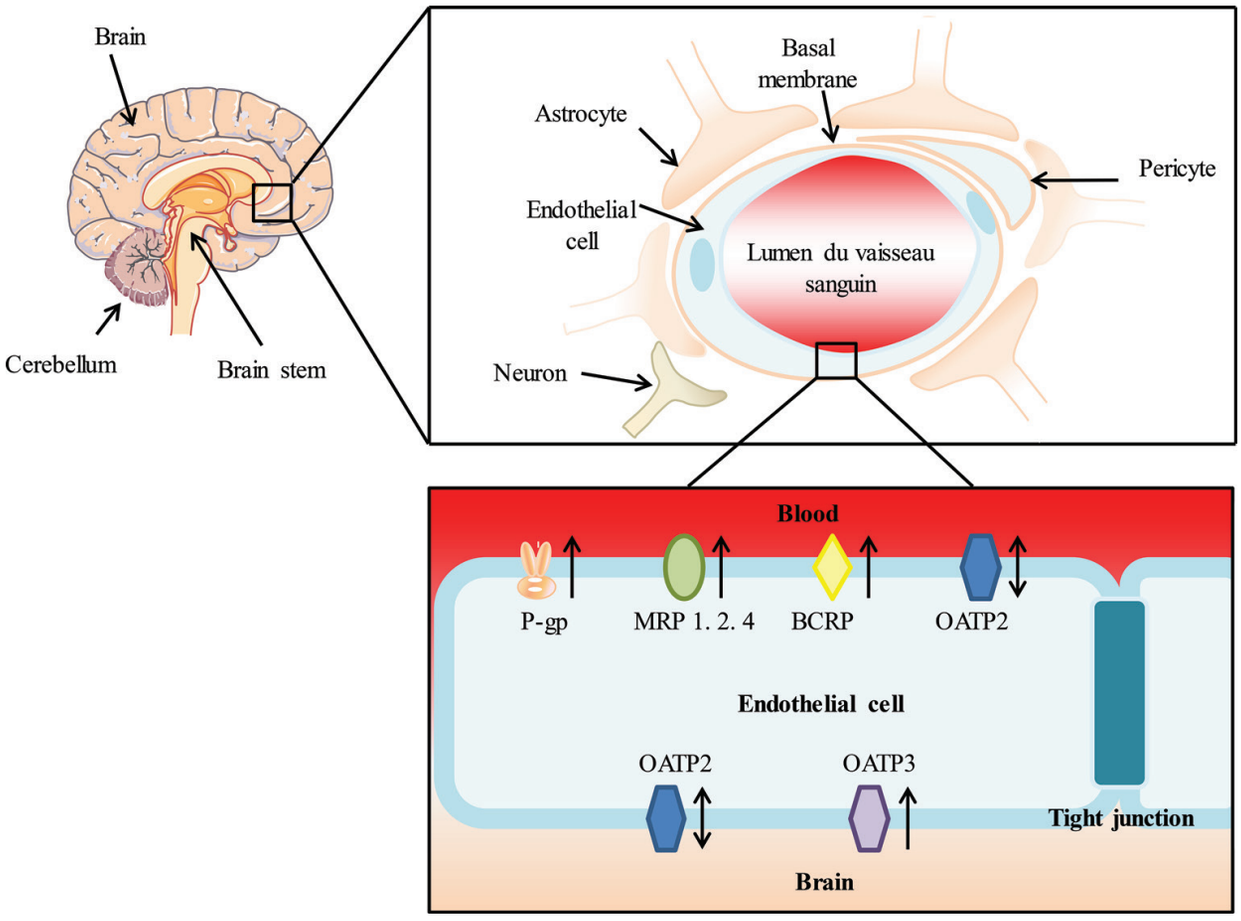

Figure 1. The blood-brain barrier. P-gp = P-glycoprotein, MRP = Multidrug resistance-associated proteins, BCRP = Breast cancer resistance protein, and OATP = Organic anion transporter polypeptide.

transporters at the BBB that limits the brain uptake of a variety of endogenous and exogenous compounds, including relatively lipophilic therapeutic compounds [20]. Most of these efflux transporters belong to the ATP-binding cassette (ABC) transporters super family. ABC transporters are transmembrane proteins that use the energy from the ATP hydrolysis to drive the efflux of their substrates. Based on three critical defining criteria, multi-specificity, location, and energetics; P-glycoprotein is considered to be the most important ABC efflux transporter at the BBB [21].

3. The P-glycoprotein

The expression of P-glycoprotein (P-gp) on endothelial cells at the human BBB was first described in 1989 by Cordon-Cardo et al. and Theibaut et al. [22, 23]. Since then, P-gp has been found at the luminal membrane of the endothelial cells lining the capillaries of the brain [4, 24], in neurons and in astrocytes [25, 26]. The P-gp is also localized at the apical surfaces of the epithelial cells that constitute the ventricular exposed surface of the human choroid plexus [27]. The P-gp was also observed in primary brain tumors [28]. The relevance of the P-gp at the BBB has been properly illustrated in knockout mice lacking the P-gp isoform mdr1a (mdr1a (−/−) mice). The mdr1a (−/−) mice were healthy and fertile and appeared phenotypically normal, but they accumulated much higher levels of P-gp substrates in the brain. A clear example was the increased sensitivity to the centrally neurotoxic pesticide ivermectin [29]. Knockout mice accumulated 100-fold higher concentrations of ivermectin in the brain as compared to wild-type mice; consequently, knockout mice developed a severe neurotoxicity and died [29]. More recently, selamectin, another pesticide also demonstrated to be a P-gp substrate [30]. Meanwhile, pharmacokinetic studies in knockout mice were rapidly extended to therapeutic drugs. Thus, the absence of mdr1a in mice led to highly increased levels of vinblastine, digoxin, and cyclosporin A in the brain [31]. Tissue distribution studies demonstrated that the relative brain penetration of radiolabeled ondansetron and loperamide is increased 4- and almost 14-fold, respectively in mdr1a (−/−) mice. Moreover, a pilot toxicity study showed that the oral administration of loperamide gains potent opiate-like activity in the CNS of mdr1a (−/−) mice. Oral domperidone also showed neuroleptic-like side effects in mdr1a (−/−) mice [31]. Using the same *in vivo* model, it was suggested that antidepressants like levomilnacipran, vilazodone, and escitalopram are P-gp substrates [32], while the modern antiepileptic topiramato is only a weak P-gp substrate [33]. These observations are strongly supported by brain distribution and disease models not only for the above drugs but also for a large list of them [2].

