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Review

Colloidal carriers and blood–brain barrier (BBB) translocation: A way to deliver drugs to the brain?

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Abstract

The major problem in drug delivery to the brain is the presence of the blood–brain barrier (BBB) which limits drug penetration even if in certain pathological situations the BBB is partly disrupted. Therefore, various strategies have been proposed to improve the delivery of drugs to this tissue. This review presents the status of the BBB in healthy patients and in pathologies like neurodegenerative, cerebrovascular and inflammatory diseases. The second part of this article aims to review the invasive and non-invasive strategies developed to circumvent the BBB and deliver drugs into the brain. The use of nanotechnologies (liposomes, nanoparticles) is especially discussed in the ultimate part of the review evidencing their potentiality as non-invasive technique in the brain delivery of drugs with the possibility to target specific brain tissue thanks to ligand linked to carrier surface.

Keywords: Blood–brain barrier; Brain pathologies; Drug delivery; Colloidal carriers

Contents

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1. The status of the blood–brain barrier in the healthy patients

Drug delivery to the brain is a challenge, because this tissue benefits from a very efficient protective barrier. The same mechanisms that protect the brain from foreign substances also restrict the entry of many potentially therapeutic agents. The blood–brain barrier (BBB) is the major barrier to the passage of active molecules from the blood compartment to the brain. It is located at the level of the brain capillaries, where there is a convergence of different cell types: endothelial cells, pericytes, astrocytes and microglias (perivascular macrophages). The brain microvessel endothelial cells (BMEC) that form the BBB, display important morphological characteristics such as the presence of tight junctions between the cells, the absence of fenestrations and a diminished pinocytic activity, that together help to restrict the passage of compounds from the blood into the extracellular environment of the brain. Tight junctions provide significant transendothelial electrical resistance (TEER) to BMEC and impede the penetration of potential therapeutic agents such as oligonucleotides, antibodies, peptides and proteins (Lo et al., 2001). Furthermore, BMEC express a variety of enzymes, both cytosolic and on the extracellular membrane which also contribute to the restrictive nature of the BBB (Bodor and Buchwald, 1999). P-glycoprotein (P-gp) is also present in the luminal plasma membrane of BMEC. This is an ATP-dependent efflux pump and a member of a family of intrinsic membrane proteins. P-gp is known to prevent the intracellular accumulation of an extensive variety of chemotherapeutic agents and hydrophobic compounds (Terasaki and Hosoya, 1999). Under normal conditions the BBB acts as a barrier to toxic agents and safeguards the integrity of the brain. Nevertheless, several disorders and diseases can affect the brain leading to some loss of BBB integrity.

2. The status of the BBB in disease

The major neurological diseases affecting the brain may be categorized as neurodegenerative, cerebrovascular, inflammatory (infectious or autoimmune) and cancerous.

2.1. Neurodegenerative diseases

2.1.1. Alzheimer disease (AD)

Alzheimer's disease is a prevalent form of adult onset dementia. It results in the progressive deterio-

ration of cognitive ability and memory, which is related to the degeneration of basal forebrain cholinergic neurons. Amyloid β (Aβ), a heterogeneous 39–43 amino acid peptide is the main constituent of the senile plaques and cerebrovascular deposits, the primary lesions in AD. The origin of the Aβ deposited in cerebral vasculature and brain is uncertain. According to the "neuronal theory", Aβ is produced locally in the brain. On the contrary, the "vascular theory" proposes that Aβ originates from the circulation and that circulating Aβ could contribute to neurotoxicity by crossing the BBB (Zlokovic, 1997). Transport of several peptides and proteins through the BBB is possible via receptor-mediated transcytosis (Pardridge, 1995). In this way, Mackinc et al. (1998) suggest that the receptor RAGE is involved in the transcytosis of a synthetic peptide (^{125}I-sAβ_{1-40}) homologous to human Aβ and it could play an important role in the development of AD. Currently, the only specific pharmacological therapeutic option available for AD patients is the treatment with cholinesterase inhibitors, which provides moderate benefits in a subset of patients for a limited period (Bickel et al., 2001). Additionally, recent studies have shown that nerve growth factor (NGF) may also be useful to prevent cholinergic neuron death following acute trauma (Shoichet and Winn, 2000).

2.1.2. Parkinson's disease (PD)

Parkinson's disease is characterized by the behavioral symptoms like akinesia/bradykinesia, rigidity and tremor (Lindner and Emerich, 1998). At the cellular level, PD manifests in a progressive loss of midbrain dopaminergic neurons of the *substantia nigra* over several years and a concomitant development of a dopaminergic deficit in the projection area, the *striatum*. Among the factors suspected of contributing to the preferential vulnerability of dopaminergic neurons, the oxidative stress is associated with dopamine metabolism. Although current drug therapy of PD is more successful compared to treatments of AD, it does not stop the degenerative process, and pharmacotherapy unfortunately looses effectiveness with progression of the disease (Hughes et al., 1993). Drugs aimed at the reduction of the dopaminergic deficit (L-DOPA, monoamine oxidase B inhibitors, dopamine agonists) remain the mainstay of symptomatic drug treatment. In addition to a loss of effect over time, therapy is accompanied by an increase in frequency and severity of side effects (Bickel et al., 2001).

2.2. Cerebrovascular diseases

The integrity of the BBB in cerebrovascular disease due to hypertension or cardiac bypass is variable, because the underlying cerebral ischemia varies with respect to the mechanism, the severity and the duration (Neuwelt, 2004). After ischemia, drastic reductions in cerebral blood flow in the core of the lesions typically result in rapid cell death within minutes. Activation of cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) and the upregulation of cell adhesion molecules can also be observed during this disease. Lymphocytes can then penetrate the BBB, releasing proteases, particularly metalloproteinases, which induce the opening of BBB. By virtue of this opening, migration and adhesion of neutrophils, monocytes and macrophages, to the site of injury, occur (McIntosh et al., 1998).

2.3. Inflammatory diseases (ID)

2.3.1. Infection

Central nervous system (CNS) infection caused by bacterial, viral, fungal or parasitic pathogens can lead to devastating neurological disability and death. The exact mechanism by which these organisms traverse the BBB is not totally known. It has been postulated that microbial pathogens may overcome the BBB and enter the CNS through paracellular, transcellular and/or "Trojan horse" mechanisms. These mechanisms can operate individually or together but the mechanism can be dependent of infective agent (Huang and Jong, 2001).

