



HAL
open science

Central nervous system delivery of molecules across the blood-brain barrier

Fabien Gosselet, Rodrigo Azevedo Loiola, Anna Roig, Anna Rosell, Maxime Culot

► **To cite this version:**

Fabien Gosselet, Rodrigo Azevedo Loiola, Anna Roig, Anna Rosell, Maxime Culot. Central nervous system delivery of molecules across the blood-brain barrier. *Neurochemistry International*, 2021, 144, 10.1016/j.neuint.2020.104952 . hal-03282235

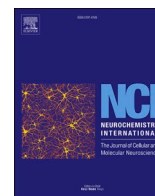
HAL Id: hal-03282235

<https://hal.science/hal-03282235>

Submitted on 8 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Central nervous system delivery of molecules across the blood-brain barrier

Fabien Gosselet^{a,*}, Rodrigo Azevedo Loiola^a, Anna Roig^b, Anna Rosell^c, Maxime Culot^a

^a Univ. Artois, UR 2465, Laboratoire de la Barrière Hémato-Encéphalique (LBHE), F-62300 Lens, France

^b Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), 08193 Bellaterra, Catalonia, Spain

^c Neurovascular Research Laboratory, Vall d'Hebron Institut de Recerca, Universitat Autònoma de Barcelona, 08035 Barcelona, Catalonia, Spain

ARTICLE INFO

Keywords:

Blood-brain barrier
Nanoparticles
Central nervous system drug delivery
Cyclodextrins
Brain diseases
PLGA

ABSTRACT

Therapies targeting neurological conditions such as Alzheimer's or Parkinson's diseases are hampered by the presence of the blood-brain barrier (BBB). During the last decades, several approaches have been developed to overcome the BBB, such as the use of nanoparticles (NPs) based on biomaterials, or alternative methods to open the BBB. In this review, we briefly highlight these strategies and the most recent advances in this field. Limitations and advantages of each approach are discussed. Combination of several methods such as functionalized NPs targeting the receptor-mediated transcytosis system with the use of magnetic resonance imaging-guided focused ultrasound (FUS) might be a promising strategy to develop theranostic tools as well as to safely deliver therapeutic molecules, such as drugs, neurotrophic factors or antibodies within the brain parenchyma.

1. Introduction

Brain parenchymal cells are isolated from the rest of the body by several barriers protecting them from neurotoxic molecules, pathogens, and circulating blood cells (Abbott et al., 2010). In parallel, these barriers allow nutrient supply and strictly regulate brain ionic composition (Abbott et al., 2010). The blood-brain barrier (BBB) is located at the brain microvessel level and represents the largest interface for blood–brain exchange with an estimated total area for exchange in the brain of between 12 and 18 m² for the average human adult (Cecchelli et al., 2007). Other barriers are blood-cerebrospinal fluid (B-CSF), blood-retinal (BRB), and blood spinal barriers, each displaying different barrier properties and cellular composition (Abbott et al., 2010; Francisco et al., 2020).

Neurodegenerative diseases (NDs), including Alzheimer (AD) and Parkinson (PD), represent a major threat to human health and have a huge impact on society and economy. Worldwide, it is estimated that nearly 45 million people have AD (Association, 2019) and 8 million live with PD (Dorsey et al., 2018). These age-related disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years, but also because of the evolution of lifestyles. Indeed, lifestyle greatly affects vascular functioning, and BBB dysfunction or loss of integrity have been reported in several of these NDs (Montagne et al., 2015; van de Haar et al., 2016; Ham et al., 2014).

Other neurodegenerative diseases are the lipid storage diseases (LSDs) regrouping more than 50 types of inherited metabolic disorders in which harmful amounts of lipids accumulate in various tissues. Because the brain is the most cholesterol-rich organ of the body, some LSDs but not all, have devastating effects on neuron survival, development, and functionality (Lachmann, 2020). According to the National Organization for Rare Disorders (NORD), LSDs are believed to have an estimated frequency of about one in every 5000 live births.

Currently, there is no cure for any of these neurodegenerative diseases. Memantine and a combination of memantine and donepezil are approved for treatment of moderate to severe AD whereas L-DOPA is given to PD patients, but these treatments only affect the neurotransmitter levels and do not slow down or reverse the neurodegenerative process. The difficulty to find a cure for these NDs is at least partly due to the presence of the BBB that impedes the distribution of promising drug candidates within the central nervous system (CNS) (Cecchelli et al., 2007; Sweeney et al., 2019).

In the case of the LSDs, the enzyme therapy replacement (ERT), consisting in intravenous injection of the deficient enzyme to patients, has been used effectively for almost 30 years and helped stabilizing organ function or slowing down disease progression (Barton et al., 1991). Unfortunately, the benefits of ERT are limited for LSDs patients with CNS dysfunctions since the injected enzyme cannot efficiently cross the BBB to reach neurons and to delay or stop the neurodegenerative

* Corresponding author. Laboratory of the blood-brain barrier, Sciences Faculty Jean Perrin, Artois University, Lens, France.

E-mail addresses: fabien.gosselet@univ-artois.fr (F. Gosselet), rodriguez@gmail.com (R.A. Loiola), roig@icmab.es (A. Roig), anna.rosell@vhir.org (A. Rosell), maxime.culot@univ-artois.fr (M. Culot).

<https://doi.org/10.1016/j.neuint.2020.104952>

Received 21 October 2020; Received in revised form 15 December 2020; Accepted 23 December 2020

Available online 2 January 2021

0197-0186/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

processes.

Consequently, for several decades some approaches have been developed to improve the CNS delivery of drugs, antibodies, peptides, enzymes, neurotrophic factors, nucleic acids, etc. The transient opening of the BBB with chemical methods has been used in clinical practice for several years (Neuwelt et al., 1986). Encapsulation of therapeutic molecules into nanoparticles (NPs) based on biomaterials could be used to increase its half-life into the human body (Schroeder et al., 2000). These NPs can be modified and functionalized to specifically target the BBB endothelial cells, thus promoting CNS delivery of the cargo. Therefore,

the combination of these 2 approaches (i.e NP encapsulation and transient opening of the BBB) might be a promising strategy to further increase the efficiency of drug delivery to the CNS.