4. Circumventing the BBB

Disruption of the BBB has been observed in various CNS pathologies such as stroke, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) [34]. From these scenarios, it is known that disruption of the BBB leads to increased extravasation of immune cells and

poorly regulated flux of molecules and ions across the BBB with consequent neuroinflammation, neurodegeneration, or infections [35]. Additionally, clinical disruption of the BBB is expensive and requires hospitalization [7]. Therefore, in diseases where the BBB represents an obstacle to attain significant brain concentrations of therapeutic compounds, a less aggressive alternative is by modulating the activity of the P-gp. Nonetheless, we cannot negligate that the P-gp protects the brain from intoxication by endogenous and exogenous harmful lipophilic compounds that otherwise could penetrate the BBB by simple diffusion without any limitation [36]. Therefore, the ideal approach should inhibit the P-gp at the BBB to let the P-gp substrate (therapeutic compound) enter into the brain and then re-induce the P-gp-mediated efflux to hamper the entry of harmful compounds. The development of third-generation P-gp modulators, which transiently and directly inhibit the transport of P-gp substrates, has been a promising approach to modulate the P-gp [37, 38]. Unfortunately, clinical studies suggest high doses of these compounds. These high doses by themselves or in co-administration with P-gp substrates may predict toxic profiles, thus limiting the use of these agents [39]. Several studies have already proposed the use of natural products, the designs of peptidomimetics, and dual activity ligands as a fourth-generation of P-gp modulators [40]. In spite of the countless studies, the effective and safe inhibition of the P-gp at the human BBB is not yet a reality. A non-invasive strategy that takes advantage of the CNS physiology involves nanomedicine [41]. This innovative strategy uses mainly nano-scale drug delivery systems (DDSs) such as liposomes, polymeric nanoparticles, lipid nanocapsules, and polymersomes. Hereafter, DDSs transport small doses of poorly soluble drugs through the body and by-pass the P-gp at the BBB to finally target the brain, thus reducing toxicity in peripheral tissues [5]. A synergistic strategy that had obtained optimistic *in vivo* results tackling the P-gp at the BBB is the concomitant loading of a P-gp substrate and a P-gp inhibitor in the same nanocarrier [42]. Nanomedicine also offers the possibility to transport diagnostic tools as well, thus providing clear benefits to diagnose and treat defiant diseases [43]. Owing to their unique characteristics like biocompatibility, biodegradability, non-inmunogenicity, and less toxicity, liposomes have been the most studied and clinically recognized among nanocarriers [11].

5. Liposomal strategies to target the central nervous system

Nanotechnology is the understanding and control of matters having dimensions roughly within the 1–100 nm range. However, in nanomedicine, particles smaller than 10 nm are quickly cleared by the kidney or through extravasation and particles bigger than 200 nm are efficiently filtered by liver, spleen, and bone marrow, thus a size between 10 and 200 nm would enable liposomes to circulate in the bloodstream [44]. Due to their structure, liposomes have demonstrated their ability as nanocarriers for CNS targeting of hydrophilic or lipophilic cytotoxics, neuroprotectants, antiepyleptics, anti-ischemia, antiretroviral and antifungals drugs among others, and diagnostic agents. Basically, liposomes deliver their cargo across the BBB through passive and active targeting (**Figure 2**). Nonetheless, active targeting goes further, opening a stimuli-responsive strategy.

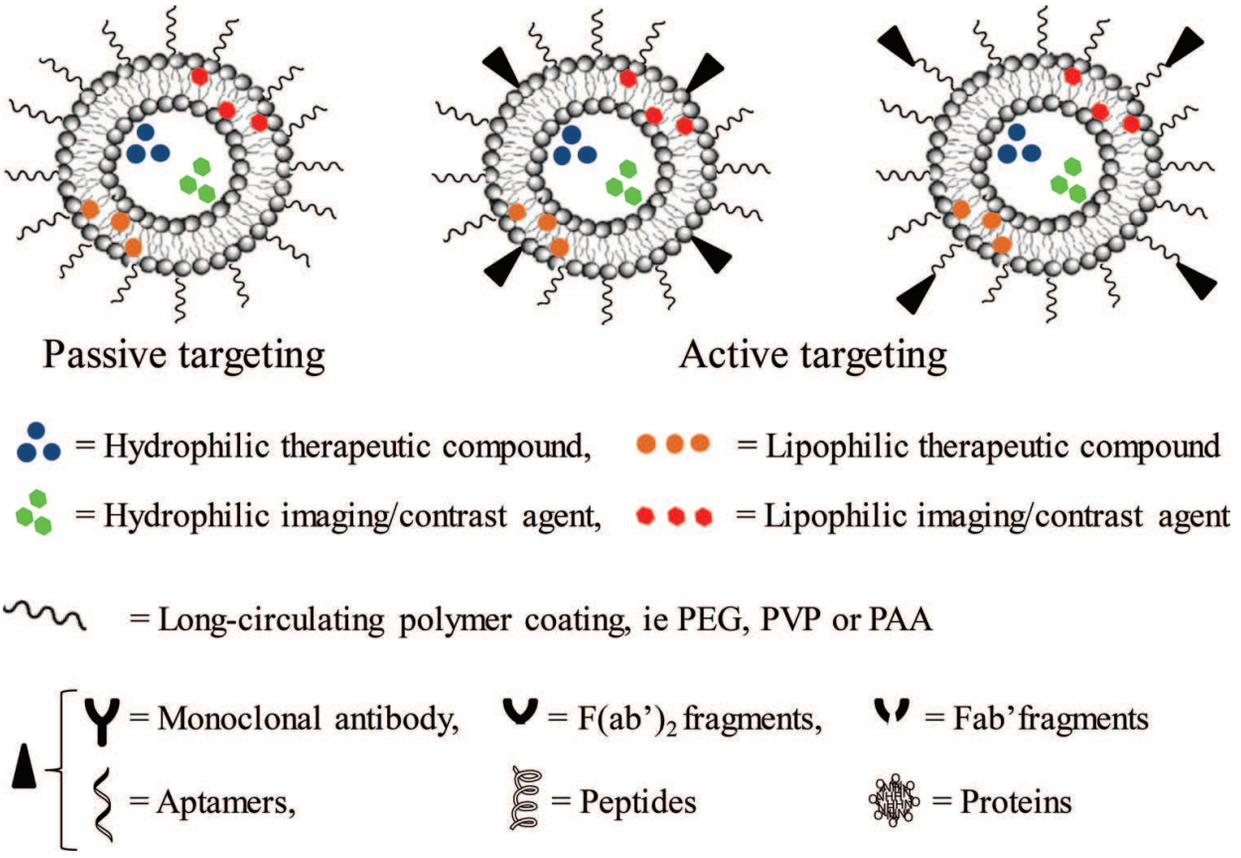

Figure 2. Liposomal strategies for passive and active targeting.