2.3.1.1. Cellular pathways to explain how microbial pathogen cross the BBB

Transcellular penetration. Certain infectious agents have been shown to invade the brain directly by passing through the BBB without altering its permeability. Transcellular invasion of BBB has been demonstrated for bacterial and viral pathogens including *Citrobacter freundii* (Badger et al., 1999), *Escherichia coli* (Wang and Kim, 2002), *Streptococcus pneumonie* (Ring et al., 1998) and human immunodeficiency virus type 1 (HIV-1) (Liu et al., 2002; Bobardt et al., 2004). In the case of *C. freundii*, vesicular transport

has been suggested to explain the intracellular location of individual and multiple bacterial cells within single membrane vacuole-like structures, suggesting that *C. freundii* invades vacuoles, replicates itself, and transcytoses through brain endothelial cells (Badger et al., 1999). Specific microbial ligand/BMEC–receptor interaction has been recently suggested as a mechanism of transcellular penetration for microbial agents. Thus, the efficient penetration of *E. coli* K-1 across the BBB is mediated by multiple factors such as specific bacterial proteins (Wang and Kim, 2002).

In the same way, recent evidence suggests that the HIV-1 virus penetrates across the BBB by inducing gp120-mediated adsorptive endocytosis, a vesicular mechanism providing a mode of entry into BMEC (Banks et al., 2001). Given that HIV-1-proteoglycan interactions are based on electrostatic contacts between basic residues in gp120 and sulfate groups in proteoglycans, HIV-1 may exploit these interactions to rapidly enter and migrate through the BBB to invade the brain. These findings were supported by the fact that heparinase and chondroitinase treatment of human BMEC reduced HIV-1 attachment, and gp120-deficient virus fails to bind and to perform transcytosis through human BMEC (Bobardt et al., 2004). Virus entry was completely blocked by heparin, suggesting that HIV-1 binds to cell membrane by way of proteoglycans. Moreover, observations of HBMEC by transmission electron microscopy (TEM) have shown important concentrations of cytoplasmic vesicles of various sizes (from 150 nm to 5 μm) incorporating from a simple virion of HIV-1 to as many as thousand of virions (Liu et al., 2002).

Paracellular entry. A very common feature of an infection of the brain is an increase of BBB permeability. The immune response is characterized by the rapid production and release of inflammatory mediators such as cytokines, chemokines, cellular adhesion molecules, and matrix metalloproteinases at the site of infection. These mediators have been shown to alter the structure and function of the BBB (Wolka et al., 2003). Perturbations of tight and adherent junctions have been shown to play a role in the pathogenesis. The cytokines, predominantly TNF-α and IL-1 (Wolka et al., 2003), and metalloproteinases contribute to open the BBB (Petty and Lo, 2002). The increase of BBB permeability has been attributed to a loss of the tight junctions proteins, occludin and ZO-1, and a redistribution of the adherent junction protein, vinculin (Bolton et al., 1998). Thus, it has been suggested that *S. pneumonie* (Tsao et al., 2002) and HIV-1 (Fiala et al., 1997) take advantage of this modification to penetrate across the BBB.

Trojan horse mechanism. The "Trojan horse" mechanism postulates that infected macrophages cross-activate brain endothelial cells to take up residence in the CNS as infected microglial cells. BMEC and immune cells, activated by cytokines, overexpress adhesion molecules and their ligands, which promotes the binding of circulating immune cells to brain vasculature. Such binding could be the first step in diapedesis, the passage of immune cells across the BBB. This proximity could also facilitate the passage of viral particles between the infected immune cell and the brain endothelial cell, analogous to the transfer of virus between infected immune cells (Liu et al., 2000). Macrophages infected by HIV-1 could cross the BBB allowing this virus to gain entry into the CNS (Persidsky et al., 1999). CNS tissue from HIV-patients has shown via immunohistochemical analysis of the tight junction proteins (occludin and ZO-1) and mononuclear cell-specific antigen CD68, alterations in the BBB integrity and monocyte infiltration. Tight junction disruption in blood vessels has been consistently associated with CD-68 positive mononuclear cell aggregates and/or microglial nodules (Huang and Jong, 2001).

2.3.2. Multiple sclerosis (MS)

Multiple sclerosis is one of the most common inflammatory disorders of the CNS. Its pathological hallmarks are demyelination and cellular infiltration of T cells and macrophages. Experimental autoimmune encephalitis (EAE) is used as an animal model to study the pathogenesis of human MS. However, EAE differs significantly from MS since: (1) there is a pronounced demyelination in MS, while in EAE demyelination is rather sparse and (2) EAE is a monophasic disease, while MS follows a relapsing-remitting or a chronic-progressive pattern. The use of EAE as a model for MS became more valid by the development of chronic-relapsing models (Polman et al., 1988). The initial role of the BBB in the development of this disorder can be explained by two hypotheses: (1) the brain endothelial cells act as sentinels of the inflammatory response, the initiating event causing the secretion of IFN-γ leading to the induction of MHC class II on the luminal surface of the endothelium; then T cells may traverse the BBB upon recognition of neural antigens presented

by MHC class II; (2) T cell activation in the periphery induces expression of adhesion molecules such as integrins and selectins that facilitate interaction with endothelium and migration of T cells across the BBB (Noseworthy et al., 2000). Moreover, glial cells such as microglia, astrocytes and oligodendrocytes play a role in the onset of the disease. Microgial cells act as antigen presenting cells which increases the inflammatory demyelination process. Upon stimulation, astrocytes have been shown to produce a variety of immunoregulatory molecules such as IL-1, IL-3, prostaglandin E, interferons, TNF. A second immunoregulatory function of astrocytes is their ability to express MHC class II antigens after stimulation with IFN-γ. Oligodendrocytes are the cells that synthesize and maintain the myelin in the CNS. However, during MS, cytokines such as TNF can deteriorate these cells, initiating the inflammatory demyelination (Owens and Sriram, 1995). Despite long-term immunotherapy, relapses occur which are commonly treated by repeated intravenous injections of high doses of glucocorticosteroids (GS) as potent antinflammatories. The main goal is to prevent ongoing tissue destruction with loss of oligodendrocytes, axons and neurons leading to permanent functional deficits (Schmidt et al., 2003b).