The first objective of this review is to summarize our current knowledge on the development of different approaches to overcome the BBB to deliver therapeutic agents to the CNS, as well as imaging agents to diagnose NDs. Particular attention will be focused on the use of some types of NPs (PBCA, PLGA, cyclodextrins) able to target the receptor-mediated transcytosis (RMT) or to be coupled with BBB opening methods.

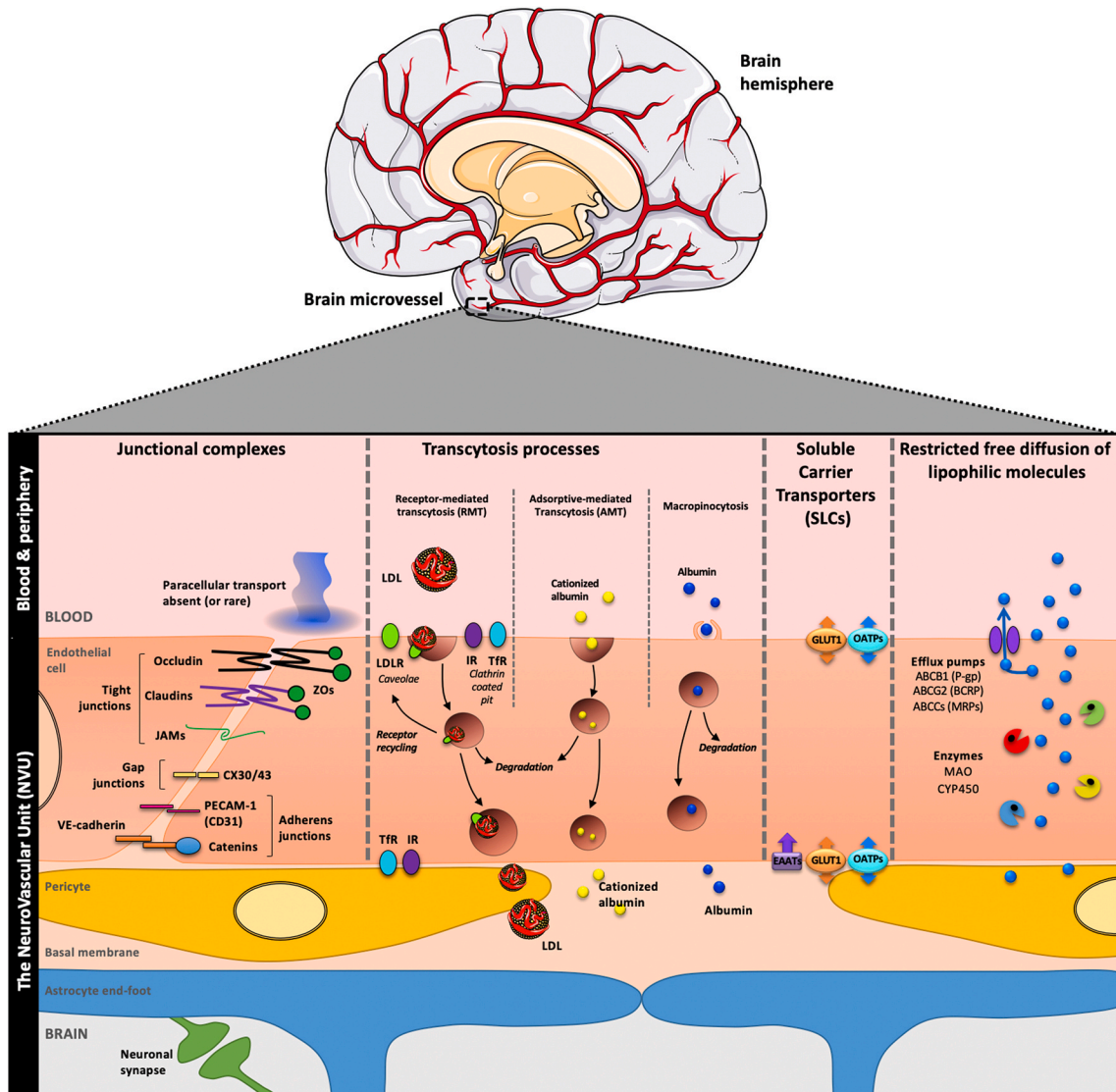


Fig. 1. The blood-brain barrier (BBB): The barrier phenotype is beared by the endothelial cells forming the brain microvessels. Contrary to the peripheral endothelial cells, the BBB endothelial cells (i) possess tight junctions, composed of occludin and claudins, both associated to intracellular scaffolding proteins (Zonula occludens), (ii) display low pinocytotic activity, and (iii) lack fenestrations. The delivery of essential nutrients for brain functioning is then strictly controlled by the presence of several receptors and transporters expressed at the luminal (blood-side) face of the endothelial cells. The passive diffusion of hexoses, monocarboxylic acids, fatty acids or amino acids (a completer) following their concentration gradient is possible through the interaction with specific transporters belonging to the solute carrier (SLC) family like LAT1 and MCT1, responsible for large neutral amino acids and glucose transport, respectively. Largest molecules such as proteins (iron-linked transferrin, insulin, etc) or low-density lipoproteins (LDL) are delivered by the receptor-mediated transport (RMT) pathway or by unspecific adsorptive-mediated transcytosis pathway (AMT). Formation of endocytosis vesicles follows the interaction of the molecules with a receptor in the case of the RMT or with the cell membrane in the case of AMT. Then, these vesicles can follow different trafficking routes including degradation by lysosomes, recycling to the apical membrane, or be transcytosed to the basolateral side where the molecules can be released. Diffusion of lipophilic molecules is restricted by the presence of ATP-binding cassette (ABC) transporters such as P-gp, BCRP or MRPs. Furthermore, harmful xenobiotics or waste products might be degraded by enzymes expressed by the BBB endothelial cells. Expression of these receptors, transporters, enzymes and efflux pumps as well as TJ functioning largely depends on the brain needs and are daily regulated by the interaction between BBB endothelial cells and other neural and mural cell types such as brain pericytes, astrocytes, neurons, oligodendrocytes, and microglial cells, thus forming the neurovascular unit (NVU).