5.1. Passive targeting strategy

Passive targeting is mainly based on the enhanced permeability and retention (EPR) effect. In 1986, Maeda and co-workers named EPR effect to the mechanism in which macromolecules with a high molecular weight, 15000 to 70000 daltons, such as polymers and proteins precipitate and accumulate effectively in tumor tissues [45]. Such high accumulation usually last more than 24 hours [45]. This phenomenon was attributed to the hypervasculature and enhanced vascular permeability in solid tumors, which is due to the overproduction of vascular mediators including bradykinin, nitric oxide (NO), vascular endothelial growth factor (VEGF) and carbon monoxide (CO) [46]. Moreover, solid tumors have defective blood vessels with large gaps, up to 1.0 μm between endothelial cells, whereby macromolecules pass to the tumor [14]. Since solid tumors lack adequate lymphatic drainage, there is therefore a poor circulatory recovery of the extravasated macromolecules, resulting in their accumulation in the tumor microenvironment for long periods [14]. This phenomenon was not observed in healthy blood vessels [45]; hence, it constituted a promising strategy to treat selectively cancer solid tumors using nanocarriers like polymer-coated liposomes [47]. Prolonged blood circulation may allow a longer interaction time between liposomes and the target. The incorporation of soluble, hydrophilic, flexible and biocompatible polymers such as polyethylene glycol (PEG) or its derivatives within the bilayer of conventional liposomes leads to the formation of a protective and hydrophilic layer on their surface. This prevents the recognition

of liposomes by opsonins and reduces their clearance by the reticuloendothelial system (RES) and consequently extends the liposomal half-life [48]. Other prominent synthetic polymers with stealth properties are poly(vinyl pyrrolidone) (PVP) and poly(acryl amide) (PAA) [11]. Liposomes size is another parameter with high impact on the passive targeting through the EPR effect. Long-circulating liposomes also called stealth liposomes should possess a size inferior to 400 nm for effective extravasation [14, 48]. Different studies demonstrated that passive targeting provides promising therapeutic outcomes in diseases where there is a BBB disruption like stroke [49, 50].

5.2. Active targeting strategy

In the last few years, more sophisticated liposomes were designed to actively target the brain. Active targeting lies on the coupling of targeting moieties including small-molecule ligands, peptides, aptamers, monoclonal antibodies (mAbs) or their fragments on the liposomal surface. Then these functionalized liposomes are able to target the brain after recognizing the biochemical transport systems expressed at the brain endothelial cells. Such systems are the adsorptive-mediated endocytosis (AME), the carrier-mediated transport (CMT), and the receptor-mediated endocytosis (RME). Brain targeting through AME is based on the electrostatic interaction between a positively charged moiety and the negatively charged sites on the luminal surface of plasma membrane and brain capillaries. For instance, cationized human serum albumin conjugated to PEGylated liposomes showed a rapidly time-dependent response taken up by cultured porcine brain capillary endothelial cells and by intact brain capillaries [51]. However, *in vivo*, AME also occurs to a large extent in other organs like liver and kidneys, thus decreasing brain specificity [6]. The CMT systems are localized at the brain capillary endothelium and mediate the passage of small molecular weight nutrients across the BBB. The most studied are the transporters for D-glucose (GLUT1), large neutral amino acid (LAT1), small neutral amino acids (EAAT), cationic amino acids (CAT1), monocarboxylic acids (MCT1), and organic cations (OCT) [52]. Although a possible competition with endogenous ligands is predicted, liposomes decorated with LAT1 were able to penetrate the BBB penetration *in vivo* [53]. RME is one of the major mechanisms by which various DDSs can deliver their cargo across the BBB. RME systems require the binding of a ligand to a specific receptor located on the luminal membrane of the BBB [6]. Then, the receptor-ligand binding induces the internalization of receptor-ligand complexes within an endocytic vesicle. From there forward, receptors may mediate different processes including: (1) transcytosis of the ligand from blood to brain, (2) reverse transcytosis from brain to blood, or (3) only endocytosis into the brain capillary endothelium without net transport across the endothelial cell [52]. Specific receptors of the brain capillary endothelium have been identified for low-density lipoproteins (LDL), low-density lipoprotein receptor-related protein 1 (LRP-1), insulin, insulin-like growth factors (IGF-I, IGF-II), interleukin-1 (IL-1), folic acid (FA) and transferrin (Tf) [52]. Hence, attachment of these endogenous ligands to the surface of liposomes has generated promising results [54]. Besides binding endogenous ligands, these receptors also bind mAbs or their fragments (Fab', $F(ab')_2$), which could be grafted on the liposomes surface [6]. The most successful mAb that has been studied and used for brain targeting is OX26 [55, 56], which specifically targets brain capillary endothelial cells, thanks to the high concentration

of the transferrin receptor (TfR) expressed on their luminal side [57]. Thus, OX26 may be able to cross the BBB via the receptor-mediated transcytosis. OX26 does not bind the TfR on the transferrin-binding site but uses another epitope [58]. Given that high doses of insulin are required to target the insulin receptor and that an overdose of insulin could cause hypoglycemia, some studies effectively promote grafting of liposomes with the murine 83–14 mAb to target the BBB via the insulin receptor [59]. The low-density lipoprotein receptor (LDLR) and the LDLR-related protein (LRP) can bind multiple ligands. Among them, the apolipoprotein E grafted to liposomes favored the internalization of the DDS via the LDLR in porcine brain capillary endothelial cells and the rodent cell line RBE4 [60]. Moreover, increased expression of epidermal growth factor receptor (EGFR) [61], vascular endothelial growth factor receptor (VEGFR) [62] and integrins [63] in the brain environment is associated with brain injury or blood-brain barrier (BBB) dysfunction, thus providing more targets in these pathological episodes.

5.3. Stimuli-responsive strategy

Usually the challenge is to formulate liposomes which have the right size and structure to entrap their cargo with high efficiency and in such a way that they do not leak out. On the other hand, it is important to play on the fluidity of the liposomal membrane. A too high liposomal stability is rather disadvantageous than desired. Remaining inside the stable liposomes, the encapsulated compounds are not delivered to the targeted tissue. At that point, it is necessary to find the right balance between stability in the bloodstream and a high delivery of liposomal cargo in the target. In general, the chemical and biophysical properties of lipid molecules primarily dictate the development of tunable (stimuli-sensitive) liposomes [64]. The various types of stimuli that could trigger liposomal cargo release can be classified into internal or intrinsic to the target tissue (changes in pH, temperature, redox condition, or the activity of certain enzymes) and external or artificially applied (magnetic field, ultrasound, and various types of irradiation) [15]. In one example, the lower pH, the higher temperature, and overexpression of several proteolytic enzymes of the tumor microenvironment should trigger the cytotoxic release when liposomes are exposed [14]. More exhaustive literature about stimuli-sensitive liposomes was formerly described [14, 15].