2.3.3. Brain tumors

Gliomas are the most frequent primary CNS tumors in humans. They are classified into four clinical grades, grade 4 or glioblastoma multiforme (GBM) being the most aggressive. The different types of glioma can be differentiated by histological characteristics reflecting cellular differentiation lineages: astrocytomas, oligodendrogliomas and mixed oligoastrocytomas (Dai and Holland, 2001). The vascular microenvironment determines the pathophysiological characteristics of gliomas, such as edema formation, tumor cell invasiveness. Indeed, endothelial cells, pericytes, and the basement membrane of tumor vessels reveal significant abnormalities when compared to cerebral vessels. An increase in vessel wall thickness is one common feature of glioma vasculature and is attributed to endothelial cell hyperplasia, leading to an increase in non-selective transendothelial transport. Tight junction opening is functionally the most important abnormality and becomes more pronounced with increasing malignancy. Thus, compared with normal human brain, astrocytomas fail to express or express a non-functional form of occludin. Moreover, fenestrations and increase in the number and size of pinocytic vacuoles has been reported (Schlageter et al., 1999; Martin and Jiang, 2001; Papadopoulos et al., 2001a, 2001b; Vajkoczy and Menger, 2001). The gliomas present a particular therapeutic problem because of their poor response to chemotherapy. The resistance of tumors to therapeutic intervention may be due to cellular mechanisms, which are categorized in terms of alterations in the biochemistry of malignant cells. They comprise altered activity of specific enzymes, altered apoptosis regulation, or transport based mechanisms, like the P-gp efflux system, responsible for multi-drug-resistance (MDR). The understanding of structural and functional characteristics of vascular microenvironment in gliomas is essential for the design of successful future therapeutic strategies against this type of tumor. Anticancer drugs are toxic to both tumor and normal cells and the efficacy of chemotherapy is often limited by important side effects (Brigger et al., 2002a).

It is clear from previous sections that the delivery of drugs into the brain is limited in normal and also pathological conditions. Progress in pharmacology and the neurosciences has resulted in greater knowledge of CNS diseases and of potential therapies, but it has also made evident the urgent need to develop new strategies to improve drug delivery to this vital tissue.

3. Strategies for drug delivery to the brain

The diffusion of drugs from the blood into the brain depends mainly upon the ability of the biologically active molecule to traverse lipid membranes. Therefore, numerous drugs do not have adequate physicochemical characteristics such as high lipid solubility, low molecular size and positive charge which are necessary to succeed in crossing BBB. This is the reason why several strategies have been developed to overcome the BBB including invasive and non-invasive techniques.

3.1. Invasive techniques

3.1.1. Disruption of the BBB

One of the earliest techniques to circumvent the BBB for therapeutical purpose and the first to be used in humans was developed by Neuwelt et al. (1979).

The idea behind this approach was to break down the barrier temporarily by injecting a sugar solution (mannitol) into arteries in the neck. The resulting high sugar concentration in brain capillaries sucks water out of the endothelial cells, shrinking them thus opening tight junctions. In current practice, the effect lasts for 20–30 min, during which time drugs that would not normally cross the BBB diffuse freely. This method allows the delivery of chemotherapeutic agents in patients with malignant glioma, cerebral lymphoma and disseminated CNS germ cell tumors, with a subsequent decrease in morbidity and mortality compared with patients receiving systemic chemotherapy alone (Miller, 2002). However, this approach, also causes several undesired side effects in humans, including physiological stress, transient increase in intracranial pressure, and unwanted delivery of anticancer agents to normal brain tissue. In addition, this technique requires considerable expertise for administration.

Beside, vasoactive molecules such as bradykinin (Bartus et al., 1996), leukotriene C4 (Hashizume and Black, 2002) and cereport (Borlongan and Emerich, 2003) have been employed to increase the permeability of brain tumor capillaries but not of healthy brain capillaries. This biochemical modulation strategy involves selective increase in blood–brain tumor barrier (BBTB) permeability to anticancer drugs without affecting the normal BBB. It is based on the divergence of properties between the BBB and the BBTB, because the normal brain capillaries are rich in γ-glutamyltranspeptidase (γ-GTP) – which acts as an enzyme barrier, rapidly degrading the leukotriene – whereas receptors against bradykinin (BK type 2) are lacking which limits the penetration of this molecule in the BBB (Black and Ningaraj, 2004). Cereport has been co-administered with active agents such as carboplatin, showing, in gliomas, a successful reduction in tumor volume and stabilization of tumor growth (Cloughesy et al., 1999).

However, disrupting the BBB even for brief periods leaves the brain vulnerable to infection and damage from toxins. Even substances that circulate harmlessly through the peripheral bloodstream, such as albumin, can have deleterious effects if they enter the brain (Miller, 2002).

3.1.2. Direct drug delivery

3.1.2.1. Alternative pathways to CNS drug delivery. One strategy to overcome the BBB that has been used extensively in clinical trials is the direct administration of drugs by intraventricular and intracerebral. The drugs can be infused intraventricularly using a plastic reservoir (Ommaya reservoir) implanted subcutaneously in the scalp and connected to the ventricules within the brain via an outlet catheter (Chauhan, 2002). Unfortunately, there are several problems, apart from the surgical intervention required. Firstly, in the human brain the diffusion distances from cerebrospinal fluid (CSF) to a drug target site may only be several centimeters, and for drugs relying only on diffusion for penetration, insufficient concentration of drug may reach the target site. Secondly, the microvessels of the brain secrete interstitial fluid at a low but finite rate, generating a flow towards the CSF spaces, which also works against diffusive drug penetration. Finally, the high turnover rate of the CSF (total renewal every 5–6 h in humans) means that injected drug is being continuously cleared back into the blood. In practice, drug injection into the CSF is a suitable strategy only for sites close to the ventricles. For drugs that need to be at elevated levels for long periods for an effective action, continuous or pulsatile infusion may be necessary (Chamberlain et al., 1997).

Intracerebral drug administration differs from systemic drug administration in terms of pharmacokinetic characteristics determining brain tissue concentration, where the available dose reaching the target organ is 100% (Grondin et al., 2003). However, there are large gradients inside the tissue with very high local concentrations at the site of administration and zero concentrations at some distance for macromolecules (Misra et al., 2003).