2. Blood-brain barrier (BBB)

2.1. Properties of the BBB

As mentioned above, the BBB is localized at the endothelial cells composing the brain microvessels. It represents more than 650 km (370 miles) of exchange length between blood and brain and are therefore, very heterogeneous. Indeed, this complex microvessel network is composed of pre-capillary arterioles, capillaries, and post-capillary venules displaying each different anatomical organization, size, and barrier phenotype (Ge et al., 2005; Saubamea et al., 2012). For example, diameters of pre-capillary arterioles and post-capillary venules are comprised between 10 and 50 μm , whereas the capillary diameters do not exceed 10 μm .

Another important point to be highlighted is the absence of barrier phenotype in few vascularized spaces of the brain such as at the circumventricular organs (CVOs) and choroid plexus (CP). Fenestrated microvessels of CVOs and CP allow diffusion of molecules to the brain parenchyma (Abbott et al., 2010). Interestingly, neurogenic niches have been reported in CVOs and around lateral ventricles where brain microvessels also show leaky characteristics, suggesting that it might be possible, in theory, to easily promote neurogenesis without the necessity to develop sophisticated strategies aiming to cross the BBB.

Elsewhere, BBB endothelial cells display a barrier phenotype represented by an absence of fenestrations, a decrease/absence of pinocytotic activity, the presence of tight junctions (TJ) associated with adherens (AJ) and gap junctions between adjacent cells (Fig. 1) (Saint-Pol et al., 2020). TJ are constituted of transmembrane proteins that include occludin, claudin 1, -3 and -5 as well as junctional associated molecule (JAM), closely associated with cytoplasmic scaffolding proteins zonula occludens 1, 2 and 3 (ZO1, ZO2 and ZO3). Moreover, BBB endothelial cells express specific enzymes, receptors, and efflux pumps allowing to supply CNS with nutrients and to eliminate waste products (Abbott et al., 2010). However, it is important to keep in mind that BBB permeability and rate of pinocytosis are heterogeneous along the vascular tree, and show different pattern in BBB endothelial cells from arterioles, venules and capillaries, in relation with their barrier phenotype (Ge et al., 2005).

Noteworthy, these barrier properties are modulated in response to neuronal activity and demands. This process is tightly regulated by crosstalks between BBB endothelial cells and other neural and mural cell types. Brain pericytes are embedded in the basal lamina of the brain capillaries, whereas smooth muscle cells surround the brain biggest vessels such as arterioles and venules (Sweeney et al., 2019). Brain pericytes cover almost 30% of the abluminal face of the brain capillaries and actively participate in the BBB formation and maintenance (Dane-man et al., 2010). Astrocytes-end feet processes unsheath the microvessels, also maintaining the BBB integrity and properties (Berezowski et al., 2004). In conjunction with the basal membrane, microglial cells, oligodendrocytes and neurons, this structure forms the neurovascular unit (NVU) (Neuwelt, 2004).

2.2. Crossing of the BBB for small molecules

Due to the presence of TJs which seal the paracellular space, very few molecules can diffuse across two adjacent BBB endothelial cells (Banks, 2009). Therefore, small lipophilic molecules (<500 Da) might passively diffuse through the membranes of endothelial cells of the BBB following their concentration gradients (Irudayanathan et al., 2017). This phenomenon strongly depends on several physico-chemical parameters including the molecular weight of the molecule, its lipophilicity, charge, the number of hydrogen bond donors and the number of hydrogen bond acceptors and its polar-surface area (Wager et al., 2010). Some hydrophilic or lipophilic molecules or gas use the transcellular route directly through the BBB endothelial cells (Banks, 2009). This transcellular route occurs freely across the biological membranes for compounds such as

oxygen or carbon dioxide, or can be facilitated by the interaction with a specific transporter, as this is the case for hexoses, monocarboxylic acids, fatty acids, or amino acids. This very specific system is named solute-mediated carrier and involves a huge family of solute carrier (SLC) transporters, ubiquitously expressed in the body but most abundant at the physiological barriers such as the BBB. Among these transporters, glucose transporter isoform 1 (GLUT-1/SLC2A1) and large neutral amino acid transporter 1 (LAT1/SLC7A5) are responsible for the brain supply of glucose and essential amino acids (Sweeney et al., 2019; Singh and Ecker, 2018), respectively. Monocarboxylate transporter-1 (MCT1) transports from blood into CNS the lactate released from skeletal muscles and ketone bodies derived from liver metabolism of fatty acids (Versele et al., 2020). Expressions of these transporters at the BBB are modulated depending on the CNS needs, but also in diseases like AD or brain cancers (Sweeney et al., 2019; Singh and Ecker, 2018). This passive and facilitated (i.e mediated by a SLC transporter) diffusion across the BBB endothelial cells, does not directly require adenosine triphosphate (ATP) consumption and follows the concentration gradients.

However, this diffusion might be hindered by interaction with efflux pumps or by enzymatic degradation, especially for lipophilic molecules that interact with cellular membranes. Efflux pumps belong to the ATP-binding cassette (ABC) family and mediate the efflux of a large range of endogenous or exogenous substrates from the CNS. The most expressed efflux pumps at the human BBB are P-glycoprotein (P-gp, aka ABCB1) and breast cancer resistance protein (BCRP, aka ABCG2) (Fig. 1) (Uchida et al., 2011). Preferentially expressed at the luminal side of the endothelial cells of the BBB, these efflux pumps transport a variety of molecules with immense structural diversity, thus showing a significant overlap in their substrates (Pahnke et al., 2008). Other efflux pumps, the multidrug-resistance associated proteins (MRPs, aka ABCCs) are also reported to be expressed at the BBB but their expression pattern remains conflicting and seems to depend on the animal strain or cellular model used (Soontornmalai et al., 2006).