The encapsulation of nanoparticles in liposomes not only adds more stimuli for delivery but also provides more useful properties. For instance, by encapsulating PEG-coated quantum dots (QDs) in the internal aqueous phase of liposomes, a more extensive fluorescent staining is observed in a solid tumor model compared to free PEGylated QDs [65]. Meanwhile, superparamagnetic iron oxide nanoparticles (SPIONs) loaded in liposomes demonstrated to serve as a magnetic resonance imaging *in vivo* tool [66].

6. Liposomes for drug delivery to the central nervous system

More recent and relevant studies applying different liposomal targeting strategies for the treatment of major CNS diseases are summarized herein.

6.1. Stroke

An ischemic stroke occurs because of an obstruction, by a blood clot, within a blood vessel supplying blood to the brain [67]. Ischemic stroke accounts for 87% of all stroke cases. The cerebral ischemic area is composed of the ischemic core, a zone of irreversibly damaged tissue, and the ischemic penumbra, a surrounding zone of less severe and reversible damaged tissue [67]. To date, the only Food and Drug Administration (FDA) approved treatment for ischemic stroke is tissue plasminogen activator (tPA), a proteolytic enzyme. tPA enhances the conversion of plasminogen to plasmin, which subsequently degrades the fibrin matrix in the clot and improves blood flow to the ischemic region [68]. However, its short half-life, 2–6 minutes and therapeutic time window, less than 4.5 hours, elicite its administration in high doses which might lead to significant hemorrhagic complications [69, 70]. Interestingly, hemorrhage was reduced when tPA was loaded in actin-targeted liposomes and intravenously administered by the internal carotid artery in an *in vivo* model bearing clots injected [71]. Other therapeutic approaches have focused on protecting neurons from the main pathogenic mechanisms causing ischemic injury in the penumbra, such as excitotoxicity, oxidative stress, inflammation, or apoptosis [67]. Certainly, loading of these neuroprotective agents in liposomes may return improved results. Various studies emphasized on passive targeting strategies because during stroke the BBB is disrupted. Therefore, the effect of intravenous administration of empty [^{3}H]-labeled PEG-liposomes in a stroke rat model was investigated [50]. One hour after middle cerebral artery occlusion (MCAO), rats received the liposomal formulation and one hour after, reperfusion was started (t-MCAO) [50]. [^{3}H]-labeled PEG-liposomes accumulated in the ischemic brain in a time-dependent manner. Such accumulation at 3 hours post-dosing was significantly higher compared to the one in the non-ischemic side. These results were attributed to the disruption of the BBB and the leakage of liposomes to the brain parenchyma, where they gradually accumulated in the ischemic region via the EPR effect. Usually, once reperfusion is started, secondary cerebral damage known as ischemic/reperfusion (I/R) injury is observed [50]. In the same study, intravenous administration of PEGylated liposomes loaded with tacrolimus, a neuroprotective agent and a P-gp substrate [2] before (I/R) injury significantly suppressed cerebral cell death. While the damage volume for PEG-liposomes encapsulating tacrolimus was about 0.2 cm^3; for free tacrolimus and PBS, it was ~0.3 and ~0.4 cm^3, respectively. This formulation also suppressed superoxidative anions induced-damage in the brain and improved motor function deficits compared to free tacrolimus [50]. Fasudil, a Rho-kinase inhibitor is an approved drug for cerebral vasospasm after subarachnoid hemorrhage but thanks to its neuroprotective properties, it could be a promising candidate for the treatment of ischemic stroke. Phase III clinical trials showed fasudil usefulness and safety [72], however, the clinical trials were finished because of fasudil poor clinical efficacy, short permanence in the bloodstream and difficulty to penetrate the BBB [49]. Hence, it was encapsulated in PEG-liposomes and intravenously administered immediately after reperfusion in t-MCAO rats [49]. Fasudil-loaded PEG-liposomes diffused and accumulated in the I/R region, from an early phase after administration up to 24 hours. Moreover, the aforementioned formulation significantly suppressed the volume of damaged brain tissue, obtaining ~0.2 cm^3, compared to free fasudil, ~0.3 cm^3, and PBS, ~0.4 cm^3. Fasudil-loaded PEGylated liposomes also reduced in a significant manner neutrophil invasion and improved the motor functional

disorder [49]. The success of this study was basically due to PEGylation and liposome size. Using the same stroke *in vivo* model, it was confirmed that~100 nm PEG-liposomes got a high accumulation on the ischemic side, ~200 nm PEG-liposomes showed a lower accumulation and no accumulation was observed for ~800 nm PEG-liposomes [49]. Xenon is a pleiotypic cytoprotective gas, which rapidly diffuses across the BBB. Although xenon has few clinical adverse effects, its administration by inhalation requires intubation and ventilation with a large xenon concentration that reduces the maximum fraction of inspired oxygen [73]. Thus, an ultimate study encapsulated xenon into echogenic liposomes and determined its benefits after systemic administration in t-MCAO rats. Different dosage schemes demonstrated that this formulation effectively reduced ischemic neuronal cell death and improved neurological function. Undoubtely, ultrasound triggered additional liposomal xenon release obtaining still better therapeutic results [73]. Other studies accentuate on the benefits of actively targeted strategies over passively targeted strategies during stroke (**Table 1**). For instance vascular endothelial growth factor (VEGF) was loaded in PEGylated liposomes decorated with transferrin and intravenously administered two days after inducing a t-MCAO model [74]. VEGF confers neuroprotection, promotes neurogenesis and cerebral angiogenesis, and transferrin is an iron-binding glycoprotein with high affinity for the transferrin receptor (TfR) at the BBB [75]. While the damage volume for VEGF-loaded PEGylated liposomes coupled to transferrin was about ~2.5 cm^3, for VEGF-loaded PEGylated liposomes was ~3.0 and for saline ~3.5 cm^3, respectively. VEGF-induced neovascularization in the penumbra zone was significantly higher for the actively targeted formulation (245,873 microvessels per field), than for the passively targeted formulation (139,801.3) and for saline (102,175.5) [74].