3.1.2.2. Intracerebral implantation of controlled-release systems. Drug delivery directly to the brain interstitium using polymeric devices release unprecedented levels of drug directly to an intracranial target in a sustained fashion for extended periods of time. The fate of a drug delivered to the brain interstitium from the biodegradable polymer was based on: (i) rates of drug transport via diffusion and fluid convection; (ii) rates of elimination from the brain via degradation, metabolism and permeation through capillary networks; (iii) rates of local binding and internalization (Guerin et al., 2004). Brem's group has demonstrated the feasibility of polymer-mediated drug delivery by using the standard chemotherapeutic agent 1,3-bis(2-

chloroethyl)-1-nitrosourea (BCNU) and showed that local treatment of gliomas by this method is effective in animal models of intracranial tumors. This led to clinical trials for glioma patients, and subsequent approval of Gliadel™ by the FDA (Wang et al., 2002).

Benoît's group has developed a new concept of drug targeting into the CNS by stereotactic implantation of biodegradable microspheres (Benoit et al., 2000). Because of their size, these microparticles can easily be implanted by stereotaxy in functional areas of the brain without damaging the surrounding tissue. Compared to large implants, microparticles do not need open surgery. The feasibility of microencapsulation of glial cell line-derived neurotrophic factor (GDNF) (Aubert-Pouessel et al., 2004; Jollivet et al., 2004) and 5-fluorouracil (5-FU) (Fournier et al., 2003a, 2003b) has enabled local delivery into neurodegenerative lesions and brain tumors, respectively. A phase I pilot study of the local and sustained delivery of 5-fluorouracil (5-FU) was carried out in eight patients with newly diagnosed glioma. Microspheres were implanted after a complete macroscopic surgical resection of the tumor. Patient median survival was doubled and one patient is still in disease remission today (Menei et al., 2004). It is noteworthy that the diffusion distances for microspheres in this case are very reduced.

Unfortunately invasive techniques have been associated with increased risk of infection and high neurosurgical cost (Abbott and Romero, 1996).

3.2. Non-invasive techniques

Non-invasive techniques of delivery may be of a chemical or biological nature.

3.2.1. Chemical methods

The chemical methods involve the use of prodrugs. The chemical change is usually designed to improve some deficient physicochemical property, such as membrane permeability or solubility. For example, esterification or amidation of hydroxy-, amino-, or carboxylic acid-containing drugs, may greatly enhance lipid solubility and hence, entry into the brain. Generally, the conversion to the active form is realized via an enzymatic cleavage.

Going to extremes on the lipophilic precursor scale, a possible choice for CNS prodrugs is to link the drug to a lipid moiety, such as a fatty acid, a glyceride or a phospholipid. Such prodrug approaches were explored for a variety of acid-containing drugs, like levodopa (Misra et al., 2003). In order to target the diseased site and to release the active compound in this environment, exploiting the pH or an enzymatic process may be theoretically possible, but in the pathological conditions there are some modifications in enzymatic concentration or pH provoking the possibility of reactive metabolites. Other problems associated with prodrugs are the poor selectivity and poor tissue retention of some of these molecules (Davis, 1997). Besides, the lipidization strategy involves the addition of lipid-like molecules through modification of the hydrophilic moieties on the drug structure. Lipid-soluble molecules are believed to be transported through the BBB by passive diffusion but the lipidization of molecules generally increases the volume of distribution, due in particular to plasma protein binding which affects all other pharmacokinetic parameters. Furthermore, increasing lipophilicity tends to increase the rate of oxidative metabolism by cytochrome P450 and other enzymes. While increased lipophilicity may improve diffusion movement across the BBB, it also tends to increase uptake into other tissues, causing an increased tissue burden (Temsamani et al., 2000; Misra et al., 2003).

3.2.2. Biological methods

Biological approaches include the conjugation of a drug with antibodies. The conjugate can then be directed towards an antigen residing on or within the target tissues. The OX26 antibody, the 8D3 MAb or the R17-217 MAb which are all antibody to transferrin receptor (TfR), were able to undergo receptor-mediated trancytosis across the mouse BBB via the endogenous TfR (Pardridge, 2002). Unfortunately, it is difficult to find target tissues bearing specific antigens that will provide a unique targeting effect. For example, in cancer chemotherapy tumor specific antigens are rare: tumor-associated antigens can be present not only within the target tissue but also elsewhere in the body. Other biological methods for targeting exploit ligands in the form of sugar or lectins, which can be directed to specific receptor found on cell surfaces (Davis, 1997).

3.2.3. An alternative route of administration

An alternative route to CNS drug delivery is the intranasal administration. Intranasal drug administration offers rapid absorption to the systemic blood avoiding

first-pass metabolism in the gut wall and the liver. This route of administration has been shown to present a safe and acceptable alternative to parenteral administration of various drugs. Further, several studies have shown a direct route of transport from the olfactory region to the CNS in animal models, without prior absorption to the circulating blood (Chou and Donovan, 1998; Wang et al., 1998; van Laar et al., 1999; Dahlin et al., 2000; Chow et al., 2001; Fisher and Ho, 2002; Bagger and Bechgaard, 2004). Since drugs absorbed via the olfactory route do not have to cross the BBB it may be possible to deliver substances to the CNS that would otherwise have been blocked from entering via the systemic circulation. However, the quantities of drugs reported to access the brain are very low indeed, with concentrations in the CSF and olfactory lobes quoted as nM or from 0.01% to 0.1% bioavailability (Illum, 2004). In order for a drug to travel from the olfactory region in the nasal cavity to the CSF or the brain parenchyma, it has to transverse the nasal olfactory epithelium and, depending on the pathway followed, also the arachnoid membrane surrounding the subarachnoid space. In principle, one can envisage three different pathways across the olfactory epithelium: (i) transcellularly especially across the sustentacular cells, most likely by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion, the latter pathway most likely for more lipophilic drugs; (ii) paracellularly through tight junctions between sustentacular cells or the so-called clefts between sustentacular cells and olfactory neurons; (iii) by the olfactory nerve pathway where the drug is taken up into the neuron cell by endocytosic or pinocytotic mechanisms and transported by intracellular axonal transport to the olfactory bulb (Illum, 2000).

These different strategies have shown interesting results but also some drawbacks. The linking of a lipophilic moiety or a ligand on the drug can provide a loss of therapeutic effect. The use of direct drug delivery will be difficult to develop on a large scale and the nasal route is for the moment experimental. Another promising strategy could be to associate drugs without any modification to colloidal carriers. These vehicles could deliver numerous drug molecules at specific site by coupling ligands to the surface of the colloids which can be administered intravenously for chronic treatment.