Although it is often predicted that a lipophilic molecule with a molecular weight below 500 Da will easily cross the BBB endothelial cells by a transcellular route, this is not true in practice due to the expression level and activity of these efflux pumps. Besides, lipophilic molecules can also be degraded by specific enzymes expressed by BBB cells, thus forming a metabolizing barrier (Shawahna et al., 2013). Indeed, an array of enzymes responsible for phase I and phase II reactions often render substrates sufficiently polar to be excreted from the CNS or to be deactivated. Phase I enzymes comprise Monoamine oxydases A and B (MAOs) and Cytochromes P450 (CYP450), whereas phase II include, but are not limited to, UDP-glucuronosyltransferases and methyltransferases.

2.3. Transcytosis of large molecules across the BBB

For the largest molecules such as proteins, lipoproteins or peptides, the transcellular route across the BBB endothelial cells requires their interaction with specific receptor and transcytosis mechanisms (Fig. 1). Transferrin, lactoferrin, insulin, and lipoproteins use this receptor-mediated transcytosis (RMT) (Freskgård and Ulrich, 2017; Candela et al., 2008). This process is highly specific and requires endocytosis vesicles formation. However, intracellular transport of these molecules between the brain and blood remains poorly understood. For a recent and full overview of this topic, see De Bock et al., 2016 (De Bock et al., 2016).

Briefly, the most highly characterized internalization pathway is clathrin-mediated endocytosis (CME) intervening in transferrin, lipoproteins, and insulin transcytosis across the BBB. After the interaction between ligand and receptor, clathrin-coated endocytic vesicles are produced via complex modular protein machinery involving more than 50 different proteins. This process shapes the membrane into a vesicle with sizes ranging from 70 to 150 nm (Kaksonen and Roux, 2018).

Other RMT mechanisms also exist at the BBB level, for example, the caveolae pathway. Caveolae are 50–100 nm flask-shape vesicles constituted by caveolin-1 at the endothelial cells. Low-density lipoproteins have been identified as able to cross the BBB using the caveolae-mediated pathway (Candela et al., 2008). Depending on the technique and model used, caveolin-1 expression at the BBB was reported as absent, low or, on the contrary, very high (De Bock et al., 2016; Strazielle and Ghersi-Egea, 2013; Smith and Gumbleton, 2006). Recently, quantitative targeted absolute proteomic analysis of brain capillaries isolated from wild-type mice clearly demonstrated that caveolin-1 is highly expressed at the mouse BBB level and that its expression increases from birth until 56 postnatal days (Omori et al., 2020). Further works are required to better decipher clathrin and caveolae involvement in RMT at the healthy and diseased BBB level.

Nonetheless, after the endocytosis process, all newly internalized vesicles are then intracellularly trafficked to early endosomes to subsequently allow delivery or degradation of the cargo. In the first case, the early endosomes can fuse with the late endosomes that then release the cargo into the brain parenchyma under the forms of exosomes or free cargo. For the molecules that are destined to be degraded, the late endosomes transfer the cargo to acidic lysosomes (De Bock et al., 2016).

The adsorptive-mediated pathway (AMT) also allows the transcytosis of large molecules through the BBB endothelial cells but does not require any interaction with a receptor. This non-specific process is mediated by the negative charge of the surface layer of glycans and glycoproteins that cover cell membranes in the body. This anionic layer, named glycocalyx, enables the unspecific binding of cationic molecules, which are then endocytosed to be trafficked across the endothelial cells. Therefore, conjugation of NPs to cationic protein such as albumin has been used to improve its delivery to the brain using this unspecific transcellular pathway (Lu et al., 2005). In mice, this glycocalyx is observed in the luminal face of brain capillaries with an area of coverage estimated at 40% whereas only 15% and 4% were reported in cardiac and pulmonary capillaries, respectively (Ando et al., 2018). Caveolae are often referred to as endocytotic vesicles mediating AMT.

Lastly, macropinocytosis represents another clathrin-independent endocytic route (reviewed in (Smith and Gumbleton, 2006; Lim and Gleeson, 2011)) and consists of the internalization of extracellular fluid and molecules. This pathway is therefore also referred to as fluid-phase endocytosis and, as for the AMT, the uptake of molecules *via* macropinocytosis occurs in a nonspecific manner. Albumin, when non-cationized, might also be endocytosed by this pathway. Macropinocytotic vesicles have no apparent coat structures and are heterogeneous in size but larger than clathrin-coated and caveolae-derived vesicles with sizes ranging from 200 to 600 nm (De Bock et al., 2016).

3. CNS delivery of drugs or therapeutic molecules

3.1. Modifying the physicochemical properties of the drugs

Since the presence of TJ between adjacent endothelial cells occludes the paracellular space at the BBB, the permeability of small molecular drugs relies on their transcellular transport across the BBB which mainly occurs by passive diffusion. Five key physicochemical parameters (molecular weight, lipophilicity, polar surface area, hydrogen bonding, and charge) require optimization to improve BBB permeability by passive diffusion (Lipinski et al., 2001). An empirical approach for selecting CNS compounds based on these parameters, known as ‘the rule of 5’ was developed by Lipinski in 2001 (Lipinski et al., 2001) and is still widely used in the early phase of drug discovery (i.e. molecule with a molecular mass less than 500 Da, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and an octanol–water partition coefficient log P not greater than 5).

Although optimizing the physicochemical properties of the drugs to enable the passive permeation across the BBB is possible, there have been only few successful cases of this approach. As an example, we can

cite the chlorambucil derivative, chlorambucil-tertiary butyl ester, that achieves a 7-fold greater concentration in brain than chlorambucil following the administration of equimolar doses (Greig et al., 1990). But modifying the physicochemical properties of the drugs does not only affect its passive diffusion across the BBB but also influence all ADME processes (e.g. distribution to other organ and binding to plasmatic proteins). Many CNS drug discovery programs have tried to improve the CNS delivery of hydrophilic CNS-active compounds by lipidization of a polar parent molecule and although that increased lipophilicity enhanced drug delivery to the brain, it did not correlate with an improved *in vivo* efficacy of these compounds. This is, at least partly due to the fact, that increasing lipophilicity of the compound also increases its non-specific binding to brain tissue (i.e. reducing its free brain fraction) and thereby reduces its availability for its therapeutic target within the brain parenchyma.