6.2. Cancer

Globocan 2012 revealed that the worldwide brain and CNS cancer incidence and mortality in both sexes was 3.4 and 2.5 per 100,000 people, respectively [76]. In parallel, the World Cancer Research Fund International (http://www.wcrf.org) estimated that 256,000 new cases of brain and CNS cancer were diagnosed in 2012. Gliomas are tumors that arise from glial or precursor cells and include astrocytoma, glioblastoma, oligodendroglioma, ependymoma, mixed glioma, malignant glioma, and a few rare histologies. Glioma accounts for 27% of all tumors and 80% of malignant tumors, and among these, glioblastoma is the most common accounting for 46.1% [77]. Unfortunately, the efficacy of conventional chemotherapy is always limited due to the poor specificity in targeting cancer, low circulation time, reduced penetration in the tissue, and most importantly the toxic side effects of anti-cancer drugs [78]. Thus, nanotechnology appeared to help chemotherapy to be reborn and Doxil®, liposomal doxorubicin, received the first approval as a nano-drug in 1995 [79]. Nowadays, several research groups are developing nanocarriers to encapsulate anti-cancer drugs and fight against cancer tumors. Owing to the leaky nature of the tumor-associated blood vessels and lack of adequate lymphatic drainage, nanocarriers may take advantage of the EPR effect to target tumors [14]. However, in the case of brain tumors, nanocarriers must first overcome the BBB, which remains intact at the early stage of the brain tumor development. Only when the tumor grows to a certain volume and angiogenesis begins, the BBB is impaired and the blood-brain tumor barrier (BBTB) then becomes the main obstacle that nanocarriers must

Disease	Year	Size (nm)	Targeting ligand	Drug/diagnose agent	Administration route	Results	Refs.
Stroke	2015	~100	HAIYPRH (T7) peptide	ZL006	Intravenous	Increased liposomal transport across the BBB, reduced infarct volume and improved neurological deficit	[117]
	2013	~160	Anti-NR1-receptor antibody	Superoxide dismutase enzyme	Intracarotid	Reduced infarct volume, inflammatory markers, and improved *in vivo* behavior	[118]
	2010	60–90	*p*-aminophenyl-α-d-mannoside	CDP-Choline	Intravenous	Reduced ischemia-reperfusion in young and aged animals	[119]
	2010	~105	Transferrin	VEGF	Intravenous	Reduced infarct volume and increased neovascularization	[74]
	2003	200–250	Antiactin antibody	tPA	Intravenous	Reduced tPA-induced hemorrhage	[71]
	2013	~100	Anti-HSP72 antibody	Citicoline/gadolinium or rhodamine	Intravenous	Increased liposomal transport across the BBB and reduced infarct volume	[120]

Disease	Year	Size (nm)	Targeting ligand	Drug/diagnose agent	Administration route	Results	Refs.
Cancer	2016	~110	PTD peptide	Epirubicine and celecoxib	Intravenous	Increased liposomal transport across the BBB, survival time and anti-vasculogenic mimicry effects	[121]
	2015	100-120	R8-dGR peptide	Paclitaxel	Intravenous	Increased survival time	[122]
	2015	~110	R8-c(RGD)	Paclitaxel	Intravenous	Increased survival time and anti-vasculogenic mimicry and anti-brain cancer stem cells effects	[123]
	2014	~95	Glutathione	Doxorubicin	Intravenous	Increased doxorubicin levels in brain, tumor growth inhibition, and increased survival	[81]
	2014	~100	Glutathione	Doxorubicin	Intravenous	Increased doxorubicin levels in brain	[83]
	2014	105	WGA	Daunorubicin, quinacrine and tamoxifen	Intravenous	Increased liposomal transport across the BBB and survival time	[124]
	2014	~120	RGD and transferrin	Paclitaxel	Intravenous	Increased liposomal transport across the BBB and antiproliferative activity against C6 cells	[125]
	2013	~180	Transferrin and folate	Doxorubicin	Intravenous	Increased tumor growth inhibition, survival time, and apoptotic activity in glioma cells	[54]
	2009	100-110	WGA	Topotecan and tamoxifen	Intravenous	Increased survival time	[86]
	2015	~100	pH-sensitive valve	Gd-DTPA	Intravenous	Improved cargo release in tumor microenvironment	[126]
	2014	~120	Endoglin	Gd	Intravenous	Increased signal emmited by Gd in tumor periphery	[127]
	2016	~180	(RGD-TPGS	Docetaxel/QD	Intravenous	Increased docetaxel levels and QD fluorescence in brain	[128]

Disease	Year	Size (nm)	Targeting ligand	Drug/diagnose agent	Administration route	Results	Refs.
AD	2015	~200	Transferrin	α-Mangostin	Intravenous	Increased α -mangostin levels in brain	[129]
	2015	~110	Glutathione	Amyloid-targeting antibody fragments	Intravenous	Increased amyloid-targeting antibody fragments levels in plasma and brain	[130]
	2013	~180	CPP	Rivastigmine	Intranasal	Increased rivastigmine levels in hippocampus and cortex	[92]
	2012	~150	DSPE-PEG_{3400}-XO4	DSPE-PEG_{3400}-XO4	Intravenous	Liposomal transport across the BBB and binding to Aβ plaques	[131]
PD	2012	~120	OX26	GDNF	Intravenous	A partial rescue of nigra-striatal neurons	[104]
	2011	~110	Chlorotoxin	L-dopa	Intraperitoneal	Increased dopamine levels in substantia nigra and striata and attenuated behavioral disorders	[102]

Abbreviations: CDP-Choline = cytidine 5′ diphosphocholine, VEGF = vascular endothelial growth factor, tPA = tissue plasminogen activator, PTD = glycine-arginine-lysine-lysine-arginine-arginine-glutamine-arginine-arginine-arginine-cysteine-glycine-NH_2 peptide, RGD = arginine-glycine-aspartic acid peptide, WGA = wheat germ agglutinin, Gd-DTPA = gadolinium-diethylendiaminopentaacetic acid, Gd = gadolinium, RGD-TPGS = arginine-glycine-aspartic acid peptide-D-alpha-tocopheryl polyethylene glucol 1000 succinate, QD = quantum dots, CPP = cell penetrating peptide, DSPE-PEG_{3400}-XO4 = 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy-XO4-(polyethylene glycol-3400)] sodium salt and GDNF = glial-derived neurotrophic factor.