4. Colloidal drug carriers

In general, colloidal drug carriers include micelles, emulsions, liposomes and nanoparticles (nanospheres and nanocapsules). It is noteworthy that only liposomes and nanoparticles have been largely exploited for brain drug delivery. The aim in using colloidal carriers is generally to increase the specificity towards cells or tissues, to improve the bioavailability of drugs by increasing their diffusion through biological membranes and/or to protect them against enzyme inactivation. Moreover, the colloidal systems allow access across the BBB of non-transportable drugs by masking their physico-chemical characteristics through their encapsulation in these systems.

The fate of colloidal particles after intravenous administration is determined by a combination of biological and physico-chemical events that need to be considered in the design of efficient drug carrier systems. After intravenous administration, all colloidal systems, indeed, dramatically interact with plasma proteins, especially with immunoglobulins, albumin, the elements of the complement, fibronectin, etc. This process, known as “opsonization” is crucial in dictating the subsequent fate of the administered colloidal particles. Thus, colloidal particles that present hydrophobic surface properties are efficiently coated with plasma components and rapidly removed from the circulation, since the macrophages of the liver and the spleen own their specific receptors for these opsonins. However, colloidal particles that are small and hydrophilic enough can escape, at least partially, from the opsonization process and consequently remain in the circulation for a relatively prolonged period of time. Additionally, the concept of “steric hindrance” has been applied to avoid the deposition of plasma proteins either by adsorbing at the surface of the colloids some surfactant molecules (such as copolymers of polyoxyethylene and polyoxypropylene) or by providing a sterical stability by the direct chemical link of polyethyleneglycol (PEG) at the surface of the particles (Peracchia et al., 1998, 1999a,b). In addition, active targeting can be achieved by the attachment of a specific ligand (such as a monoclonal antibody) onto the surface of the colloidal particle, preferentially at the end of the PEG molecules since the targeted colloidal particles will be much more efficient if they are also sterically stabilized.

4.1. Polymeric micelles

Polymeric micelles as drug delivery systems are formed by amphiphilic copolymers having an A–B diblock structure with A, the hydrophilic (shell) and B, the hydrophobic polymers (core). The polymeric micelles are thermodynamically and kinetically stable in aqueous media. They have a size range of several tens of nanometers with a considerably narrow distribution. This narrow size range is similar to that of viruses and lipoproteins.

Several reviews have analyzed in great details the properties of the different copolymers used in the preparation of the polymeric micelles (Adams et al., 2003) as well as the physical chemistry of these systems (Jones and Leroux, 1999), which may influence their properties such as their size distribution and stability, their drug loading capacity, the drug release kinetics, their blood circulation time and biodistribution (Allen et al., 1999).

Earlier studies by Kabanov et al. (1992) have shown that poloxamer (Pluronic^{TM}) micelles conjugated with antibodies may improve brain distribution of haloperidol, a neuroleptic agent; this approach has resulted in a dramatic improvement of drug efficacy. This result indicates that Pluronic^{TM} micelles provide an effective transport of solubilized neuroleptic agents across the BBB. However, recent investigations made by the same group demonstrated that only Pluronic^{TM} unimers allowed cell penetration in bovine BMEC monolayers of molecules such as rhodamine 123 (Batrakova et al., 2001a), digoxin (Batrakova et al., 2001b) or doxorubicin (Alakhov et al., 1999) by inhibition of the P-gp-mediated drug efflux system. Other studies performed by Witt et al. (2002) have shown an increased analgesic effect when enkephalin, biphalin or morphine were administered as a cocktail with Pluronic P-85 at a concentration of 0.01%. It is noteworthy that the analgesia was lower with a higher concentration of Pluronic P-85 (0.1%) due to micellar trapping, which reduces the free drug concentration available for transcellular flux.

4.2. Liposomes

Liposomes are small vesicles composed of unilamellar or multilamellar phospholipids bilayers surrounding aqueous compartments. They are composed of biocompatible and biodegradable lipids similar to biological membranes. Their biophysical properties, such as size, surface charge, lipid composition and amount of cholesterol, are various and able to control distribution, tissue uptake and drug delivery.

Liposomes have been considered for brain targeting in several pathologies through both intracerebral and intravenous administrations. An enhanced transport of liposome-encapsulated drugs has been observed in several reported studies. Table 1 summarizes the applications of liposomes in the treatment of several diseases of the CNS. Most of the studies have focused on tumor therapies to deliver doxorubicin and other antineoplastic agents with the aid of either cationic or pegylated liposomes (i.e. liposomes sterically stabilized by a coating of PEG). In general, these treatments have let to long-term survival and inhibition of tumor growth in patients (Siegal et al., 1995; Koukourakis et al., 2000; Fiorillo et al., 2004; Saito et al., 2004).

4.2.1. Cationic liposomes

Recent advances in liposomal formulations include cationic liposomes used to entrap genetic material. Encapsulation of genetic material into cationic liposomes confers a protection from the extracellular environment and provides a mechanism for genetic material transfer to target cells. In gene therapy, delivery of plasmid DNA to the endosome will be without benefit, since it is an inappropriate cellular compartment for DNA function. Therefore, it is essential that the genetic material escapes from the endosomal compartment into the cytoplasm and is presented in an episomal fashion within the nucleus allowing expression (Davis, 1997). Each of the different factors involved in liposome targeting for gene therapy (size, surface recognition of the ligand and cell uptake of the liposomes, endosomal escape, episomal presentation and gene expression of the DNA) could be essential for a successful outcome. The ability of cationic liposomes to mediate transfection was attributed to certain properties such as spontaneous electrostatic interactions between the positively charged liposomes and the negatively charged DNA, which results in an efficient condensation of the nucleic acids. A variety of mono or multivalent cationic lipids are currently available for gene transfer, such as DOTMA (*N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) or DOTAP (1,2-dioleoyl-3-trimathylammonium-propane). These