The new optimizing approach for CNS compounds thus concentrates on finding drug candidates with the right balance between free fraction in plasma, passive diffusion through the BBB and free fraction in the brain (Reichel, 2009). Due to the challenge of developing small CNS drugs with an optimal mix in physicochemical properties to cross the BBB without losing their efficacy within the brain parenchyma, other approaches consisting of improving their delivery through the BBB without affecting their physicochemical properties by encapsulating it into NPs have been developed for treating CNS diseases but also for allowing early detection of these diseases.

3.2. Use of NPs to cross the BBB

3.2.1. PBCA-NPs to deliver dalargin

Since the early 1990s, NPs have been used to improve drug delivery to the brain and have immediately shown promising results. The official definition of NPs given by the European Commission is an organic or inorganic object with only one of its characteristic dimensions to be in the range 1–100 nm, even if its other dimensions are outside that range. Usually, in biomaterials and biological fields, it is widely considered that NPs are colloidal carriers with sizes between 1 and 1000 nm. Synthetic NPs may be prepared from polymeric (bio)materials such as poly(butyl cyanoacrylate) (PBCA), oligosaccharides (cyclodextrins), poly(ϵ -caprolactone) (PCL), poly (lactic-co-glycolic acid) (PLGA), polyesters (poly (lactic acid)) (PLA) or from inorganic materials such as gold or silica (Fisher and Price, 2019). The drug or molecule to deliver is dissolved, dispersed, encapsulated, entrapped, or attached to the NP. Liposomes, micelles, and extracellular vesicles (EVs) are also colloidal carriers, but are not discussed in this review since they have their own physical properties and were discussed elsewhere (Saint-Pol et al., 2020; Naqvi et al., 2020), including in this Special Issue.

The first molecule to be designed with NPs to target the brain was dalargin, a hexapeptide of \approx 700 Da with analgesic properties. When adsorbed at the surface of PBCA nanoparticles coated with polysorbate 80 (PS80), a strong CNS analgesic effect was observed in mice after intravenous injection, but none when administrated alone or in the form of a mixture of drugs, nanoparticles, and surfactant (Kreuter et al., 1995; Schröder and Sabel, 1996). The uptake by the BBB endothelial cells was unspecific and was described as a ‘phagocytotic-like process’ by the authors. Quickly, this approach showed also promising results with other molecules unable to cross the BBB such as loperamide, doxorubicin, or tubocurarine (Alyautdin et al., 1997, 1998; Gulyaev et al., 1999) and was extended to other surfactants such as polysorbate 85 or poloxamer 188, and NPs such as solid-lipid NPs (SLN) (Yang et al., 1999). In addition to the BBB crossing, these coated PBCA-NPs also showed an increased efficiency when they were orally given to the animals suggesting that these NPs can be also used as an oral drug delivery method (Yang et al., 1999; Schroeder et al., 1998).

When the total biodistribution of injected dalargin-loaded and coated NPs were more deeply investigated in mice, it was observed that increased NPs brain delivery was the consequence of the decrease of the

liver uptake, and thus a decrease of the plasmatic clearance of dalargin (Schroeder et al., 2000). Indeed, NP brain delivery could be compared based on the percentage of injected dose able to reach the brain (% ID/g of brain). The % ID/g of a NPs reflects its ability to cross the BBB but is also related to its plasma concentration. Therefore, in all these studies, PBCA-NPs increased the half-life of dalargin in plasma, and promoted NPs interaction with cellular membranes of the BBB endothelial cells (Borchard et al., 1994; Ramge et al., 2000), but this interaction also occurred for all endothelial cells of the mice body. Despite the apparent efficiency of this method to deliver more molecules into the CNS, an increased quantity of NPs and dalargin were also measured in almost all organs. This phenomenon was later explained by the same team, demonstrating that coated NPs adsorbed plasmatic apolipoproteins such as ApoE or ApoA-I from the blood after injection and mimicked lipoprotein particles, which were then uptaken by the endothelial cells via RMT and the LDL receptor (Kreuter et al., 2002). Then, bound drugs could be further transported into the brain by transcellular pathways before being released into CNS parenchyma. This interaction between plasmatic proteins and NPs is now known as opsonization, and more than 70 different serum proteins forming a “corona” around the NPs were first reported by label-free liquid chromatography-mass spectrometry tandem (Walkey et al., 2012). Recently, more than 235 proteins were found to interact with different types of PLGA-NPs (Monge et al., 2020) among which, unsurprisingly, were the most abundant proteins in the plasma (i.e. albumin, proteins of the complement and apolipoproteins) (Monge et al., 2020).