Table 1. Recent studies based in active targeted liposomes for CNS diseases.

http://dx.doi.org/10.5772/intechopen.70055

circumvent. Hence, the presence of receptors on the BBB and BBTB provides a pathway to actively target brain tumors (**Table 1**) [80]. A recent *in vivo* study showed the difference between passive and active brain targeting of doxorubicin, a P-gp substrate [2]. PEGylated liposomal [^{14}C]-labeled doxorubicin similar to Doxil®/Caelyx® was used as the passive targeting formulation, whereas Glutathione PEGylated liposomal [^{14}C]-labeled doxorubicin (2B3-101) was assessed as the active delivery system [81]. Glutathione is an endogenous tripeptide currently used as a drug-targeting ligand because among the nutrient transporters in mammalian species, glutathione transporter has a preferential expression at the BBB [82]. After intravenous administration, both liposomal formulations displayed a similar doxorubicin pharmacokinetic profile and brain exposure during the first 24 hours. However, 4 days post-dosing, the brain doxorubicin concentration as well as its brain-to-plasma ratio was higher for Glutathione PEGylated liposomes. Compared to passive liposomes, active liposomes resulted in a significant inhibition of tumor growth and two animals of this group showed a complete tumor regression. Moreover, the active delivery system exhibited an increase of 16.1% in the median survival compared to the passive delivery system and an increase of 38.5% compared to saline [81]. Later, the same research group, using the sophisticated cerebral open flow microperfusion (cOFM) brain sampling technique, found that Glutathione PEGylated liposomes enhanced doxorubicin concentration in the brain extracellular space ~5-fold relative to PEGylated liposomes [83]. 2B3-101 was recently investigated in a phase I/IIa clinical study in patients with solid tumors, brain metastases, or recurrent malignant glioma (www.clinicaltrials.gov). Another approach emphasized on doxorubicin loaded liposomes dually functionalized with transferrin and folate to actively target an *in vivo* brain C6 glioma-bearing model [54]. While transferrin binds the TfR at the BBB [75], folate or folic acid binds the folate receptor (FR) [84], which is over-expressed in a wide variety of human tumors and whose density increases as the stage of cancer worsens [84]. After four intravenous administrations in 17 days, the mean survival time was 30 days for rats treated with this active targeting formulation, 27 days for doxorubicin-loaded liposomes, 24 days for doxorubicin solution and 20 days for saline. Doxorubicin-loaded liposomes functionalized with transferrin and folate also exhibited the least tumor area and the highest apoptotic activity in the glioma cells among all the treated groups [54] and did not modify liver enzyme levels or heart histology [54]. Due to its active targeting mechanism of receptor-mediated endocitosis and its high affinity for the cerebral capillary endothelium, wheat germ agglutinin (WGA) showed to be a good candidate to target the BBB [85]. Grafted to the surface, WGA favored the transfer of topotecan-tamoxifen-loaded liposomes across the BBB and then targeted brain tumors [86]. Among the four types of topotecan liposomes with or without the P-gp modulator tamoxifen and/or WGA, the one modified with tamoxifen and WGA exhibited the strongest cytotoxic effect against murine glial tumor (C6) cells [86]. Likewise, this formulation achieved the highest inhibitory effect against C6 cells after crossing an *in vitro* BBB (murine brain microvascular endothelial cells/rat astrocytes) model [86]. Moreover, after one week of treatment with the different formulations, the mean survival time of an *in vivo* brain C6 glioma-bearing model was 26 days for topotecan liposomes modified with tamoxifen and WGA, 20 days for topotecan liposomes, 19 days for free topotecan and 15 days for saline. A mean survival time of 31 days was achieved with two weeks of treatment with topotecan liposomes modified with tamoxifen and WGA [86].

6.3. Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative dementia and contributes to 65% of all cases. AD is substantially increased among people aged 65 years or older, leading to progressive decline in memory, thinking, language, and learning capacity [87]. The pathophysiology of AD is related to the injury and death of neurons caused by the progressive production and accumulation of insoluble proteins aggregates, such as amyloid-β (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau [88]. Currently, there is no drug to treat AD; only four FDA-approved compounds are known to relieve AD symptoms. These are donepezil, galantamine, memantine, and rivastigmine [89]. Once donepezil, a weak P-gp substrate [90], was encapsulated in PEGylated liposomes and administered by intranasal route in experimentation animals, it exhibited higher plasma and brain concentrations than free donepezil administered by the same or oral route [91]. In addition, histopathological examination showed that PEGylated liposomal donepezil was safe and non-toxic [91]. Rivastigmine was encapsulated in PEGylated liposomes functionalized with a cell penetrating peptide (CPP), whose proved internalization pathway across the cell membrane is via transduction or endocytosis [92, 93]. This formulation administered by intranasal route demonstrated its capacity to improve rivastigmine distribution and retention in the hippocampus and cortex, which are CNS regions highly affected by AD. This is in comparison with the intravenous administration of rivastigmine solution [92]. The clinical utility of galantamine, which is also a P-gp inhibitor [94], is hampered by its intricate transport across the BBB and its poor retention in the CNS. Hence, galantamine was loaded in PEGylated liposomes functionalized with a synthethic peptide, Lys-Val-Leu-Phe-Leu-Ser [95]. The selected peptide possess a 75% similar sequence to the serpin enzyme complex-receptor (SEC-R), which is expressed on the surface of neural (PC12) cells and may interact with soluble and non-toxic Aβ-peptides [96]. Thus, fluorometry and confocal microscopy confirmed that actively targeted liposomes significantly facilitated a higher uptake and accumulation of galantamine in PC12 neuronal cells related to non-targeted PEGylated liposomes [95]. The utility of neuroprotective agents in AD was also optimized by encapsulating them in actively targeted liposomes (**Table 1**).

6.4. Parkinson's disease

Parkinson's disease (PD) is the second most common progressive neurodegenerative brain disorder of insidious onset. This chronic disease is caused by a selective degeneration of dopaminergic neurons in the substantia nigra pars compacta, which consequently results in a reduction in striatal dopamine levels [97]. PD is generally characterized by primary motor symptoms such resting tremor, bradykinesia, rigidity, and postural instability. Non-motor symptoms experienced by PD patients may include cognitive impairment, mood disorders, and sleep disturbances [98]. Up-to-date, there is no cure for PD, the only available treatment, dopamine, is focused on the signs and symptoms. Since exogenous dopamine cannot cross the BBB, the gold standard therapy for PD is based on the administration of the natural precursor of dopamine, L-dopa, to restore dopaminergic transmission [99]. Because L-dopa is a P-gp substrate [100], it only crosses the BBB to a certain extent and once in the brain, it is

converted to dopamine. However, L-dopa cannot be administered alone because it is catalyzed to dopamine by peripheral dopamine-decarboxylase enzyme and causes peripheral side effects, such as nausea, sleepiness, and dyskinesia [99]. Thus, L-dopa was encapsulated in PEGylated and chlorotoxin-functionalized liposomes and studied in an *in vivo* 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP)-induced PD model [101]. Chlorotoxin (ClTx) is a 36-amino acid peptide that exhibits high affinity for brain gliomas and other tumors of neuroectodermal origin [102] but it is also able to bind proliferating vascular endothelial cells [103]. After intraperitoneal injection, the aforementioned formulation significantly increased the distribution of dopamine in the substantia nigra and striata and attenuated the behavioral disorders. Besides, it diminished the MPTP-induced loss of tyrosine hydroxylase-positive dopaminergic neurons as compared with L-dopa in PEGylated liposomes and free L-dopa [101]. Glial-derived neurotrophic factor (GDNF), a rescue of nigra-striatal tract agent was encapsulated in PEGylated liposomes functionalized with the mAb OX26 and administered in a 6-hydroxydopamine-induced PD *in vivo* model [104]. Authors explained the partial rescue of the nigra-striatal tract through the two weeks delayed and single liposomal intravenous administration, thus suggesting that future studies should increase and timely synchronize dosing administration with the onset of the disease [104].