Table 1
Examples of liposomes used as brain drug delivery during CNS diseases

Molecule tested (reference)	Liposome type	CNS diseases	Model of study	Via of administration	Relevant results
CM-Dil (fluorescent molecule) (Omori et al., 2003)	PEG-liposomes coupled with transferrin	Ischemia	Rat with middle cerebral occlusion (ischemic rat)	Intravenously	Increased brain accumulation
Amphotericin B (Groll et al., 2000)	Liposomes (commercial formulation ambisone; Fujisawa, USA, Deerfiel)	Infection	Rabbit model of hematogenous *candida albicans* meningoencephalitis	Intravenously	Antifungal efficacy
[^{3}H]-prednisolone (Schmidt et al., 2003a)	Pegylated liposomes	Experimental autoimmune encephalitis	Rat	Intravenously	Effective restoration of the BBB integrity, reduction of cellular inflammation and macrophage infiltration
Doxorubicin (Koukourakis et al., 2000; Hau et al., 2004)	Pegylated liposomes (commercial formulation Caelyx®)	Brain Tumor	Clinical study	Intravenously	Non-severe toxicity, higher drug accumulation in glioblastoma than in metastatic tumors and long-term survival
IFN-β gene plasmid (Yoshida et al., 2004)	Cationic liposomes	Brain tumor	Clinical study	Intratumoral	Inhibition of growth tumor of resistant malignant glioma and aggressive tumor
Recombinant adenoviral vector: herpes simplex virus thymidine kinase (Mizuno et al., 2002)	Cationic liposomes	Brain tumor	Mouse glioma model	Intratumoral	Reduction of immunogenicity and maintained anticancer activity
Antisense epidermal growth factor (Sugawa et al., 1998)	Cationic liposomes	Brain tumor	Human malignant glioma cell lines (U-87M6, TM-1 and TM2)	-	Inhibition of tumor growth

cationic lipids are frequently mixed with the neutral lipid dioleoyl phosphatidylethanolamine (DOPE), which is known to enhance transfection efficacy due to its ability to form hexagonal phases that may contribute to the destabilization of the endosomal membrane. The cholesterol also increases the levels of transfection and can potentially reduce the destabilization of the liposomes in the presence of serum (da Cruz et al., 2004). It is necessary to notice that interactions between cationic liposomes and nucleic acids do not form true liposomal structure. Hexagonal structures have been found in these systems which are called "lipoplexes" (Artzner et al., 2000).

Cationic liposomes have been used to realize plasmid-mediated transfection of murine brain cells (Roessler and Davidson, 1994). When these liposomes were injected/infused directly into the brain of mice, the expression of transgene could be observed for at least 21 days in the caudate putamen region. In order to deliver genetic material to disseminated tumor sites, two approaches have been used. The first one is the reinforcement of the effectiveness of the immunotherapy. In this view, the studies on interferon (IFN)-β gene therapy using cationic liposomes have shown interesting results in the treatment of brain tumors. This gene therapy was based on four antitumors mechanisms induced by IFN-β gene transfer: (i) apoptosis of tumor cells; (ii) growth inhibition of the tumor; (iii) induction of immune response; (iv) increased secretion of cytokines (Yoshida and Mizuno, 2003). Thus, the primary study developed by Norimoto et al. (2003) through morphological analyses of tumor cells following liposomal IFN-β gene transfection has demonstrated that approximately 20% of the 203G (mouse glioma cell line) cells underwent morphological changes consistent with apoptosis produced by the liposomal formulation. In this way, Yoshida et al. (2004) have developed a protocol to determine the safety and effectiveness of cationic liposomes containing the human IFN-β gene after tumor removal in five patients with recurrent malignant gliomas. The second strategy is based on the sensibilization of cancerous cells to drugs. This therapeutic approach involves liposomal delivery of the herpes simplex virus thymidine kinase (HSV-tk) gene into the glioma to improve cell sensitization to ganciclovir. The administration of HSV-tk gene associated with cationic liposomes and followed by ganciclovir treatment was found to reduce antigenicity and to maintain antitumor activity in GL261 glioma model (Mizuno et al., 2002).

To achieve an efficient transfer of the cationic liposomes content into cells, fusogenic liposomes have been prepared using fusogenic lipids (Shangguan et al., 1998), by conjugation of fusogenic molecules to liposome membranes (Kono et al., 2000) or by incorporation of viral fusion proteins to bilayers. In this way, Matsuo et al. (2000) have reported the feasibility to introduce oligodeoxynucleotides (FITC-ODN) into MBEC4 cells (mouse brain-capillary endothelial cells) by utilizing the hemagglutatinin virus of Japan (HVJ)-liposomes with fusogenic activity.

Unfortunately, cationic liposomes normally require an invasive way of administration to transfer genes into the brain.

4.2.2. Pegylated liposomes

Pegylated liposomes have proven their ability to deliver the drugs owing to their long blood circulating times and their reduced clearance by the RES system. This allows them to selectively extravasate in pathological sites, like tumors or inflamed regions with a leaky endothelium. The earlier studies in animals demonstrated an enhanced drug exposure and improved therapeutic activity (Gabizon, 1992; Siegal et al., 1995). Now, a liposomal formulation of pegylated liposomes encapsulating doxorubicin (Caelyx®) is used in clinical practice, showing effectiveness in glioblastomas and metastatic tumors (Koukourakis et al., 2000; Hau et al., 2004).

Experimental autoimmune encephalitis is another brain disease in which liposomes have been found useful for drug delivery. In inflammatory conditions, it is believed that the disruption of BBB allows the free diffusion of liposomes. Thus, prednisolone entrapped into pegylated liposomes has demonstrated an effective restoration of the BBB integrity; macrophage infiltration was diminished in the treated animals. Additionally, the use of liposomes may reduce systemic side effects and could be employed for the treatment of multiple sclerosis (Schmidt et al., 2003b).

4.2.3. Active targeting by liposomes

Active targeting can be achieved by complexing the liposomes with an antibody or a ligand that will be recognized by cell surface receptor in the targeted tissue. This approach may be the most striking advance

in BBB targeting and translocation. Monoclonal antibodies (MAb) have enabled brain targeting of pegylated liposomes. The MAb are able to attach a receptor expressed on the BBB and to trigger a receptor-mediated transcytosis across the BBB. The targeting MAb acts as a molecular Trojan horse to ferry the liposomes across biological barriers in the brain via endogenous transport systems (Zhang et al., 2004). In this regard, Huwyler et al. (1996) have shown that specific OX26-mediated targeting of daunomycin to the brain may be successfully achieved by the use of pegylated MAb-liposomes. This system was synthesized using thiolated monoclonal antibodies and a bifunctional 2000 Da PEG which contains a lipid at one end and a maleimide at other end. The encapsulation of digoxin within pegylated OX26-liposomes was found to enhance brain endothelial cell uptake.

