# Nano particle transport across the Blood Brain Barrier

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REVIEW

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# Nano particle transport across the Blood Brain Barrier

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**Keywords:** BBB, drug delivery, drug targeting, liposome, nanoparticle, nanocarrier

**Abbreviations:** BBB, Blood brain barrier; BCB, Blood CSF barrier; CBB, CSF-brain barrier; CNS, Central Nervous System; CSF, Cerebrospinal fluid; GLUT1, Glucose transporter-1; LAT1, Large neutral amino-acid transporter type 1; NP, Nanoparticle; P-gp, P-glycoprotein; Tf, Transferrin; TJ, Tight junction

While the role of the blood-brain barrier (BBB) is increasingly recognized in the (development of treatments targeting neurodegenerative disorders, to date, few strategies exist that enable drug delivery of non-BBB crossing molecules directly to their site of action, the brain. However, the recent advent of Nanomedicines may provide a potent tool to implement CNS targeted delivery of active compounds. Approaches for BBB crossing are deeply investigated in relation to the pathology: among the main important diseases of the CNS, this review focuses on the application of nanomedicines to neurodegenerative disorders (Alzheimer, Parkinson and Huntington's Disease) and to other brain pathologies as epilepsy, infectious diseases, multiple sclerosis, lysosomal storage disorders, strokes.

## Introduction

### The blood brain barrier (BBB)

The central nervous system (CNS) is protected from toxins and metabolic fluctuations by barriers, among which the blood-brain barrier (BBB) plays a key role in maintaining homeostasis. The BBB is composed of four major cellular components, the endothelial cells of the brain capillaries, pericytes, astrocytes, and the basement membrane. Together, they build a functional unit, the 'Neurovascular unit', mediating exchange between neurons, capillaries and glia. That way, the BBB supplies the brain with necessary nutrients, glucose and oxygen to sustain normal neural functioning. At the same time, the BBB plays a protective role against neurotoxic substances. Together, the BBB creates the neural microenvironment that is crucial for healthy brain function[2].

To fulfill this function, the brain has a very close-packed microvasculature.[74,76] This enables very short distances between the nearest blood capillary and neuronal cells,[99] which allows a fast delivery of substances after crossing the BBB. Further, the capillaries in the brain are quite different from capillaries found in the peripheral organs. Microvessels of the BBB are formed by non-fenestrated endothelial cells that contact each other forming tight junctions (TJs) (or zonula occludens). TJs prevent the passive diffusion of hydrophilic solutes via paracellular transport, the transfer of substances from the blood into brain parenchyma by passing through the intercellular space between the cells. Thus, water-soluble substances have to cross the BBB by mechanisms of transcellular transport that involve active transport via specific carriers.

In addition, brain capillaries are surrounded by pericytes protruding into the basement membrane, and astrocytic end feet encapsulating the surface (Grabrucker et al., 2014). Astrocytes release inductive signals that regulate the BBB [43] and play a role in maintaining water, ionic, and amino acid homeostasis within the CNS.[1] With an electrical resistance estimated to be ≈ 1500–2000 $\Omega cm^2$, brain endothelium possesses a much higher electrical resistance than other systemic endothelia with ≈3–33 $\Omega cm^2$ [17] further limiting diffusion of certain molecules across the BBB. However, the BBB is a highly dynamic structure and its formation, maturity and integrity controlled by a collective action of all of its constituents. In addition, neurons have been shown to release growth or nutritional factors maintaining BBB functioning.

The consequence of this intricate interplay is a controlled and restricted transport of molecules across the BBB. Only very small (<400 Da) and lipophilic molecules can diffuse through the BBB, while the crossing of lipid insoluble or larger hydrophilic molecules is very limited. However, additionally to lipophilicity, other factors such as the affinity toward available transport system further control BBB crossing. For example, the transport of glucose through the BBB is mediated by glucose transporter-1 (GLUT-1).[60]

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The effectiveness of the BBB function depends on several factors such as the controlled expression of the major constituents of TJs, such as occluding proteins and proteins of the claudin family.[2] Further, molecules released during neural activity may act on receptors and transporters of endothelial cells and/or contacting astrocytes. Examples include neurotransmitters such as glutamate and serotonin, but also calcium, and adrenaline.[75] Through these modulatory agents, several parameters of the BBB such as capillary diameter (and thus local blood flow), expression of specific transporters, and the tightness of TJs can be regulated (Peppiatt et al., 2006; [14,27,47]; Zlokovic, 2008). Therefore, disrupted signaling between endothelial cells, pericytes and/or astrocytes may affect the integrity of the BBB.[9,13,28]

In addition to regulating exchange of molecules between the peripheral blood circulation and the brain by its anatomical properties, the BBB is a metabolic barrier. A variety of degrading and metabolizing enzymes can be found at the membrane of brain capillary endothelial cells[6,32] along with a large number of transporter proteins that strictly regulate the influx and efflux of substances.

Taken together, these unique features of the BBB control the homeostasis of a large variety of molecules in the brain. However, it is the same features that impose a major obstacle to successful delivery of drugs into the brain. For example, various phase l and phase ll enzymes of the drug metabolism system have been found in endothelial cells. The major drug efflux transporters include P-glycoprotein (P-gp),[24] ATP binding cassette (ABC), multidrug resistance-associated proteins (MRPs) and Breast Cancer resistance proteins (BCRP). Although in some conditions such as trauma, multiple sclerosis and Alzheimer Disease, the anatomical and functional state of the BBB may be altered, the delivery of active compounds into the CNS needs effective mechanisms to facilitate BBB crossing.

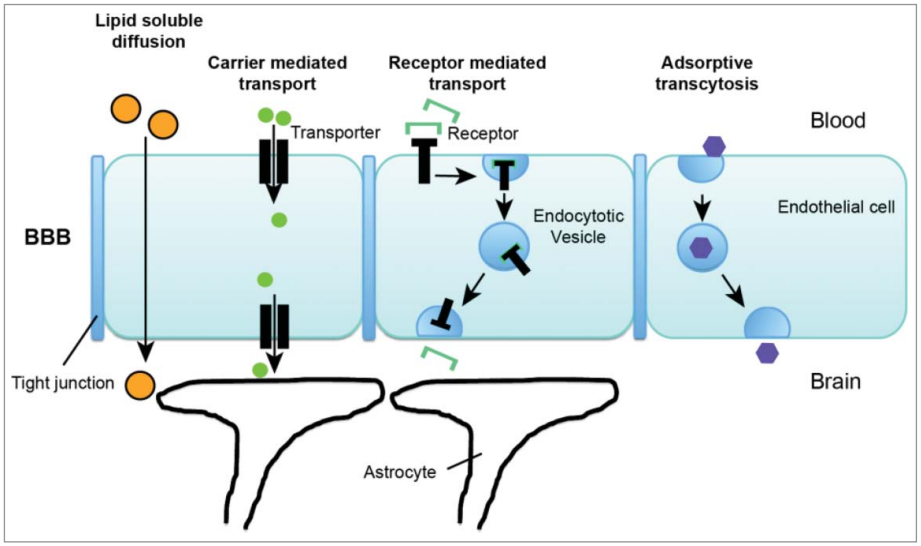


**Figure 1. Transport pathways across blood brain barrier.** Under physiological conditions, substances may cross the BBB by passive diffusion, carrier mediated transport, receptor mediated transport, and adsorptive transcytosis.

Although other barriers such as the blood-cerebrospinal fluid (CSF) barrier (BCB) and CSF-brain barrier (CBB) exist, the surface area of the BBB is far greater than the surface area of the BCB. Thus, the BBB is considered the main site for crossing of endogenous substances into the CNS. In addition, even despite the entrance of a drug into the CSF, it does not necessarily results in its penetration into the brain parenchyma. The penetration of substances from CSF to brain parenchyma is facilitated through diffusion. However, the large distances create a diffusion barrier that can be referred to as the CSF-brain barrier (CBB). In addition to the aforementioned external barriers, after crossing the BBB, the presence of further internal barriers creates another obstacle for effective drug delivery, such as fast clearing mechanisms by glial cells.

**Mechanism of BBB crossing by endogenous substances**

Despite the restricted transport of molecules across the BBB, the brain has high demand for energy and nutrients. Therefore, multiple mechanisms exist that mediate the uptake of specific endogenous substances. Some of these provide a useful basis also for the development of strategies for drug delivery. In general, under physiological conditions, substances may cross the BBB by passive diffusion, carrier mediated transport, receptor mediated transport, and adsorptive transcytosis (Fig. 1).