6.5. Epilepsy

Epilepsy is a chronic and often progressive brain disorder, characterized by recurrent seizures, which are brief episodes of involuntary movement involving a part or the whole body. These episodes are caused by excessive electrical discharges from cortical neurons, which can cause visual disturbances, loss of control of bowel or bladder function, and consciousness [105]. According to the World Health Organization, epilepsy affects about 50 million people worldwide but only 70% of patients can be successfully treated while about 30% of patients are resistant or refractory to current available antiepileptics [106]. Earlier research has shown that mainly the activity of the P-gp at the BBB is directly related to anticonvulsants resistance [107]. This so-called medically intractable epilepsy is often associated with a poor prognosis, increased morbidity and mortality in patients, and a negative social impact in the life of patients and their family environment [106]. Unfortunately, only few studies have tried to improve the epilepsy therapy by applying nanotechnology. In a pilocarpine-induced seizure *in vivo* model, nimodipine, a neuroprotective agent [108] and a P-gp substrate [109] encapsulated in liposomes prevented epileptic seizures and mortality compared to free nimodipine [110]. In the meantime, curcumin, another neuroprotective agent [111] with ability to inhibit the P-gp [112], encapsulated in liposomes delayed the onset and decreases the duration of epileptic seizures in a pentylenetetrazole-induced seizure *in vivo* model [113]. In the same way, the anticonvulsivant activity of gossypin, a bioflavonoid isolated from *Hibiscus vitifolius* and possible P-gp inhibitor [114], was significantly improved when it was entrapped in liposomes. Liposomal gossypin succeeded in increasing seizures threshold and latency of current electroshock seizures pentylenetetrazole-induced seizure *in vivo* model [115]. Earlier studies demonstrated that liposomal anticonvulsivants as valproic acid and phenytoin exerted more prominent therapeutic efficacy than free drugs [116].

7. Liposomes for diagnostics of central nervous system diseases

Liposomes by themselves do not have any imaging property but various efforts have enabled liposomes to entrap and deliver diagnostic agents in pathological tissues. Most investigations deal with diagnosis of cancer.

7.1. Cancer

By attaching synthetic pH-responsive chemical modulators to an *Escherichia coli* mechanosensitive ion channel of large conductance (MscL), it was properly converted in a pH-sensitive valve able to gate at acidic environments such as solid tumors, sites of inflammation, endosomes, and lysosomes [132]. The sensitivity and pH interval for channel opening were tuned by varying the hydrophobicity and pK_a of the pH modulators. At a pH lower than the pKa of the modulator, the channel acquires a charge in the pore of the channel, which tends to the opening [132]. Later, these pH-sensitive valves were incorporated in PEGylated liposomes loaded with paramagnetic chelate gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA), which is detectable *in vivo* by magnetic resonance imaging (MRI). The aforementioned formulation, Gd-DTPA-pH-sensitive PEGylated liposomes was compared to Gd-DTPA-pH-insensitive PEGylated liposomes in mice implanted with a C6 glioblastoma tumor which has a pH between 6.6 and 7.0 [126]. While Gd-DTPA-pH-sensitive PEGylated liposomes started to release their cargo ten minutes post-dosing and lasted up to forty minutes, the Gd-DTPA-pH-insensitive PEGylated liposomes showed a slow initial release that only stabilize after ten minutes and was significantly lower than the pH-sensitive formulation. This study demonstrated that only few ion channels per liposome are sufficient to induce the release of content [126]. The strategy used herein is advantageous and highly supported over other pH-sensitive liposomes containing high amounts of negatively charged lipids, polymers, or unsaturated lipids. These materials make liposomes prone to fast bloodstream clearance affecting basically their pharmacokinetic properties and thus those of the encapsulated cargo [126]. Usually, the targeting moiety is conjugated to the liposomal surface and the whole formulation is assessed *in vitro* or *in vivo*. Nonetheless, a two-step active targeting is also possible and it was the case for molecular imaging of delineating tumor margins in a C6 glioma-bearing model [127]. Herein, the biotin-streptavidin ligation technique was used for its reability to attach antibodies on liposomes surface [133]. Gadolinium was used as the imaging agent and endoglin (CD105), a protein involved in angiogenesis, was used as the targeting moiety. Hence, experimentation animals received an intravenous injection of biotin-endoglin and after 24 hours, injection of streptavidin-PEGylated liposomes loaded with gadolinium. In this way, in the tumor periphery, the signal emitted by gadolinium from the two-step targeting was about 59% higher than those obtained for the usual one-step targeted liposomes and non-targeted liposomes [127].

8. Liposomes for theranostics of central nervous system diseases

A major achievement of nanomedicine in the last few years was the development of theranostic delivery systems, which integrate imaging and therapeutic functions in one single but

complex structure, thus providing a powerful approach to improve disease-specific detection, treatment, and follow-up monitoring [134]. The flexible composition of liposomes enables them to be engineered to adsorb, entrap, encapsulate, or conjugate different imaging agents and therapeutic compounds [134].

8.1. Stroke

Citicoline (CDP-Choline), a drug used in the treatment of stroke [135], was loaded in liposomes made of phospholipids containing rhodamine or gadolinium, which enabled the nanocarriers to be traceable by fluorescence or MRI. These liposomes were functionalized with anti-HSP72 antibody, which is able to bind the HSP72 protein. HSP72 protein is a biomarker expressed for up to seven days in the peri-infarct region following cerebral ischemia [120]. This formulation was administered by intravenous route after surgery in an MCA *in vivo* model, where it achieved a damage of 30% volume smaller than the one obtained with free citicoline. This could be attributed to the 80% traceable liposomal localization on the periphery of the ischemic lesion [120].