3.2.2. Factors influencing NPs efficiency

Promising for delivering drugs to the CNS, NPs are not only able to protect their cargo from rapid degradation and clearance, but they also can be functionalized or modified to cross more easily or specifically the BBB. Indeed, NPs delivery will depend on several other factors including NPs coating, size, shape, surface charge and functionalization of the NPs. First, it is possible to decrease the clearance of NPs by the liver, spleen, and macrophages, by grafting poly(ethylene glycol) (PEG) groups on their surface, thus increasing their plasmatic circulating half-life (Nance et al., 2012; Zhang et al., 2020a). Then, several studies reported that NPs passage is inversely related to their size (Decuzzi et al., 2010) and that optimal sizes vary between 50 nm and 100 nm. These sizes are in the same range as the clathrin-coated vesicles and caveolae, 70–200 and 50–100 nm respectively (Hansen and Nichols, 2009; McMahon and Boucrot, 2011). However, some studies report NPs with sizes above 500 nm that efficiently cross the BBB, especially when they are functionalized and/or coated with PEG (Nowak et al., 2020; Kolhar et al., 2013; Kurakhmaeva et al., 2009; Karatas et al., 2009). Another important factor that needs further study is the impact of the protein corona that forms on NPs when it come into contact with biological fluids on its sizes and plasmatic half-life. Regarding the shapes of the NPs, they can be spherical, rod-like, discoid, etc. Spherical forms are the most widely used because of their facility to be synthesized and characterized. However, the first studies focusing on this question clearly demonstrated that both elongated and flattened particles attached more to the endothelial cells than the spherical ones, thus confirming that particle shape plays an important role in molecule delivery (Doshi et al., 2010). These results were later confirmed by studies demonstrating that functionalized rod-shaped polystyrene- and gold-NPs interacted more specifically with BBB cells and crossed more the barrier *in vitro* and *in vivo* than their spherical counterparts (Kolhar et al., 2013; Praca et al., 2018). Corona and Zeta potential/charge of the NPs are other important parameters that might affect NPs BBB crossing due to the high presence of the glycocalyx at the cell surface. However, it is not yet possible to identify an ideal charge of the NPs for CNS delivery as examples of NPs with positive (Fenart et al., 1999; Jallouli et al., 2007; Gao et al., 2014), neutral (Jallouli et al., 2007) and negative (Decuzzi et al., 2010; Kreuter et al., 2007; Wiley et al., 2013) zeta potentials have been reported to efficiently cross the BBB cross.

3.2.3. Cyclodextrins

To date, other NPs materials than PBCA are also used to improve CNS drug delivery in NDs. Among them, cyclodextrins (CDs) are of particular interest. Being considered as natural products in Japan, native CDs are widely used in medicine and foods in this country. Furthermore, they are approved by several official organizations such as the US food and drug administration (FDA) and the European Medicine Agency (EMA) and are therefore worldwide used in numerous industrial production processes in food, cosmetics, agriculture, environment, medicine, and chemistry. CDs are thus biomaterials widely used in the formulations of a huge quantity of marketed preparations because of their capacity to enhance drug solubility, drug bioavailability, and chemical stability. In oncology, several undergoing clinical trials aim to investigate their intravenous use to deliver anticancer drugs to several types of tumors (summarized in Table 1). However, native CDs are very-well known to induce toxicity in numerous cell lines *in vitro* due to their ability to trap cellular cholesterol (Coisne et al., 2016a) and CDs show high renal toxicity when directly injected into the bloodstream.

Native CDs represent a family of cyclic oligosaccharides prepared from enzymatic digestion of starch. They are shaped as truncated cones with an outer hydrophilic surface of (α 1 \rightarrow 4)-linked β -D-glucopyranose units and a lipophilic internal cavity (Coisne et al., 2016b) (Fig. 2A). They are usually made up of six, seven, or eight glucopyranose units defining the native α -, β - and γ -CDs, respectively. Native CDs and more specifically β -CDs are poorly water-soluble. Many derivatives have been generated to improve their water-solubility such as to add partial methylation by substitution of any of the hydroxyl groups (Table 2). Therefore, many CD derivatives display different degrees of substitution conferring specific biochemical and biological properties to the CDs. The safety and toxicity of these CDs usually depend of the type of CD used and of the route of administration. For example, intravenously injected, O-methylated CDs cause elevated levels of biomarkers of hepatic injury (Braga, 2019).

Given the high capacity of CDs to form inclusion complexes with many molecules and biomaterials (hormones, peptides, etc), several researchers have proposed to use CDs to increase the brain delivery of these molecules. To date, several pre-clinical studies demonstrated higher brain delivery of CD formulation in particular for sulfobutyl-lether- β -cyclodextrin (SBE β CD), quaternary ammonium beta-cyclodextrin (QAbetaCD) and hydroxypropyl- β -cyclodextrin (HP β CD) (Coisne et al., 2016b; Shityakov et al., 2016; Gil et al., 2009) (Table 2). However, because these CDs cross only slightly the BBB (Banks et al., 2019; Monnaert et al., 2004a), the exact molecular mechanisms of the CNS drug-releasing remains unclear. Endocytosis is still observed, but

Table 1

Few examples of undergoing clinical trials for intravenous nanoparticles PLGA, PLA and β -CD-based formulations.

Study purpose	ClinicalTrials.gov identifiers (Phase)
Target the PSMA expressed by cancer cells with PEGylated PLGA-NPs or PLA-NPs to deliver docetaxel (BIND-014) to prostate, metastatic, cervical, head and neck, non small cell lung cancers	NCT02479178 (Ph II)
	NCT02283320 (Ph II)
	NCT01812746 (Ph II)
	NCT01792479 (Ph II)
Use of formulated β -CD (CRLX301 or CRLX101) to deliver anticancer drugs to Rectal/Ovarian/Tubal/Peritoneal cancer cells	NCT01300533 (Ph II)
	NCT02187302 (Ph II)
	NCT02010567 (Ph I/II)
	NCT02389985 (Ph I)
	NCT01803269 (Ph II)
NCT01652079 (Ph II)	
NCT02769962 (Ph I)	

β -CD, β -cyclodextrin; PLA, poly (D,L-lactic acid); PLGA, poly (lactic-co-glycolic acid); PSMA, Prostatic specific membrane antigen.

BIND-014 is a PLGA and PLA-nanoparticle formulation containing docetaxel and targeting the PSMA. CRLX301 and CRLX101 are formulations based on β -CD conjugated with docetaxel or camptothecin, respectively.

Adapted from (Anselmo and Mitragotri, 2019).