The possibility for substances to passively cross the BBB by diffusion is very limited (see above) due to the BBB physiology. The exception are lipid soluble small molecules with an molecular weight of less than 400 Da. Uptake of such substances is possible by lipid-mediated free diffusion across the BBB. In contrast to diffusion through water, diffusion through lipid membranes is highly dependent on the correlating features of molecular volume, molecular weight, and surface area of a substance.[84]

High lipid solubility in turn is correlated to low hydrogen bonding. It has been shown that the ability of a substance to permeate membranes is decreased by 10-fold with each pair of hydrogen bonds.[112] This rule has been confirmed in multiple studies i.e. investigating BBB transport of steroid hormones and oligopeptides.[84]

Therefore, the majority of substances is taken up into the brain by carrier mediated transport and receptor mediated transport. Here, the structure of the molecule possesses high affinity for specific carriers or receptor found at the BBB. For example, water-soluble metabolic substrates such as glucose, are bound by specific transporters, here the glucose transporter type 1 (GLUT1) that, however, has also affinity for other hexoses, including mannose and galactose.[79] Another example is the large neutral amino-acid transporter type 1 (LAT1), which is a carrier for

phenylalanine, but also transports over 10 other large neutral amino acids and, although with lower affinity, small neutral amino acids.[82] To date, many specific transporters have been identified. Their exact cellular location at the brain microvasculature and expression level, as well as their selectivity largely determine the kinetics and turnover of the transported substances.

For example, the supply of Glucose for the brain is realized only by GLUT1. GLUT1 is enriched at the brain microvasculature only at the BBB on both luminal and abluminal membranes. This expression patterns enables not only uptake of Glucose from the blood into epithelial cells but indeed transport across the cells of the BBB. Due to the high energy - demand of the brain, the abundance of GLUT1 at the microvasculature is high, relative to other transporters, which enables rates of glycolysis in the brain that are over 100-fold greater than rates of amino-acid incorporation into brain proteins.[83] However, due to the demand of carriers, the process of carrier mediated transport of a substance across the BBB is saturable.

Carrier mediated transport is not only responsible for uptake of substances into the brain, but a process that regulates homeostasis in bi-directional manner. To that end, active efflux transporters can be found at the BBB that mediate the asymmetric efflux of metabolites from the brain back to the blood circulation. It is also these transporters that impose an obstacle to drug delivery into the brain. The classical active efflux transporter system within the BBB is P-glycoprotein (P-gp). This protein is encoded by the multidrug resistance gene 1 (MDR1) and its functional complex is not only modified by internal factors but also environmental clues such as the presence of toxins.[30]

Receptor mediated transport processes additionally occur at the BBB as mechanism regulating the crossing of endogenous substances. These peptide receptors may mediate processes such as transcytosis of ligands from blood to brain or from brain to blood, or only uptake into the brain capillary endothelium without further export into the brain parenchyma. Well investigated examples include the transport of insulin or transferrin. Insulin is transported across the BBB by binding to an endogenous BBB insulin receptor.[81] Similarly, for transferrin (Tf) a specific receptor, the Tf receptor (TfR) can be found at the BBB.[80] Similar to carrier mediated transport, the cellular location and expression levels of the receptors determine the kinetics and turnover of the transported substances. For example, the TfR mediates influx of holo-transferrin from blood to brain but also reverse transcytosis of apotransferrin from brain to blood due to expression on the luminal and abluminal membrane of the capillary endothelium.[108,130]

Finally, a mechanism of BBB crossing called adsorptive endocytosis has been described. This process requires an interaction between a ligand, such as a macromolecule or protein in the bloodstream and the cell surface. This interaction may be triggered by an electrostatic interaction between the positively charged ligand and negatively charged plasma membrane. Ultimately, this may result in adsorptive transcytosis of the ligand, which is mediated by clathrin-dependent endocytosis in most cases. This mode of BBB crossing seems to occur only unidirectional, from blood to brain. Adsorptive transcytosis has been described for some compounds and Nanoparticles (NPs). However, whether adsorptive transcytosis contributes significantly to the transport of endogenous substances across the BBB is still under investigation.[119]

Several of the mechanisms involved in BBB crossing of endogenous substances can be used for the development of strategies for drug delivery. For example, delivery of drugs that have structures that mimic the endogenous substrate of transporters at the BBB may be facilitated. The vast majority of carrier mediated transport systems has been identified and transporters can be expressed in *in vitro* systems to screen for drugs that have an acceptable affinity for a given BBB transporter. A drug that shows such an affinity for a carrier mediated transport system is L-DOPA. L-DOPA is used in the treatment of Parkinson Disease and is a dopamine precursor that is transported across the BBB via the LAT1 carrier.[55] Alternatively, drugs might be conjugated to substances that normally cross the BBB by carrier mediated processes. Further, the presence of endogenous receptors with specific ligands triggering BBB crossing mechanisms, such as the insulin receptor or the TfR, enables the engineering of peptides that attached to compounds or drugs mimic these ligands.

However, after successful crossing of the BBB, most drugs are taken up by cells. A major pathway mediating this uptake is clathrin-dependent endocytosis. This mode of uptake leads to the eventual delivery of substances into the endo-lysosomal compartments. Due to the low pH and the presence of degrading enzymes in lysosomal compartments, delivered biomolecule may be degraded creating a major obstacle in the delivery of active compounds, in particular of therapeutic proteins.

Thus, taken together, there is an urgent need to develop new strategies for the effective delivery of substances across the BBB. Therapeutic compounds need to be protected from degrading, metabolizing and inhibiting processes within the blood stream, need to be taken up into the CNS via the BBB with high efficiency, and need to be targeted to specific sites of action preventing lysosomal degradation. Here, Nanomedicines provide major advantages compared to common drug delivery systems

## Nanomedicines

In this review, we would like to summarize the most interesting applications of the last 20 y of nanoparticles and liposomes in in vivo Brain experiments. Considering the variability of in vivo models, we skip to review the "proof-of-concept," but mainly focusing on "*In vivo* disease models." The "In vivo disease models" describes the application of nanoparticles and liposomes in brain pathology, pointing the critical attention on the animal models of pathology and on the possible mechanism involvement in the treatments. This section is divided into two main underchapter: "Neurodegenerative Diseases" and "Other Brain Pathologies." In "Neurodegenerative pathologies" section, we include Alzheimer, Parkinson and Multiple Sclerosis diseases. Finally, in "Other Brain Pathologies" section we include some of the most relevant papers on convulsive models, stroke, brain infectious diseases and prion diseases.

Firstly, we have to stress the difficulty to identify a "standard" *in vivo* model for each disease, thus the possible meta-analysis of the data, which could help the neuroscientist in the basic research, is virtually impossible. In particular, to our best knowledge, in almost all neuro-pathologies conditions (such as Parkinson disease, HIV-dementia and some infectious brain diseases), the BBB permeability is seriously compromised, while in other brain diseases (stroke and brain tumors, as examples), the BBB integrity appears to be seriously damaged. As example, we must consider that the use of brain tumors (namely, gliomas) *in vivo* application must be thought as "glioma" delivery and not as brain targeting or BBB crossing exploitation, since over the progression of the pathology, it has been demonstrated that BBB is losing its integrity.[10]

Secondly, since this field of science (namely, "nanoneuroscience" or "neuro-nanomedicine") is relative new and young, the most of papers are based on "Proof-of-Concept," while the application on brain diseases models appears in constant increase, but still lower than the basic research. This evidence could be explained by the fact that a unique carrier for brain delivery does not exist, since it possible to take advantage of different ligands, receptors and mechanisms for BBB crossing and brain targeting. Then, the use of different starting polymer (poly-lactide-co-glycolide, poly-buthyl/heaxil-cyano-acrylate, albumin, chitosan, and many others) for nanoparticle preparation or lipid composition (cationic/anionic/neutral, pegylated lipids and many others) for liposome preparation, results in different brain targeting mechanisms, different biodistribution profiles, different brain delivery efficacies and overall toxicities.

Notwithstanding all of these differences in animal models and carriers, with this review, we aimed to produce a useful tool for all the neuroscientist to access a plenary of *in vivo* evidences.