8.2. Cancer

Liposomes co-encapsulating the cytotoxic and P-gp substrate docetaxel [2] and QDs and actively targeted with arginine-glycine-aspartic acid peptide -*D*-alpha-tocopheryl polyethylene glycol 1000 succinate (RGD – TPGS) were developed and tested *in vivo* for brain targeting [128]. QDs are semiconductor nanocrystals with a photostability up to 100–10000 fold greater than conventional organic dyes. Unluckily, because QDs are heavy metals, they may lead to potential toxicities, but their encapsulation in liposomes may improve their biocompatibility to become a potential tool for diagnostics in *in vitro* and *in vivo* tumor models [136]. TPGS, a derivative of the natural vitamin E (alpha-tocopherol), has shown great potential in overcoming the P-gp via inhibition of its ATPase activity [42]. RGD peptide binds preferentially the $\alpha v\beta 3$ integrin, an adhesion molecule highly expressed on activated endothelial cells, new-born vessels and some tumor cells [137]. In this context, targeting tumor cells or tumor vasculature by RGD-based strategies is a promising approach [138]. Hence, docetaxel-QDs-loaded liposomes functionalized with RGD-TPGS intravenously administered in rats, exhibited a docetaxel brain distribution ~2-fold higher than the value obtained for docetaxel-QDs-loaded liposomes functionalized with TPGS and ~7-fold higher than the value obtained for free docetaxel [128]. These data is in line with the strongest fluorescence of brain sections for docetaxel-QDs-loaded liposomes functionalized with RGD-TPGS, mild fluorescence for docetaxel-QDs-loaded liposomes functionalized with TPGS and no fluorescence for free QDs [128]. Surely TPGS inhibited the P-gp allowing a higher brain distribution of liposomal docetaxel related to free docetaxel [128]. Another study formulated magnetoliposomes co-loaded with doxorubicin and SPIONs and coated with carboxymethyl dextran (CMD), a stealth alternative to PEGylation [139]. Typically, superparamagnetic nanoparticles coated with carboxydextran are used as MRI agents to detect tumors and their microenvironment [140]. Thus, *in vitro*, this formulation demonstrated to be an efficient T_2-weighed contrast agent for MRI but also induced cytotoxicity which could be enhanced by low-frequency

alternating magnetic field [139]. Further *in vivo* data could determine the usefulness of this formulation as a potential carrier for targeting diagnostic and therapy to brain cancer.

8.3. Parkinson's disease

QDs and apomorphine, a rescue medication for Parkinson's disease were encapsulated in PEGylated liposomes [141]. Then, *in vivo* bioimaging analysis determined that the fluorescence emitted from PEGylated liposomal QDs after intravenous administration was higher in the brain than in other organs and it lasted up to 60 minutes. In contrast, the fluorescence derived from free QDs was visualized immediately following the injection, decreased rapidly in the brain but lasted up to 35 minutes in liver [141]. Likewise the brain uptake of liposomal apomorphine at 1 hour post-dosing was 2.4-fold higher than the value obtained for free apomorphine. Cell uptake studies in bEND3 cells suggested that these theranostic liposomes could enter into cells by clathrin-dependent and caveola-mediated endocytosis [141].

9. Future outcomes

Nanomedicine has emerged as the key to open the door of the medicine of tomorrow. Thanks to exponential efforts and improvements, nanomedicine has launched to the clinical field various drug-loaded liposomes for brain targeting (**Table 2**). However, regarding other CNS diseases, nanomedicine has not yet fulfilled its promise. The possible improved brain distribution of various liposomal therapeutic compounds and diagnostic agents remains to be studied. The CNS is so complex that gives us a wide variety of receptors to target. Currently, various targeting moieties such as aptamers, peptides, mAbs, or their fragments have proved their ability to target a specific receptor in the CNS. Thus, future studies should investigate their *in vivo* potential but always paying attention to the grafting itself. Attachment of targeting moieties does not alter the liposomal biodistribution; it only increases the liposomal internalization in targeted cells. Thus, the quantity of targeting

Therapeutic compound	Purpose: to assess	Patients	Phase
Cytarabine (DepoCyte®)	The safety of whole brain radiotherapy	Brain metastases	I
Cytarabine	The effectiveness in co-administration with high doses of methotrexate	SNC metastases	II
Doxorubicin	The effectiveness	Refractory solid brain tumors	I
Vincristine (Marqibo)	The safety, activity, and pharmacokinetics	Refractory solid brain tumors in children and adolescents	I/II
Doxorubicin (2B3-101)	The safety, tolerability, and pharmacokinetics alone or in combination with trastuzumab	Solid tumors and brain metastases or recurrent malignant glioma	I/IIa

Data obtained from www.clinicaltrials.gov

Table 2. Current completed clinical trials based on liposomal formulations for CNS diseases.

ligand should not compromise the liposomal long-circulating properties conferred by PEG or another polymer. The amount of surface PEG-lipid complex necessary for creating stealth liposomes varies between 5 and 10 mol% and the optimal PEG-derivative length should have a molecular weight of 2000 daltons [142]. If a PEG-derivative is used as the spacer to graft the targeting ligand, the amount of PEG molecules to guarantee steric stabilization must not be inferior to 5 mol% [143]. Since steric hindrance of the PEG chains may interfere with the targeting moiety recognition by the targeted tissue, functionalization of liposomes with two PEG chain lengths was proposed. While PEG_{2000} would confer long circulating properties, PEG_{5000} would be used as linker to overexpose the targeting ligand to targeted cells [56]. To ensure sustained cargo release, an alternative to PEGylation, is the integration of pre-encapsulated loaded liposomes within depot polymeric scaffolds. This strategy attempts to provide ingenious solutions to limitations of conventional liposomes such as short plasma half-lives, toxicity, stability, and poor control of cargo release over prolonged periods [144]. The lack of liposomal toxicity information in the pre-clinical stage is another issue that could hamper the success of these DDSs. Liposomal synthesis protocols must ensure the absence of impurities from organic solvents, free surfactants, and heavy metals, which could contribute to bias results. Because, toxic effects could arise from organic solvent residues, latest studies accentuate on liposomes formulations using organic solvents-free methods [145]. Although nanomedicine aims to prolong the half-life of the cargo incorporated in the DDS, some pharmacotherapies would need a chronic administration. In this sense, an exhaustive study of empty liposomes toxicity would help to exploit their best usage in different therapies.

10. Conclusions

Nanomedicine and especially liposomes represent a step forward to deliver diagnostic agents and/or therapeutic compounds in CNS diseases such as stroke, brain cancer, AD, PD, or epilepsy. Therefore, this manuscript outlines the most recent and relevant engineered liposomal strategies to cirvumvent the BBB and its main efflux transporter, the P-gp. Moreover, important aspects to further optimize liposomal strategies are discussed at the end of this chapter.

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