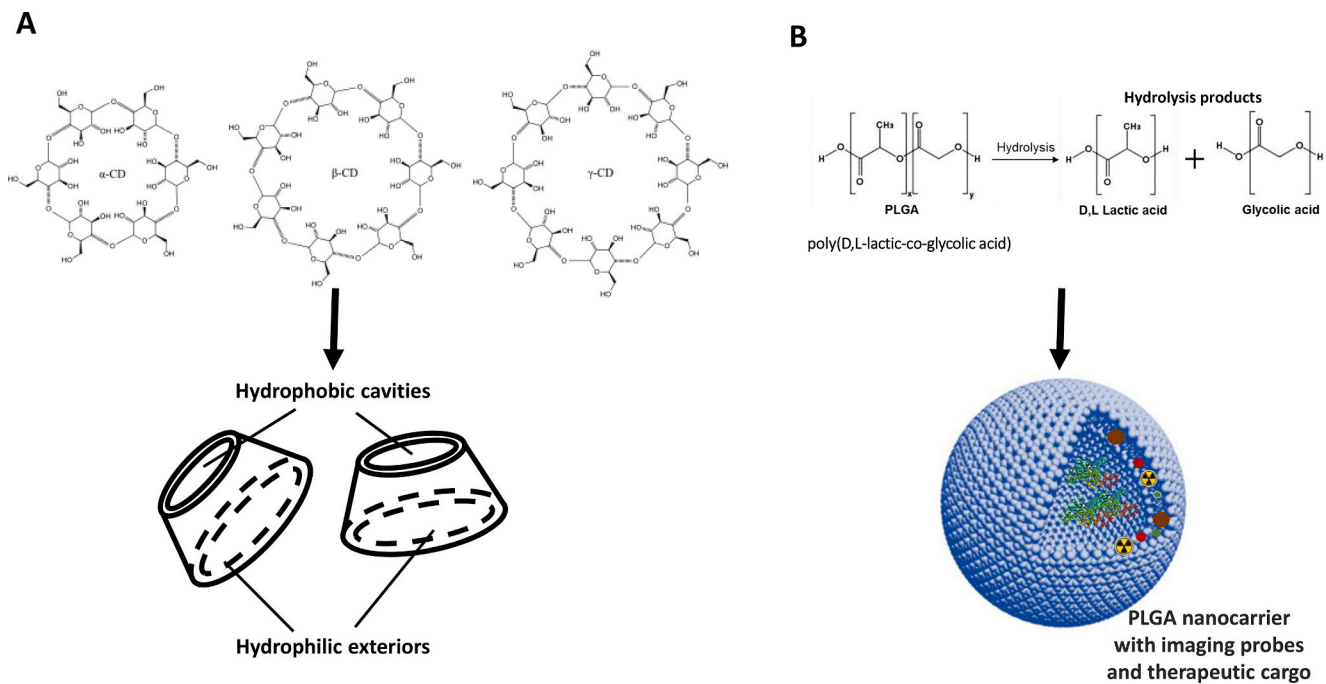


Fig. 2. Nanoparticles (NPs) summarized in this review: (A) Cyclodextrins (CDs) are cyclic oligosaccharides consisting in α -D-glucopyranoside units attached with α 1–4 links. Most common CDs used in medicine are composed by 5, 6, and 7 glucoses creating a cone shape and named α , β , and γ , respectively. (B), poly (lactic-co-glycolic acid)(PLGA)-NPs are synthesized by co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid and can be modified to include antibodies, fluorescent probes, peptides, etc. Superparamagnetic iron oxide nanoparticles (SPIONs) can also be added during the PLGA synthesis which can be used for magnetic targeting and retention and as a contrast agent for MRI. Therapeutic molecules can be added within the cavities of these NPs or can be covalently attached to the surface.

Table 2

Structure of native CDs and common modified CDs.

G = H or R

Abbreviation	N	Substituent (R)	Number of R group by CD
α -CD	6	(–)	0
β -CD	7	(–)	0
γ -CD	8	(–)	0
HP β CD	7	–CH ₂ –CHOH–CH ₃	5.6
KLEPTOSE® CRYSMEB	7	–CH ₃	4
Methyl- β -CD	7	–CH ₃	1.6
RAME β	7	–CH ₃	12.6
SBE7- β -CD	7	–(CH ₂) ₄ –SO ₃ Na	7
TRIMETHYL- β -CD	7	–CH ₃	21

CD, cyclodextrin; α -CD, α -cyclodextrin; β -CD, β -cyclodextrin; γ -CD, γ -cyclodextrin; CRYSMEB, crystalline methylated- β -cyclodextrin; HP β CD, 2-hydroxypropyl- β -cyclodextrin; Methyl- β -CD, methyl- β -cyclodextrin; RAME β , randomly-methylated- β -cyclodextrin; SBE7- β -CD, sulfobutylether-7- β -cyclodextrin; TRIMETHYL- β -CD, TRIMETHYL- β - cyclodextrin.

Adapted from (Coisne et al., 2016b).

CDs are also able to interact with and trap the cholesterol of the cell membranes (Coisne et al., 2016a). For this reason, it is suggested that CDs have fusogenicity properties and can bypass or inhibit the efflux pump activity of the P-gp and BCRP expressed by the BBB endothelial cells (Coisne et al., 2016b; Tilloy et al., 2006; Monnaert et al., 2004b; Nolay et al., 2020).

In the context of the LSDs, the ability of some CDs to trap the cellular cholesterol is of particular interest. This is the case, for the Niemann-Pick disease type C (NPC), which is characterized by intracellular

accumulation of cholesterol due to mutations in *NPC1* or *NPC2* gene. Almost 90% of NPC patients suffer from neurodegenerative symptoms. In 2009, Davidson et al. observed that the injection of HP- β -CD in NPC transgenic mice delayed the onset of the clinical disease, reduced intraneuronal storage of cholesterol, and significantly increased their lifespan (Davidson et al., 2009). Unfortunately, the beneficial effects of HP- β -CD observed in young mice were not confirmed in older animals, probably reflecting different maturation stage of the BBB in these animals (Banks et al., 2019; Monnaert et al., 2004a). Since beneficial effects of HP- β -CD were also reported in old *NPC1*-deficient mice animals following intracerebroventricular infusion, these results support the need to develop new CD formulations or to improve HP- β -CD delivery across the BBB for NPC patients. To date, the recruitment of NPC1 patients is ongoing for a clinical trial to test combined intravenous and intrathecal administrations of HP- β -CD in order to first assess liver tolerability and recovery (Table 3).