### Nanoparticles and liposomes

Generally talking, polymeric nanoparticles (NPs) are solid colloid matrix-like particles sizing from 1 to 1000 nm, made of polymers of different nature, and encapsulating molecules during the preparation process, chosen depending on both the polymers and the drug to be delivered. There are several natural or synthetic polymers often mixed during the preparation of NPs, but considering the stringent request of FDA guidelines in terms of biodegradability and biocompatibility for *in vivo* administration, the list of polymers, that could be used to prepare NPs systems, results very short with few polymers available for drug delivery system preparation and approved for systemic administration. Among them, natural (e.g. Albumin) and poly-ester (poly-lactide and derivatives) polymers are the most accepted. In particular, it is a common opinion that the toxicology of a nanodevice should be related both to morphological and structural parameters of the systems (size, surface charge, shape) and the polymers used.

Liposome (LPs) are surely the most studied and old *smart* systems, applied in different fields of medicine research, representing the first innovative approach for the treatment of challenging pathologies, like cancer, HIV, strokes and many other diseases which require selective therapies.[42,68] As described for NPs, LPs need a surface modification in order to promote specific delivery and targeting of the embedded drugs. Generally speaking, good in vitro results on LPs experimentations do not reflect in vivo confirmations, leading to a very low applicability of the liposome-approach in the treatment protocols. This event, connected with a very quick elimination and degradation when LPs are injected in the bloodstream, along with the type and properties of phospholipids which are constituents of the liposomes, was faced with different strategies such as using stabilizers or modification of preparation procedures. Particularly, the conjugation with molecules, able to protect the LPs from the degradation (GM1, poloamine, polaxamer and PEG) or able to induce an increased affinity to the diseased cells, represented one of the most applied strategies. The pegylation technology offers a potential resolution of the problem of liposome's bio-stability, connected to the liposomal intravenous administration.[50,91] Finally, in order to increase the minimal affinity to the diseased cells and to avoid the toxic effect on non diseased tissues, an active targeting due to functionalization of the surface of the liposome is hardly needed.[128]

## In vivo disease models

If in the previous examples the integrity of the BBB was maintained and the brain was considered as a "non-pathologic" brain, in the following examples, several researches have been reviewed concerning neuro-pathologies in which the BBB integrity and permeability is seriously compromised.[12,51,103,135] These pathologies could vary from neurodegenerative diseases (Parkinson, Alzheimer and Huntigton's) to epilepsy, infectious brain diseases, strokes and many others.

### Neurodegenerative disease

#### *Alzheimer disease*

Alzheimer disease (AD) is a chronic and progressive neurodegenerative disorder that begins with cognitive and memory impairments, accompanied with behavioral disturbances such as aggression, depression, hallucination, delusion, anger and agitation and eventually progresses to dementia, physical impairment and death.[26] AD is the most common form of dementia, and the greatest risk factor for AD is age. It is estimated that 10% of people over the age of 65 are affected by AD [4] and by 2050, the number of people with AD in the US will increase threefold.[45] The definite diagnosis of AD is made upon histological verification, established by biopsy or at autopsy, of two main hallmarks: extracellular amyloid plaques and intracellular neurofibrillary tangles, which enclose hyperphosphorylated tau protein.[26,102] Furthermore, the neuropathology of AD is characterized by a progressive loss of synapses and neurons in a region- and cell-type-specific manner.[95] In Alzheimer disease, microglia and astrocytes are activated by β-amyloid protein and related oligopeptides, leading to a cascade of events producing toxic molecules, neuronal damage, synaptic dysfunction and alteration of the permeability of the BBB.[10]

To study this pathology, a model of acute amnesia is induced in rats or mice by subcutaneous injection of scopolamine.[86] These animals are submitted to the passive avoidance reflex (PAR) test[118] to determine the memory changes; the PAR is based on the measurement of the retention latency times, necessaries for the animals to avoid a painful stimulus; these latencies are directly proportional to the decrease of amnesia, thus representing a useful tool to describe the effectiveness of anti-amnesia drug injected. Kurakhamaeva and colleagues[59] used Nerve Growth Factor (NGF) loaded in PBCA NPs covered with PS-80 to reduce amnestic effect. With this study, they demonstrated that brain-targeted NPs loaded with NGF allowed an improvement of amnesia with a significant increase of the latent period of 2-fold, if compared to animals treated with NGF-free solution. In another paper, Abdel-Wahab[3] demonstrated the efficacy of 2 different systems: PBCA Np covered with polysorbate-80 and PBCA Np covered with PEG5000, both loaded with tacrine (reversible inhibitor of acetylcholinesterase) able to improve cognitive function. These NPs formulations increased the latency period in treated animals of 1,2-fold and 1,4-fold, respectively, compared to the injection of tacrine-free solution (Table 1).

In our opinion, the greater effect obtained with drug loaded PBCA NPs covered with PEG5000 as compared to the drug-free solution and to the drug loaded PBCA-NPs covered with Polisorbate-80 could be due to the alteration of the BBB permeability rather than a specific targeting: in fact, the presence of PEG onto the NPs surface confers stealth properties to the NPs, allowing them to remain over a longer time in the systemic circulation, thus improving the chances to reach the pathological site.

Moreover, the role of surfactants (polysorbate 80 as well as polaxamers or PEG) as possible pathways for BBB permeability increase was deeply debated by several authors, without any effective and clear result.[7,87] The only evidence was achieved by 2-D electrophoresis (2-D PAGE) showing that PBCA NPs coated with polaxamer 188 (Pluronic F68®) and Ps80 showed a considerable adsorption of apolipoprotein A-I (ApoA-I), leading to a possible interaction with BBB receptors. This finding could induce to propose a general pathway for BBB crossing of PBCA coated Ps80 (or Pluorinc), based on a great absorption of APO A-I allowing a receptor-mediated transcytosis. This hypothesis cannot be generalized, since it should be taken into consideration the close interaction between polymer and surfactants as well as the whole NPs interaction with the BBB.

*Parkinson disease*

Parkinson disease (PD) is a multicentric neurodegenerative disease. Estimates state that 1% of people over age 60 are affected by PD, the leading cause of the movement disorder called Parkinsonism.[4] Clinical features of PD include tremor at rest, bradykinesia, rigidity and flexed posture.[48]

Pathologically, the key deficit in PD has been considered to be the loss of dopaminergic neurons in the substantia nigra, which causes consequent reduction of dopamine level.[98] Cell loss and Lewy body formation (abnormal intraneuronal aggregates of protein, predominantly a-synuclein) occur in the locus coeruleus, dorsal motor nucleus, and substantia innominata.[15] Consequently, nor-adrenergic, serotonergic and cholinergic neurons are also lost and this widespread neurodegeneration leads to the emergence of a variety of features known as "non-motor" symptoms in PD that include cognitive decline, apathy, depression, anxiety disorders, hallucinations, gait and balance disturbances, sleep disorders, sexual dysfunction, bowel problems, drenching sweats and pain.[46] At present, for this pathology no significant evidences of BBB permeability alteration are described.

As there is currently no permanent rescue and cure for Parkinson disease, and there are no known agents to slow the progression of PD, the current treatments are used to decrease the symptoms of PD.[64,122] Although symptomatic therapy of PD is effective (Levo-Dopa), novel therapeutics are required as well as advanced delivery methods, as the active substances will need to enter the CNS to exploit their effects (Table 1).

Animal PD model is obtained through injection of neurotoxins: the most used toxin is 6-hydroxydopamine (6-OHDA stereotaxical injected),[16] but also 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP i.p. injected) is used[18]: both agents destroy neurons by generating active oxygen species such as superoxide radicals. Kurakhamaeva and colleagues[59] used mice treated with MPTP to test the efficacy of NGF entrapped in PBCA NPs covered with polysorbate-80. This mice were submitted to many tests to evaluate the improvement in Parkinson symptoms: i) open field test, that allows to check the orientation-research reaction; ii) locomotor activity in the actometer, to value the quality and the quantity of movement; iii) rigidity, through the registration of the alteration of gain dynamic; iv) tremor, through the registration of intensity and duration of tremor. With all these tests, the researchers observed that the administration of targeted NPs allowed a significant improvement in PD symptoms already after 90 minute and maintained for 21 day.