Pegylated MAb-liposomes have also been used to deliver genetic material to the brain (Shi et al., 2001; Zhang et al., 2003a, 2003b, 2004; Zhu et al., 2004). These systems offer the advantage that they may be administered intravenously avoiding invasive way. The toxicity of these systems has been investigated. The results have shown a prolonged duration of gene expression without toxicity after chronic weekly intravenous administration of a tyrosine hydroxylase plasmid encapsulated in pegylated OX26-liposomes (Zhang et al., 2003b). These carriers were also employed for the encapsulation of an antisense gene directed to epidermal growth factor receptor (EGFR). This formulation was found to be efficient in reducing the growth of an EGFR-dependent glioma (Zhang et al., 2004).

Another approach is the use of the transferrin ligand. da Cruz et al. (2004) reported that cationic liposomes decorated with tranferrin resulted in a significant enhancement of luciferase gene expression activity in C6 glioma cells, primary hippocampal neurons and primary cortical neurons. However, the transfection efficiency of this system was low in comparison with pegylated MAb-liposomes, perhaps due to the fact that transferrin was just electrostatically associated to the cationic liposomes. Therefore, perhaps not all the Tf binding motifs were available to bind with the corresponding receptors at the surface of the targeted cells.

In conclusion, liposomes have been extensively investigated for the brain delivery of molecules, showing increased drug efficacy and reduced drug toxicity.

4.3. Nanoparticles

The term “nanoparticle” may be defined as a submicron drug carrier system, generally (but not necessarily) of polymeric nature (the polymer used may be or not biodegradable). Thus, this term is somewhat general since it does not take into account the morphological and structural organization of the polymer. In this respect, “nanosphere” is used to identify a nanoparticle system with a matrix character and constituted by a solid core with a dense polymeric network. In contrast, “nanocapsules” are formed by a thin polymeric envelope surrounding an oil-filled cavity. Nanocapsules may, thus, be considered as a “reservoir” system. Practically, the nanoparticles have a size around 200 nm and the drugs or other molecules may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. These systems are attractive because the methods of preparation are generally simple and easy to scale-up. Nanoparticles can be made from a broad number of materials such as poly(alkylcyanoacrylates) (PACAs), polyacetates, polysaccharides and copolymers. The methods of preparation of the nanoparticles, their characterization and medical applications have been reviewed in details earlier (Kreuter, 1992; Barratt et al., 2001; Fattal and Vauthier, 2002). We will focus here on the application for drug delivery to the brain.

The advantage of using nanoparticles for drug delivery results from their two basic properties. Firstly, due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. Secondly, the use of biodegradable materials for nanoparticle preparation, allows sustained drug release at the targeted site over a period of days or even weeks after injection (Vinogradov et al., 2002).

4.3.1. Coated nanoparticles

In earlier studies realized by Kreuter et al., molecules such as dalargin (Kreuter et al., 1995; Schroeder et al., 1998) and loperamide (Alyautdin et al., 1997) have been loaded onto nanoparticles with the aim of brain delivery. After peripheral administration, these molecules themselves do not exhibit any therapeutic effect because they do not diffuse through the BBB. But, when dalargin or loperamide were ad-

sorbed onto the surface of poly(butylcyanoacrylate) (PBCA) nanoparticles further coated with the detergent, polysorbate-80 (PS-80), a pronounced analgesic effect was obtained, reaching a maximum 45 min after administration. The mechanism behind the translocation of those nanoparticles into the brain remains, however, not fully understood. Recently, Olivier et al. (1999) have suggested that the PBCA nanoparticles coated with PS-80 displayed some toxic effect towards the BBB. In addition, it was also suggested that the nanoparticles could open the tight junctions between endothelial cells in the brain microvasculature, thus creating a paracellular pathway for nanoparticle translocation. Olivier et al. (1999) have based their arguments on the observation done in an in vitro model of the BBB consisting of a coculture of bovine brain endothelial cells and rat astrocytes. However, both in vivo and in vitro studies performed by Kreuter et al. (2003) did not demonstrate any disruption of the BBB by the presence of PS-80 coated nanoparticles since the permeability of the extracellular markers (sucrose and inulin) was not modified in the presence of 10 or 20 μg/ml of PBCA nanoparticles with and without polysorbate-80. This indicates, on the contrary to what was hypothesized by Olivier et al. (1999), no facilitation of the paracellular route by disruption of tight junctions due to nanoparticles.

In vivo experiments in mice have clearly shown that the analgesic effect of dalargin was obtained only when the drug was pre-adsorbed onto the nanoparticles, whereas a single mixture of dalargin and PBCA nanoparticles did not show any analgesic effect The enhancement of the drug transport through BBB by the coated nanoparticles can be explained by different mechanisms: (1) the binding of nanoparticles to the inner endothelial lining of the brain capillaries could provide a drug concentration gradient, thus improving passive diffusion and (2) brain endothelial cell uptake of nanoparticles may occur through endocytosis or transcytosis. Additionally, it has been reported that apolipoproteins (APO) could be involved in the brain penetration of PBCA nanoparticles overcoated with PS-80 (Kreuter et al., 2002). Indeed, a study has been performed using PBCA nanoparticles loaded with dalargin or loperamide and overcoated with the APO-A, B, C, E or J (with or without precoating with PS-80), the antinociceptive effect being measured in mice by the tail flick test. In these conditions, only dalargin or loperamide-PBCA nanoparticles coated with polysorbate-80 and/or with apolipoprotein B or E were able to achieve an antinociceptive effect. This effect was significantly higher after both PS-80-precoating and APO-B- or APO-E-overcoating. No antinociceptive effect was seen after coating with the other apolipoproteins. Interestingly, in APO-E-deficient mice, the antinociceptive effect was reduced comparatively to normal mice after injection of the PS-80-coated nanoparticles. Thus, it is suggested that the PS-80 could act as an anchor for APO-B and APO-E, at the surface of the nanoparticles which are then be able to interact with LDL receptor, before being taken up by the BMEC via receptor-mediated endocytosis (Borchard et al., 1994; Kreuter et al., 1995; Alyaudtin et al., 2001). After this the drug may be released in these cells and diffuse into the brain interior or the particles may be transcytosed.