The research group of Pardridge performed several studies using rodents 6-OHDA treated with apomorphine or amphetamine to induce rotation: the reduction in the number of rotations, promoted by administration of anti-parkinson drugs, demonstrated a improvement in PD induced syndrome. In fact, Xia and co-worker[126] prepared a LPs formulation loaded with GDNF plasmid (Glial-derived neurotrophic factor) and targeted to the brain with OX-26 (mAb against the mouse TfR). The single administration of that system to 6-OHDA rat treated with apomorphine, showed a general improvement of PD symptoms, as they observed a decrease in rotational behavior of 58% and a great increase of Tyrosine Hydroxylase (TH) activity as compared with saline control. In another paper, Zhang and colleagues improved the results obtained by Xia, using a multiple injection of the LPs,[132] achieving a decrease in the rotational behavior until 90% and an increase in the TH activity until 77%, compared with saline control. Besides, Zhang performed another test, namely the Vibrissae-Elicited Forelimb Placing Test, aiming to evaluate the forelimb response to ipsilateral facial whisker stimulation[8] This test showed an improvement of response up to 4-fold, if compared to saline control, thus allowing the authors to corroborate the results obtained before. In another article,[131] Zhang showed the results obtained with the same kind of

**Table 1.** Neurodegenerative diseases models and Nanotech applications.

| PATHOLOGY | TARGETING (BBB/tumor) | APPROACH | DRUG | MODEL | TEST | RESULTS | REF |
|---|---|---|---|---|---|---|---|
| Alzheimer | LDL Receptor | PBCA-NPs-Ps80 | NGF | Mice treated with scopolamine.[86] | PAR test.[118] | Increase of retention latency (2,12-fold vs drug free) | *Kurakhmaeva et al., 2009* |
| | LDL Receptor | PBCA-NPs-Ps80 | Tacrine | Rats injected with scopolamine | PAR test and HPLC biodistribution | Increase of retention latency (1,20-fold vs drug free; increase brain delivery (1,43 fold vs drug free) | *Abdel-Wahab et al 2009* |
| | / | PBCA-NPs-PEG500 | Tacrine | Rats injected with scopolamine | PAR test and HPLC biodistribution | Increase of retention latency (1,37 fold vs drug free; increase brain delivery (1,75 fold vs drug free) | |
| Parkinson | LDL Receptor | PBCA-NPs-Ps80 | NGF | C57Bl/6 mice treated with MPTP.[18] | Open field test (*Schmidt et al., 2001)*; actophotometer test; rigidity test *(Amende et al., 2005)*; tremor test. | Significant reduction of Parkinson symptoms; reduction of rigidity; improvement of locomotor activity | *Kurakhmaeva et al, 2009* |
| | / | LPs (CHOL:LEC: Span 80:Tween 20 1:9:2:1) | Dopamine | Rats treated with Haloperidol | Rotarod test and actophotometer test | Increase of muscular coordination activity (3,5-fold); increase of locomotor activity (3,4-fold) | [88] |
| | Transferrin Receptor | LPs (POPC:DDAB: DSPE-PEG 47:1:2)-OX26 | 877 cDna (TH gene) | Rats treated with 6-OHDA (RMFB) *(16) injected with apomorphine to induce rotation* | Count of controlateral rotation | After 3 day post i.v. decrease of rotational behavior (> 70%); decrease of TH enzyme activity during time (after 9 day is like control);TH enzyme activity is $\propto$ to injected dose. | *Zhang et al., 2003* |
| | / | LPs (PC:Chol: SA 6:3:1) | Dopamine HCl | Chlorpromazine-induced catatonia in rats | Reduction in degree of catatonia | 83% of animal obtain complete reduction of catatonia until 150 minute after injection. (0% with dopamine solution) | [53] |
| | Transferrin Receptor | LPs (POPC:DDAB: DSPE-PEG 47:1:2) -OX26 | GDNF plasmid DNA | Rats treated with 6-OHDA (RMFB) then injected with and apomorphine or amphetamine to induce rotation | Count of controlateral and ipsilateral rotation; Vibrissae-Elicited Forelimb Placing Test ([8]) | Decrease of rotational behavior (> 87–90%); increase of TH enzyme activity (>77 %); improvement of response to Vibrissae test (4-fold vs saline control) | *Zhang et al., 2008* |
| | Transferrin Receptor | LPs (POPC:DDAB: DSPE-PEG 47:1:2)-OX26 | GDNF plasmid DNA | Rats treated with 6-OHDA (RMFB) then injected with apomorphine | Count of controlateral rotation induced by apoM | Decrease of rotational behavior after 4 week post i.v. (>58 %); increase of TH enzyme activity (>19 fold vs saline control) | [126] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Transferrin Receptor | LPs (POPC:DDAB:DSPE-PEG 47:1:2)-OX26 | 877 cDna (TH gene) (10 ug/rat) | Rats treated with 6-OHDA (RMFB) then injected with apomorphine. | Count of controlateral rotation induced by apoM | Day 3 post i.v.: increase motor function (86%) and 26 fold increased TH enzyme activity Day 6 and 10 post i. v.: No great improvement | 127 |
| | | | PrgTH3 (TH plasmid) (10 ug/rat) | | | Day 3 post i.v.: No great improvement Day 6 and 10 post i.v.: increase motor function (69%); 2 and 4-fold increased TH enzyme activity | |
| | | | 877 cDna (TH gene) (10 ug/rat) and PrgTH3 (TH plasmid) (10 ug/rat) | | | Day 3 post i.v.: increase motor function (73%) and 13-fold increased TH enzyme activity Day 6 and 10 post i. v.: increase motor function (71%) and 6 −5-fold increased TH enzyme activity | |
| Huntigton's Disease | BBB-crossing glycopeptides | PLGA | Cholesterol | KO HD mice | Pharmacology Behavior Pathological evidences Confocal Microscopy | Ability of NPs to deliver cholesterol in the brain, confirmation of cholesterol localization in specific areas, rescue in KO animal models | [116] |
| Multiple Sclerosis | / | PEG-LPs (DPPC: DSPE-$PEG_{2000}$: CHOL 1.85:0.15:1) | Prednisolone | Rats with EAE induced injection of freshly activated, MBP-specific T cells *(Gold et al.,1995)* | Immunohistochemistry | Reduction in Tcells apoptosis and Tcells infiltration in the CNS (spinal cord) after 42 h of treatment; reduction of inflamation and restoring of BBB integrity | [100], *Schmidt et al., 2003b* |
| | / | PEG-LPs (EPC:CHOL: DSPE- $PEG_{2000}$ 54:41:5) | TMN | Mice with acute EAE (*Pollak et al 2003) and* chronic EAE (*Offen et al 2000)* | PCR-Real time and expression of HPRT mRNA (proinflammatory cytokines) | Inhibition of Cytokine expression in mice treated with L-TMN. | [56] |

LDL, low-density lipoprotein; NPs, nanoparticles; PBCA, poly(butylcyanoacrylate); Ps80, polysorbate80; NGF, nerve growth factor; PAR, passive avoidance reflex test; PEG, polyethylene glycol; HPLC, high performance liquid chromatography; LPs, liposomes; CHOL, cholesterol; LEC, lectine; POPC, 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine; DDAB, dimethyldioctadecylammonium bromide; DSPE-PEG, distearyl phosphatidylethanolamine polyethylene glycol; OX26, murine monoclonal antibody against the mouse transferring receptor; TH: tyrosine hydoxylase; 6-OHDA, 6-hydroxydopamine; RMFB, right medial forebrain bundle; PC, phosphatidylcholine; SA, stearylamine; GDNF, glial-derivedneurotrophic factor; apoM, apomorphine. DPPC, dipalmitoyl phosphatidylcholine; EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; EPC, egg phosphatidylcholine; TMN, tempamine. PCR, polymerase chain reaction.

targeted LPs loaded with 877 cDNA (a cDNA of TH gene). It was seen that the LPs, administered at a different dose of loaded drugs, allowed a decrease in rotational behavior of 70% until 3 day post injection, but after 6 d the TH enzyme activity decreased and after 9 d it was comparable to the control. Xia and colleagues, in a following article,[127] demonstrated that the incorporation of a TH plasmid in the same targeted systems can allow a prolongation of the neuroprotective effect but, unfortunately, without showing the effect 3 d after the injection.

Haloperidol and chlorpromazine were also used to induce extra pyramidal effects in rat or mice. In particular, Pichandy and colleagues[88] treated rats with haloperidol to test the anti-PD effect of Dopamine loaded in LPs formulated with the use of span 80 and tween 80. Both formulations allowed an increase in muscular coordination activity, tested with the rotarod test, along with in locomotor activity, tested with actophotometer. On the contrary, Jain and coworkers,[53] took advantage of rats, injected with chlorpromazine (inducing catatonia) to test non targeted LPs loaded with dopamine, showing a complete reduction of catatonia state in the 83% of animals treated 150 min after injection.