These PS-80 nanoparticles have been used to deliver other molecules to the brain, such as MRZ 2/576 which is a potent but rather short-acting anticonvulsivant drug following intravenous administration. It was observed that the administration of MRZ 2/576 loaded onto PS-80 overcoated nanoparticles prolonged the duration of the anticonvulsive activity (Friese et al., 2000). Doxorubicin was also adsorbed onto these nanoparticles for the treatment of experimental glioblastoma. Rats treated with this system have shown significantly higher survival times (Steiniger et al., 2004). Moreover, the acute toxicity of doxorubicin was reduced when it was associated with PS-80-coated nanoparticles (Gelperina et al., 2002).

4.3.2. *Pegylated nanoparticles*

In another approach, pegylated-poly(hexadecylcyanoacrylate) (PEG-PHDCA) nanoparticles have been investigated for the treatment of several CNS pathologies such as brain tumors (Brigger et al., 2002b), EAE (Calvo et al., 2002) and prion diseases (Calvo et al., 2001a). The preparation of the PEG-PHDCA copolymer was achieved by the synthesis of a cyanoacrylate monomer substituted with PEG and its co-polymerization with hexadecylcyanoacrylate in a 1:4 ratio (Peracchia et al., 1998). In this technology, the PEG is therefore covalently attached to the hydrophobic block, rather than adsorbed, which seems to be the

better choice to avoid the possibility of PEG desorption. These particles with PEG chains at the surface of the hexadecylcyanoacrylate hydrophobic core have shown long-circulating properties in vivo (Peracchia et al., 1999b).

PEG-PHDCA nanoparticles have been shown to penetrate into the brain to a greater extent than all the other nanoparticles formulations tested, including the above discussed PS-80 nanoparticles (Calvo et al., 2001b). Calvo et al. (2002) have investigated the accumulation of PEG-PHDCA nanoparticles in EAE rats. Confocal microscopy have evidenced that fluorescent-PEG-PHDCA nanoparticles were present in the epithelial cells (Brigger et al., 2002b) of the brain and spinal cord surface and in the ependymal cells of the choroid plexus. In EAE rats, PEG-PHDCA nanoparticles could reach the brain by two mechanisms: passive diffusion due to the increase of BBB permeability and transport by nanoparticles-containing macrophages which infiltrate these inflammatory tissues. This study claims that PEG-PHDCA nanoparticles had appropriate characteristics for penetration into CNS under pathological conditions, especially in neuroinflammatory diseases (Calvo et al., 2002).

After intravenous administration in rats bearing intracerebral well-established gliosarcoma, PEG-PHDCA nanoparticles have accumulated preferentially in the tumoral tissue, rather than in the peritumoral brain tissue or in the healthy controlateral hemisphere. Interestingly PEG-PHDCA nanoparticles concentrated much more in the gliosarcoma than did their non-pegylated counterparts (PHDCA nanoparticles). Based on the test of sucrose permeability, PEG-PHDCA nanoparticles did not display any toxicity towards the BBB. Using a simplified pharmacokinetic model two different mechanisms were proposed to explain the accumulation of PEG-PHDCA nanoparticles into the brain tumor and, although to a lower extend, into the normal brain tissue. Firstly, in gliosarcoma, the preferential accumulation of PEG-PHDCA nanoparticles was attributable to their reduced plasma clearance. The mechanism of diffusion/convection would explain the extravasation of the nanoparticles into the tumor site. Secondly, in normal brain, the applied pharmacokinetic model suggested an affinity of the PEG-PHDCA nanoparticles for the endothelial cells of the BBB, allowing their translocation (Brigger et al., 2002b).

4.4. Nanogel

Vinogradov et al. have developed a new family of carrier systems for the delivery of drugs and biomacromolecules to the brain (Vinogradov et al., 1999, 2002, 2004). These so called “nanogels” systems, are made from a network of cross-linked ionic polyethylenimine (PEI) and non-ionic PEG chains (PEG-cl-PEI). They have been synthesized using the emulsification-solvent evaporation method. When a biologically active macromolecule is associated to the nanogel by electrostatic interactions, the PEI chains have a tendency to collapse which results in decreased volume and size of the particles. Because of the steric stabilization of the PEG chains, the collapsed nanogel forms stable dispersions with a mean particle size of 80 nm. To realize active targeting, the surface of the nanogel could be modified with biospecific ligands. For this purpose, various coupling strategies have been used including covalent attachment of the ligand moiety to free amino groups of the PEI fragments in the PEG-cl-PEI nanogel. Another simple way to introduce ligands in the nanogel particles consists in the partial modification of PEI fragments with biotin moieties allowing attachment of ligand using standard biotin-avidin coupling chemistry (Vinogradov et al., 2002).

Nanogels have been tested as a potential carrier for oligonucleotide delivery to the brain (Vinogradov et al., 2004) by using polarized monolayers of bovine BMEC. The studies performed with that model of BBB have shown an increased transport of ODN across the cell monolayers as a result of their incorporation into the nanogel. Further increase in oligonucleotide transport was observed when the nanogel carriers were modified with insulin or transferrin ligands (Kabanov and Batrakova, 2004). Further permeability assays with mannitol indicated that the increased transport of ODN-nanogels did not result from single paracellular diffusion due to a disruption of the bovine BMEC monolayers. After intravenous injection of ODN-nanogels in mice, no adverse toxic effects were observed and increased brain and decreased liver/spleen accumulations were noted, compared to the free ODN (Vinogradov et al., 2004). These preliminary studies suggest that this system could represent a promising carrier for the delivery of ODN to the brain.

5. Conclusion

It emerges from this review that colloidal systems can easily enter brain capillaries before reaching the surface of the brain microvascular endothelial cells, under the condition that the surface of these colloids is modified in a proper way (i.e. by PEG or PS-80). The prolonged blood circulation of these surface-modified colloidal particles enhances exposure of the BBB, which favors interaction and penetration into brain endothelial cells. Colloidal systems may further be modified with specific targeting molecules aiming to enhance their binding with surface receptors of the BMEC, thus promoting their transport across the BBB. Therefore, when drug is loaded, colloidal carriers may be helpful for the treatment of brain diseases (with or without disruption of BBB), because they offer clinical advantages such as decreased drug dose, reduced drug side effects, increased drug viability, non invasive routes of administration and improved patient quality of life. However, there is an urgent need to clarify the mechanisms which manage the carrier-mediated transport of the drugs to the brain.

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