*Huntington's disease*

Huntington's disease (HD) is a genetic neurological disorder caused by a CAG expansion in the gene encoding the huntingtin (HTT) protein. HD is featured by motor, cognitive and psychiatric disturbances, neuronal dysfunction, atrophy of the striatum and other brain regions, and progressive loss of striatal medium-sized spiny neurons (MSNs) and of cortical pyramidal neurons (Vonsattel et al 1998). Several molecular and cellular dysfunctions have been identified (Zuccato et al 2010) and one affected pathway implicates brain cholesterol. Brain cholesterol biosynthesis and cholesterol level are reduced in Huntington's disease (HD) mice suggesting that locally synthetized, newly formed cholesterol is less available to neurons. This might be detrimental for neuronal function, especially given that locally synthesized cholesterol is implicated in synapse integrity and remodeling. Biodegradable and biocompatible polymeric nanoparticles (NPs) modified with glycopeptides (g7) and loaded with cholesterol (g7-NPs-Chol), which per se is not blood-brain barrier (BBB) permeable, were successfully apply in order to obtain high rate brain delivery after intraperitoneal injection in HD mice. g7-NPs, in contrast to unmodified NPs, efficiently crossed the BBB and localized in glial and neuronal cells in different brain regions. We also found that repeated systemic delivery of g7-NPs-Chol rescued synaptic and cognitive dysfunction and partially improved global activity in HD mice.[116]

*Multiple sclerosis*

Multiple Sclerosis (MS) is a disorder of the CNS with inflammatory and neurodegenerative components, and characterized by a variable clinical pattern, which ultimately leads to a progressive neurological dysfunction.[63] Its pathological hallmarks are demyelination and cellular infiltration of T cells and macrophages (Mf). Despite long-term immunotherapy, relapses occur, which are commonly treated by repeated i.v. injections of high dose (pulse) glucocorticosteroids (GS) as a potent antiinflammatory drug (Table 1).

The most applied in vivo experimental model of MS is the experimental autoimmune encephalomyelitis (EAE) with a high tissue inflammation and the partial disruption of the BBB.[73,115] Considering MS-diseased BBB, it has been shown that disease severitiy could be correlated with alteration of BBB integrity, as confirmed by loss and delocalization of brain endothelilal junction proteins, like occluding., VE-cadherin and others.[67,123] Schmidt and co-workers, in two different works, [100]; 2003b) demonstrated that long-circulating prednisolone LPs (PL) were able to localize the high tissue therapeutic levels of glucocorticalsteroid in the inflamed cerebral area of autoimmune EAE rats model[39] as compared with an equivalent dose given as free drug with a following reduction of inflammation and a significantly improvement in the disease course of (AT)-EAE. Moreover, the drug targeting by LPs appears highly effective in restoring the BBB integrity, ameliorating the disease activity of active and adoptive transfer (AT)-EAE without detectable side effects. The authors hypothesized a selective targeting, but in our opinion the altered permeability of BBB surely increases the penetration and the localization of LPs in inflamed tissue. Similarly, Kizelsztein at al.[56] demonstrated the ability of long-circulating LPs to transport a potent antioxidant (Tempamine,TMN) to the brain tissue in a concentration able to significantly attenuate clinical symptoms of EAE in the mouse model.[89] The authors hypothesized that the size and the long circulation properties of these LPs could promote their extravasation into a well permeable inflammation sites.

## Other brain pathologies

*Epilepsy*

Epilepsy is defined as a condition characterized by recurrent (two or more) epileptic seizures, unrelated to any immediate identified cause[44] Epilepsy is one of the oldest known conditions to mankind and still the most common neurological condition affecting individuals of all ages. At any given time, it is estimated that 50 million individuals worldwide have a diagnosis of epilepsy[124] ; 2001b). Recently an excellent descriptive review concerning the epidemiology of epilepsy was published.[11] Several evidences demonstrated that the epileptic seizures alter blood-brain barrier (BBB) properties and permeability.[31,54,117] Particularly, a recently in vivo study demonstrated the dynamic changes of BBB permeability during epileptogenesis. The long-lasting increased permeability of the BBB indicated that it may contribute to increased excitability in the epileptogenic foci, leading to the progression of epilepsy and seizure development.[117] Considering the application of drug delivery systems as a treatment strategy in epilepsy (Table 2), Mori and coworkers, in two different papers, compared the anticonvulsivant effect of valproic acid and γ-butyrolactone-γ-carbonyl-L-histidyl-L propynamide citrate (DN-1417) entrapped in LPs with that obtained after free drug i. v. administration in amygdaloid-kindled rats[70]; 1992b). The animal models were obtained implanting a bipolar electrodes in the right area amygdalaris anterior that are stimulated at a defined

**Table 2.** Other brain pathologies models and Nanotech applications.

| PATHOLOGY | TARGETING (BBB/tumor) | APPROCHES | DRUG | MODEL | TEST | Results | REF |
|---|---|---|---|---|---|---|---|
| Epilepsy | / | LPs (PC:CHOL: Sterylamine 7:2:1) | PHT or HRP | Rats with status epilepticus | Anticonvulsivant effect Analysis of HRP reaction products in the brain | Suppression of AM discharges by two doses of L-PHT; accumulation of HRP (by L-HRP admin.) in the AM epileptogenic focus (2 of 5 animals tested) | [71] |
| | / | LPs (PC:CHOL: Sterylamine 7:2:1) | DN-1417 | Amygdaloid kindled rats | Anticonvulsivant effect | Suppression of kindled seizure and prolonged anticonvulsivant effect after L-DN 1417 admin. than free DN 1417 | [70] |
| | / | LPs (cationic) | VPA | Amygdaloid kindled rats | Anticonvulsivant effect | Prolonged anticonvulsivant effect after cationic L-VPA admin (2 days) than anionic L-VPA admin and free VPA (1 h) | [72] |
| | LDL Receptor | PBCA-NPs-Ps80 | MRZ 2/576 | Convulsion model in mice | Anticonvulsivant effect (MES test) | Longer duration in MES test after coated Np-MRZ 2/576 admin. (up to 210 min) than uncoated Np admin. (60 min) | [36] |
| Lysosomal storage disorders | Transferrin receptor (BBB) | LPs (POPC: DDAB : DSPE-PEG2000: DSPE-PEG2000 -maleimide + 8D3 mAb) | pCMV-GUSB plasmid DNA | MPS type VII GUSB null mice | GUSB enzyme activity measurement Protein concentration determination | GUSB enzyme activity in the brain of adult mice = 13.3 ± 0.9 nmol/hr/mg protein; GUSB enzyme activity in the brain of GUSB null mice after L/plasmid DNA treatment =1.7± 0.1 nmol/hr/mg protein; GUSB enzyme activity in the brain of GUSB null mice after saline admin. < 0.2 nmol/hr/mg protein. | [132] |
| Cerebral Ischemia (Traumatic Injury) | / | LPs (DPPC:CHOL: Sterilamine 14:7:4) | CuZn-SOD | Rats cold- injury brain model *(Chan et al 1983)* | Determination of CuZn-SOD; determination of superoxide radicals; Evans Blue Permeability (BBB permeability) | Protection of drug; increase of CuZn-SOD brain level 8from 30 min to 2 h); reduction in brain level of superoxide radicals and water content and ameliorated BBB permeability | *Chan et al 1987* |
| | / | LPs (PC:CHOL: Sulfatides) | Calpain inhibitor (ALL NA1) | Gerbil model of forebrain ischemia (*Yokota et al 1995)* | Immunohistochemistry and immunoblot analysis | Dose dependent prevention after L-ALL Na1of CA1 neuronal damage | *Yokota et al., 1999* |
| | / | LPs (DPPC:CHOL: Sterilamine 14:7:4) | CuZn-SOD | Rats model of focal cerebral ischemia (*Chen et al.,1986)* | CuZn-SOD activity and measure of infarct size | Increase of CuZn-SOD activities in the brain; reduction of infarct size (33%, 25% and 18% for the anterior, middle and posterior slices, respectively) | [49] |
| | / | NPs | MRZ 2/566 | | Quantification of infarct lesion | Increased neurological score vs placebo Reduction at 53%of | [111] |

(*Continued on next page*)

**Table 2.** Other brain pathologies models and Nanotech applications. *(Continued)*

| PATHOLOGY | TARGETING (BBB/tumor) | APPROCHES | DRUG | MODEL | TEST | Results | REF |
|---|---|---|---|---|---|---|---|
| | | | | Rats models of focal cerebral ischemia | and neurological severity score | total infarct size (60% of cortical and 42% of striatal infarction) | |
| | / | PLGA-NPs | SOD | Rats models of focal cerebral ischemia -perfusion injury model | Quantification of infarct lesion and neurological severity score | 5% of survival after SOD-Np admin. at 28 d (Infarct volume after SOD-Np = 20%, saline control=56%, SOD- SOL=75%. Ischemic lesion area after SOD-Np=25%, saline control =50%, SOD-SOL =80%. Neurological severity score after SOD-Np=3.5, saline control=10, SOD-SOL=11) | 90 |
| | / | PLA-NPs | QC | Rats model of cerebral ischemia | Diene level, index of lipid peroxidation and GSSG/GSH ratio | Significant protection to endogenous antioxidant enzymes against ischemia induced oxidative damage in neuronal cells | 29 |
| Arsenic induced oxidative damage | / | PLGA-NPs | QC | Rats with neuronal cell damage after $NaASO_2$ injection | Intracellular GSH, GPx, GR, GST and G6PDH activity in the brain | QC-Np prevent the antioxidant deplection in brain cells (levels of GPx from Np 4.98 ± 0.31 vs 2.17±0.09 μmol NADPH oxidation/min/mg protein; G6PDH nmol NADPH reduced/min/mg protein 6.07 ±0.39 vs animal model 3.61 ±0.29; GR μmol NADPH oxidation/min/mg protein 17.92 ±2.33 vs 9.92 ±0.87;GST nmol product/min/mg 97.86 ±7.22 vs 69.26 ±9.88) | 38 |
| Traumatic Brain Injury | / | PANAM dendrimers | N-acetyL-cysteine, valproic acid | Traumatic brain injury, rats | In vivo administration, biodristribution and pharmacological test | PANAM localize in the injured neurons and microglia in the brain. -loading with N-acetyl cysteine and valproic acid | 69 |
| | / | self-assembling peptide nanofiber scaffold | / | Traumatic brain injury, rats | In vivo administration | Reduction of acute brain injury and brain cavity formation | 96 |
| | / | pegylated PLGA NP (100-200-800 nm) | / | Traumatic brain injury animal model | In vivo administration, brain localization | Smaller NPs are facilitated in BBB crossing | 25 |
| | / | PLGA NPs | Cerebrolysin | Traumatic brain injury, rats | In vivo administration, brain edema formation, rescue tests | Ability in delivering cerebrolysin to site of action; - reduction of brain pathology features | 94 |
| Infectious diseases | / | LPs (PC:CHOL:DSPG -AmBisome) | AMB | Candida Albicans infected rabbits | CFU in the brain tissue | Reduction of infection in the brain | 41 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | / | LPs (PC:CHOL:DSPG -AmBisome) | AMB | Murine model of cryptococcal meningitis (Cryptococcus neoformans) | CFU in the brain tissue and therapeutic activity | 20 mg/kg and 30 mg/kg of Ambisome were able to clear the yeast from the brains of the mice (44 and 78% clearance, respectively) [5] |
| | / | LPs (PC:CHOL:DSPG -AmBisome) | AMB | Murine model of cryptococcidiosis (Cryptococcus neoformans) | Therapeutic activity in the brain tissue | 10 mg/kg AmBisome = 5.69±0.38 $\log_{10}$ CFU/brain; 1 mg/kg fungizone = 6.92±0.50 $\log_{10}$ CFU/brain. Ten mg/kg Ambisome=50–60% survival; 1 mg/kg fungizone = 12–30%. [113] |
| | / | LPs (PC:CHOL: DSPG-AmBisome ) | AMB | Immuno-suppressed rabbits with Coccidioides immitis | CFU in the brain tissue and therapeutic activity | 3x15mg/kg Ambisome for 3 weeks clears the fungus from the brains (3/8 rabbits). None with other treatments. CFU reduction vs control [23] |
| | / | LPs (PC:CHOL: DSPG-AmBisome) *Association with other VCZ, CAS, MICA)* | AMB | Murine models of CNS aspergillosis | CFU in the brain tissue and therapeutic activity of animals | 1 mg/kg Ambisome from 1 to 3 d with VCZ from days 4 to 10 demonstrated higher efficacy than other combinations [22] |
| | LDLr (?) | PLA-b-PEG-NPs-Ps 80 | AMB | Cryptococcal Meningitis Bearing Mice | Fungal growth and therapeutic activity | Survival: twice than controls [92] |
| | | $CG_3R_6TAT$ NPs | $CG_3R_6TAT$ | Staphilococcus Aureus induced meningitis rabbit model | CFU in the brain tissue | Np 2 lg CFU/g tissue; control 3.7 lg CFU/g tissue; Suppression of bacterial growth in the brain, decreasing the degree of the infection [61] |
| | | $CG_3R_6TAT$ NPs | $CG_3R_6TAT$ | Criptococcal neoformans meningitis rabbit model | CFU in the brain tissue and biodistribution studies (FITC-loaded Np) | Suppression of yeast growth in brain tissue (Np treatment < 2CFU/g; control ≥15/g). [121] |

LPs, liposomes; PC, phosphatidylcholine; CHOL, cholesterol; PHT, phenytoin; HRP, horseradish peroxidase; AM, amygdala; DN-1417, γ -butyrolactone- γ-carbonyl-L-histidyl-Lpropinamide citrate; VPA, valproic acid; PBCA, poly(butylcyanoacrylate); NPs, nanoparticles; MRZ 2/576, 8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]quinoline-5-oxide choline salt; MES, prevention of maximal electroshock; POPC, 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine; DDAB, dimethyldioctadecylammonium bromide; DSPE-$PEG_{2000}$ distearoylphosphatidylethanolamine polyethylene glycol; 8D3 mAb, monoclonal antibody against the mouse transferring receptor; ; GUSB, b-glucuronidase; MPS, mucopolysaccharidosis; CuZn-SOD, CuZn superoxidase dismutase; DPPC, dipalmitoyl phosphatidylcholine; DPPS dipalmitoyl phosphatidylserine; MRZ 2/566, (8-chloro-4-hydroxy-l-oxo- 1,2-dihydropyridazino[4,5-b]quinolin-5-oxide choline salt; PANAM, polyamidoamine; PLGA, poly(D,L-lactide co-glycolide); SOD, superoxide dismutase; QC, Quercetin; GSSG, oxidized glutathione; GSH, reduced glutathione; PLA, Polylactide; $NaASO_2$, sodium arsenite; GSH, glutathione; GPx, glutathiane peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; G6PDH, glucose-6-phosphate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; DSPG, distearoylphosphatidylglycerol; CFU, colony forming units; VCZ, voriconazole; CAS, caspofungin; MICA, micofungin; Ps80, Polysorbate 80; AMB, amphotericin B; $CG_3R_6TAT$, cholesterol conjugated 3 glycine, six arginine and TAT peptide; FITC, fluorescein isothiocyanate.

time intervals and frequency as described by Löscher and co-workers.[62] In all cases, while the administration of free drug suppressed amygdaloid kindled seizure for few time, the drug encapsulated in positive charged LPs showed a prolonged anticonvulsivant effect. The authors hypothesized that LPs protected the drug from the enzymatic degradation and that LPs surface charge played an important role in allowing the rapidly cross of the BBB, by taking advantage of adsorbtive-mediated endocytosis. Recently, the same group of research evaluated the effect of phenytoin (PHT), entrapped in LPs (PHT-LPs), on the status epilepticus, induced in rats by injecting a combination of dibutyryl-cAMP (db-cAMP) and ethylenediaminetetraacetic acid (EDTA) into the amygdala (AM).

The researchers observed the suppression of AM discharges by two injections of PHT-L. The authors hypothesized that the AM epileptogenic focus created by db-cAMP/EDTA has a high affinity for LPs, and this factor participates in the local suppression of AM discharges by PHT-L. Even if, the mechanism underlying the selective augmentation of LPs in the epileptogenic AM is not known, the amount of LPs in the circulation may be important for the understanding of this finding, since two injections of PHT-L caused preferential suppression of AM discharges as well as two injections of horseradish peroxidase (HRP; to which the blood-brain barrier is impermeable) loaded LPs increased their augmentation in the AM. Since, the blood flow is reliably increased in the epileptogenic focus, it is our opinion that the possible cause of LPs local augmentation could lie in the blood-flow increase, along with the permeability of BBB increasing over the disease progression, consequently promoting the brain localization of LPs (Mori et al., 1995).

Friese and coworkers evaluated the prevention of maximal electroshock induced convulsions in mice model after i.v. administration of a novel non-competitive NMDA receptor antagonist MRZ 2/576 (potent but rather short-acting anticonvulsant, rapidly discharged from the central nervous system by transport processes that are sensitive to probenecid) bound to PBCA NPs coated with PS-80; this formulation prolonged the length of the anticonvulsive effect up to 210 min and after probenecid pretreatment up to 270 min compared to 150 min with probenecid and MRZ 2/576 alone.[36] Reasonably, the NPs operated as drug delivery systems, able to protect and to prolong the release of drugs to the CNS. Moreover, the coating of NPs with PS-80 seems to be necessary to target and to achieve an uptake of the particles into the brain. It is also important to consider that the BBB properties can be altered by the treatment performed to achieve animal model, thus promoting and enhancing NPs BBB crossing.

*Lysosomal storage disorders*

The lysosomal storage diseases (LSDs) are a heterogeneous group of disorders that affect 1/7,000 live-born infants, the majority of which develop CNS disease. Each LSD results from a deficiency of a single lysosomal enzyme, pivotal for degrading macromolecules that must be turned over in lysosomes.[110] More than 40 LSDs have been described. The neurological compromission is generally refractory to treatment, which will require long-term correction of lesions dispersed throughout the central nervous system to be effective. A promising approach is enzyme replacement therapy (ERT) or gene therapy by gene transfer methods enable the treatment of these diseases by transferring a wild-type copy of the gene of interest to some target organ, where the production and export of the protein proceed for extended periods of time.[109] In this contest, Zhang and co-workers[133] treated a mice model for type VII mucopolysaccharidosis (MPS-VII), deficient for the β-glucuronidase (GUSB) lysosomal enzyme, with GUSB expression plasmid (p-CMV GUSB) encapsulated in LPs and superficially engineered with the rat 8D3 MAb to the mouse transferrin receptor (TfR) to achieve BBB crossing (Table 3). The results demonstrated that sub-normal, yet therapeutic levels of GUSB enzyme activity, are achieved in brain following the i.v. administration of the TfR mAb-targeted LPs encapsulating p-CMV GUSB plasmide. This kind of strategy, even if interesting, will required repeat administrations, considering the chronic progression of disease and the inability of the plasmid delivered by LPs to integrate into the host genome.[21]

*Stroke*

After heart disease and cancer within the developed world, stroke is the third largest killer, second most common cause of neurologic disability after AD.[78] There are over five million deaths/year from stroke and over nine million stroke survivors. Between the ages of 45 and 85, 20-25% of men and women, respectively, can expect to have at least one incident of stroke. Atherosclerosis, heart disease, hypertension, diabetes, and lifestyle habits are risk factors correlated with disease. Unlike AD and PD, many of the risk factors for developing this disease can be modified. The etiology of stroke is brain vascular occlusions (thrombotic stroke) or rupture (hemorrhagic stroke). The neuropathological hallmarks of stroke are necrotic infarcts of variable size with inflammatory gliosis.

The effect of hypoxia-ischemia on the BBB has been extensively investigated[57]; Bolton et al., 1998;[34,65]: this disease sets in motion a series of events, which leads to disruption of TJ and increased BBB permeability, probably mediated by cytokines, VEGF, and NO. Moreover, elevated levels of proinflammatory cytokines, IL-1β, and TNF-α have been demonstrated in animal brains after focal and global ischemia[33] and in cerebrospinal fluid of stroke patients.[114]

The potential of LPs and NPs in protecting and modulating the delivery of un-stable drug during the ischemic insult was deeply investigated in several researches with interesting positive results (Table 2): the success of the use of nanocarriers is not to be correlated to the need of an active targeting, but better to the facilitate diffusion of these systems through the compromised and broken BBB. LPs and NPs were used to deliver drugs able to prevent ischemic neuronal damage. Several researchers used rats as animal models, in which the focal cerebral ischemia was produced by the method described by Chen and colleagues[20] based on an extensive, but reproducible infarct. In an old study, Chan and coworkers[19] described the ameliorated condition of animals manifesting the reduction in brain level of superoxide radicals when treated with superoxide dismutase (SOD) loaded into LPs. This success could

be firstly searched both in the protection exploited by LPs along with their ability to operate a prolonged delivery of drug. More recently, Imaizumi and co-workers, using the same animal model, demonstrated that LPs facilitate the delivery of SOD, thus elevating SOD activities into the brain, not only in the infarct, but also in the non-infarcted subcortical areal. The authors try to justify these results hypothesizing the penetration of SOD loaded LPs across the leaky BBB in the ischemic zone and subsequently LPs uptake by adjacent neurons and microglia extending the area of action; otherwise, SOD loaded LPs could exert their action on extracellular superoxide on the luminal side of capillaries, without penetrating the BBB.[49] More recently, Reddy and coworkers demonstrated the ability of PLA NPs to encapsulate and to stabilize SOD from degradation and to provide a localized modulated brain delivery that assures the protective effect of the enzyme by neutralizing the deleterious effect of reactive oxygen species which are the mediators of reperfusion injury.[90] By using focal cerebral ischemia model rats, Sopala and co-workers[111] evaluated the neuroprotective activity of the novel glycine-β site NMDA (N-methyl-D-aspartate) receptor antagonist MRZ 2/576, formulated in NPs that provide a system able to protect and to modulate the delivery of the drug to the CNS.

In another study, citicoline-loaded LPs were administered in ischemic reperfused mice; the LPs formulations produced an increase in rat survival rate of about 24% and a reduction of 60% in diene levels (index of lipid peroxidation) compared to the free drug.[35] Yokota and colleagues (Yokota et al., 1999) administered a calpain inhibitor to gerbils to rescue ischemic neuronal damage, but with a little beneficial effect due to BBB crossing inability of the inhibitor. On the contrary, the calpain inhibitor-LPs complex was able to produce a potently and selectively inhibition of calpain activation. LPs protect the drugs and maintain a higher concentration in the brain than free drug.

Querciein is an interesting herbal compound that exhibits antioxidant properties against many neurological diseases. Initially, Das and coworkers[29,97] proposed the use of LPs encapsulating quercetin, administered i.v. to rats (pre-treated with arsenic) as a good formulation to attenuate the oxidative damage induced by ischemia reperfusion injury in the rat brain. Recently, they proposed the use of PLGA NPs as quercitin carriers in combating arsenic induced oxidative damage in brain of rats. A singular dose of sodium arsenite was sub-cutaneously injected in rats to obtain a model of arsenic toxicity. Nanoencapsulated quercitin, orally administered before the arsenite injection, prevented the arsenic cerebral oxidative damage.[38] The authors hypothesized the ability of these NPs to cross the BBB via particle uptake mechanism based on an endocytotic pathway by the brain capillary endothelial cells. It is not clear if the treatment of rats with arsenic maintained the BBB integrity, thus it is difficult to give an absolute conclusion on the real efficacy and BBB crossing pathway. The increased efficacy of these formulations should be found in their ability to stabilize the drug and to prolong the brain delivery.

*Traumatic brain injury*

Traumatic brain injury (TBI), happens when an external force traumatically injures the brain. TBI can be classified on severity, mechanism (closed or penetrating head injury) and area (specific or widespread area). TBI is now considered as one the major major causes of death and disability worldwide, especially in children and young adults.. It could vary on severity depending on the casues, but the common features are mainly consiting on alterations in cerebral blood flow and the pressure within the skull, contributing substantially to the damage from the initial injury. In this kind of pathology, blood-brain barrier features are strongly altered, as permeability and transport across the BBB are dramatically changed.

Several attempts in prevention and "quick" interventions were performed in the past years, also dealing with nanomedicines, able to transport and deliver efficiently in the traumatic areas those drugs needed to restore "altered" parameters.[105,107]

In particular, a recent study[69] demonstrates that the administration of polyamidoamine (PAMAM) dendrimers could be taken up in the brain of injured animals and selectively localize in the injured neurons and microglia in the brain. Besides, these dendrimers were also able to deliver two clinically approved drugs, N-acetyl cysteine (attenuating neuroinflammation) and valproic acid (attenuating excitotoxicity), building on positive outcomes in a rabbit model of perinatal brain injury.

In another elegant research,[96] self-assembling peptide nanofiber scaffold were used to reduce acute brain injury and brain cavity formation, and to improve sensorimotor functional recovery. SAPNS serves as biocompatible material in the hemorrhagic brain cavity. Local delivery of this nanomaterial may facilitate the repair of ICH related brain injury and functional recovery.

Besides, in another paper,[25] the impact on the size of nanomedicines (pegylated PLGA NPs sizing 100nm, 200 nm and 800nm) on distribution in TBI animal models was studied. The results highlighted that smaller NPs are facilitated in BBB crossing while bigger NPs may result in a lower permeation. This finding would be extremely important in order to understand the rationale in planning nanomedicine for this kind of interventions and may help in designing NPs with a higher performance in targeting traumatic brain areas depending on the size and on the BBB permeability under TBI events.

In the treatment of TBI, the use of mixture of peptides activating growth factors was deeply studied in the last 5 y[106] Cerebrolysin is a peptide mixture able to ameliorate symptomatology and to delay progression of neurological disorders such as Alzheimer disease and dementia. Cerebrolysin loaded PLGA NPs were therefore designed, formulated, tested on stability over time in plasma and finally in animal models, where they were able to deliver cerebrolysin to reduce brain pathology following traumatic brain injury.[94]

*Infectious disease*

Brain inflammatory diseases such as meningitis and encephalitis are among the top ten infection causes of death; they are caused by bacteria (such as Bacillus anthrax, Staphylococcus aureus), fungi (ex Candida albicans, Cryptococcus neoformans) or viruses (Jafari et al. 1994;[77,93] Despite the general efficacy of antibiotic treatment, high mortality and morbidity is frequently

recovered due to the difficulty of drugs to the BBB and access the brain (Table 3).

The LPs approach in brain infectious therapy could be mainly refereed to the use of Ambisome®, a liposomal formulation of amphotericine B in which the drug is strongly associated with the bilayer structure of unilamellar LPs.

Several works, using different animal models, provided evidences of AmBisome effectiveness against brain infections. Groll and colleagues[41] evaluated groups of un-infected and Candida albicans-infected rabbits treated with AmBisome and the other commercially formulation of amphotericine B (Amphotec®), showing AmBisome treatment able to completely clear Candida albicans from the brain of infected animals. Several years ago, Albert and colleagues[5] described a mouse model of meningitis caused by Cryptococcus neoformans; also in this case, the AmBisome therapy (20 and 30 mg/kg) produced a disappearance of Cryptococcus in the brain. Several years later, Takemoto and co-workers[113] treated the same animal model with a lower dose of Ambisome (10 mg/kg) in comparison with other antifungal drugs as fungizone. AmBisome exhibited greater efficacy and, more importantly, it is distributed into the brain as the penetration enhanced by the progression of cryptococcal meningitis correlating with the in vivo activity. Clemons and colleagues administered AmBisome to treat different brain infections: in a first study, AmBisome was compared with other drugs (as fluconazole or amphotericin B alone) to treat coccidioidal meningitis in rabbits. The animal model was represented by immune-suppressed rabbits, challenged intra-cisternally with Coccidioides immitis[23] Recently, the authors studied the efficacy of AmBisome, micafungin (MICA), caspofungin (CAS), amphotericin B, voriconazole (VCZ), given alone or in combination, after administration in immune-suppressed mice, infected intracerebrally with Aspergillus fumigates. In the first study, the brain infection was significantly reduced by Ambisome treatment if compared with fluconazole and amphotericine B treatments. On the contrary, only the combination therapy at suboptimal doses of AmBisome and VCZ improved the outcome in CNS aspergillosis.[22] Thus, AmBisome seems to have an important role in treating brain infections, in which the inflammation, due to pathogens, alters the permeability of BBB; in fact AmBisome could allow to deliver high and active concentrations of drug in the tissue, reducing or eradicating the fungal burden.

Only in the last two years, polymeric NPs were investigated as an alternative treatment in brain infection diseases. Liu and coworkers tested a new kind of NPs in rabbit models to treat the brain infections disease. Amphiphilic cholesterol-conjugated G3R6TAT peptide (CG3R6TAT), which contains TAT sequence, was designed and assembled into core/shell NPs. These NPs possessed a broad spectrum of strong antimicrobial activities, much stronger than the hydrophilic peptide, incapable of forming NPs. The Nps were firstly examined for in vivo activity against Staphylococcus Aureus in comparison with vancomycin, a therapeutic agent widely used for the prophylaxis, suppression, and treatment of S. Aureus meningitis. The NPs were found to be as efficient as vancomycin in treating the meningitis in rabbits (Liu at al., 2009). In another experiment, CG3R6TAT NPs showed a good antimicrobial activity against the brain infections caused by Cryptococcus neoformans in rabbit model, in which the meningitis was induced inoculating intracisternally the yeast suspension. The authors demonstrated that the NPs crossed the BBB and suppressed the yeast growth in the brain tissues with similar efficiency as amphotericin B did.[121] They hypothesized that cationic peptide-decorated NPs may be taken up by the BBB through adsorptive endocytosis, diffusing into the brain tissue and suppressing bacterial growth in the brain.

Finally, starting from the hypothesis that[37,58] in NPs coated with polisorbate 80 seem to be uptaken by the brain capillary endothelial cells due to specific interaction with LDL receptors, Ren and co-workers prepared polysorbate 80 coated amphotericin B/PLA-b-PEG NPs to target cryptococcal meningitis-bearing mice. The results confirmed the therapeutic efficacy of these formulations in fungal infection in the brain, decreasing the speed of colony growth and the count of colony, thus increasing the survival time of animals.[92]

## Conclusion

The interest in developing targeted systems, able to reach the CNS, represented one of major section of neuroscience, over the last 20 y The pathologies affecting the "Brain" are surely the most difficult to be treated and the less favorable in term of rescue of the integrity of the neuronal structures. These pathologies, meaning gliomas, PD and AD, neurodegenerative diseases as MS, or infectious and stroke diseases, all have similar features, which are common and could represent a possible target for innovative therapies. Moreover, the presence of the BBB is usually a great limitation for drugs, which are normally unable to enter in the CNS.

Thus, the application of nanotechnology to CNS diseases (namely Nano-Neuroscience) is surely representing one of the most innovative strategy to improve the current treatments and, possibly, to find newer and, most of all, more efficacious therapies. Both NPs and LPs have been deeply investigated with several in vitro and in vivo models. In this review, we tried to summarize the most interesting and valuable in vivo evidences, since we do consider extremely risky to translate in vitro results (on BBB co-coltures) to in vivo outlooks of efficacy.

Moreover, in some of the cited pathologies, the integrity of BBB is seriously compromised, such as in glioma or stroke, as well as in other diseases, like in AD and PD, the permeability across the inflamed BBB is actually implemented. In these cases, a brain targeting has not to be considered as BBB crossing goal, but better as "pathology-targeted" aim.

Several experiments on polymeric NPs and LPs have been reviewed considering a general aim of understanding both the rate of brain delivery and the biodistribution; in particular, the last aim is pivotal for the scale-up of neuro-nanomedicine, since scientists must strongly assess both brain delivery efficiency and safety of nanocarriers. Moreover, beside a number of proof-of-concept experiments, relatively few pre-clinical data have been reached by using in vivo models (mice and rats, mainly), assessing

the efficacy against brain disease of active molecules (gene, drugs, enzyme, etc..) by means of nanocarrier-mediated delivery. These results pointed out a batch of data which would be useful for improving, developing and finalizing this new, non-invasive and efficacious strategy for the treatment of the brain diseases, such as AD and PD, gliomas, infectious disease, MS and LSD.

A final remark should be given regarding the possible development of nanocarriers for CNS and hopefully to drive a complete research project for neuro-nanomedicine.

Firstly, the choice of biodegradable and biocompatible starting materials is pivotal in speeding up the acceptability and FDA approval of new nanocarriers; secondly, the targeting strategy (meaning surface engineering with CNS targeting ligands) should be chosen in function of the pathology, since the BBB integrity and permeability is varying from disease to disease. It could be possible that the need for BBB crossing would not be required, especially in the case of stroke and gliomas, while it is fundamental in other BBB-healthy pathologies (such as AD or MS). Third, the choice of the carriers should be considered as function of the kind of drugs to be delivered: in particular, polymeric NPs could be useful in both hydrophilic and hydrophobic active substance encapsulation, while LPs would better contribute to an effective gene therapy. Finally, the overall fate of carriers and, most of all, their stability in the bloodstream should be accurately investigated a-priori and the proper corrections and tricks should be taken in great consideration, to definitively allow nanocarriers to exploit their job at their best.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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