3.2.4. PLGA

PLGA-NPs are another promising type of NPs for improving CNS drug delivery and as theranostic platform. Even though PLGA-NPs may suffer from low drug loading and high drug release, they are still widely used because they show optimal properties for the encapsulation of a large variety of therapeutic agents such as proteins or DNA. Biocompatible and biodegradable, PLGA-NPs have already been approved by FDA and EMA for some intravenous applications and therefore are extensively employed in nanomedicine (summarized in (Park et al., 2019)). For example, some PLGA-NPs targeting one specific antigen are currently under investigation in several clinical trials for their ability to deliver docetaxel to cancer cells (summarized in Table 1).

Additionally, it is possible to easily control their size (from 100 to 350 nm) and charge and they can be covalently coupled with fluorophores, superparamagnetic iron oxide particles or with other ligands in order to reduce possible toxicity and/or to improve cell targeting.

For example, PLGA-NPs were recently coupled with different

Table 3

Few examples of clinical trials showcasing the progress of CNS-drug delivery approaches.

Study purpose (Clinical trial ID)	Starting Year	Phase
Use of nanoparticles in Neurodegenerative diseases Intrathecal administration of VTS-270 (HP- β -CD) in patients with Niemann-Pick C disease (NCT02534844)	2015	Phase II/ III
Use of the receptor-mediated system to deliver drugs Cerebral Enzyme replacement therapy in patients with mucopolysaccharidosis I using the insulin receptor-mediated transcytosis (AGT-181) (NCT03053089, NCT03071341)	2015/2016	Phase I// II
Cerebral Enzyme replacement therapy in patients with mucopolysaccharidosis II using the transferrin receptor-mediated transcytosis (JR-141) (NCT03128593 & NCT04573023)	2017 & 2021 (expected)	Phase II & III
BBB opening to deliver drugs Treatment of brain cancers with a combination of mephalan and BBB disruption mediated by mannitol (NCT02340156)	1998	Phase I
Brain delivery of Temozolomide in patients with high grades gliomas after Regadenoson administration (NCT02389738)	2015	Early Phase I
Determining exact dose of Regadenoson to transiently alter blood-brain barrier permeability in patients with high grade gliomas (NCT03971734)	2020	Phase I
Study designed to evaluate the safety of BBB opening in Patients with early AD using MRgFUS (NCT02986932)	2017	Phase I
Feasibility, safety and efficiency of repeated (MRI)-guided FUS for BBB opening in AD patients (NCT03739905)	2018	Phase II
Improvement of the delivery of Cerezyme to PD patients with the use of (MRI)-guided FUS (NCT04370665)	2020	N/A
BBB opening by (MRI)-guided FUS and PET tracking in AD patients to deliver therapeutics into the brain (NCT04118764)	2020	Phase I

imaging moieties thus forming a promising multimodal theranostic tool allowing to realize several imaging techniques including MRI, fluorescence at different emitting wavelengths (blue and NIR) or ^{89}Zr -labeling enabled positron emission tomography (PET) imaging (Fig. 2B) (Zhang et al., 2020b). However, this multimodal NPs showed no brain penetration. Coating of these NPs with poloxamer 188 should improve their interaction with BBB cells and thus their brain delivery. Indeed, intravenous injection of PLGA coated with poloxamer 188 was successfully used to increase the brain delivery of brain-derived neurotrophic factor (BDNF) in mice with brain trauma (Khalin et al., 2016), and promoted recovery of the brain deficits caused by the traumatic injury. Another system recently developed to improve the brain delivery of PLGA-NPs in mice and rat is the grafting of a glycosylated peptide named g7, derived from an analog of enkephalin. Enkephalin and its derivatives are antinociceptive peptides that poorly cross the BBB (Dhanasekaran and Polt, 2005). Interestingly, glycosylation of these analgesic peptides significantly improved their ability to cross BBB endothelial cells. Tosi and collaborators reported an improved brain delivery of different molecules (e.g loperamide, zinc, etc) or fluorescent probes by encapsulation into PLGA_{g7}-NPs in wild-type or transgenic mice (Tosi et al., 2007, 2011a, 2020; Vilella et al., 2018). The crossing mechanism of the NPs is still unclear but *in vitro* and *in vivo* investigations demonstrated that PLGA_{g7}-NPs interacted more with BBB cells and were quickly endocytosed, probably by adsorptive pathway (Tosi et al., 2011b). It might also be hypothesized that the glycosylated peptide coated on the PLGA-NP surface could interact with GLUT-1 highly expressed by the BBB endothelial cells.

Altogether, these results suggest that PLGA-NPs coated with poloxamer 188 or coupled with different peptides or antibodies specifically targeting the BBB endothelial cells might represent a safe and powerful

tool for early diagnostic of NDs and for promoting neuronal repair.

3.3. Functionalization of NPs: the Trojan horse system

In order to improve the brain delivery of NPs, it possible to hijack the RMT system. The best-studied macromolecule transport into the brain is the transferrin receptor (TfR)-mediated transcytosis pathway. When present in blood plasma, ferric iron rapidly interacts with apo-transferrin to form holo-transferrin. Then, holo-transferrin, which is present at relatively high concentrations in blood, interacts with the TfR, highly expressed at the cell surface of the BBB endothelial cells (Jefferies et al., 1984). Transferrin is then shuttled across the cells and iron is released into brain parenchyma. TfR is then recycled and redirected at the membrane of the cells. TfR is the RMT system the most widely used as a Trojan horse strategy to deliver NPs, recombinant proteins, and monoclonal antibodies within CNS. For example, when functionalized with antibodies targeting TfR, gold, SLN-, PLGA- or PLA-NPs are uptaken by BBB endothelial cells *in vitro* and *in vivo* (Johnsen et al., 2019; Li et al., 2020; Ramalho et al., 2018; Loureiro et al., 2017). Their cargos are delivered to brain parenchyma of mouse models of AD or brain tumors (Johnsen et al., 2019; Li et al., 2020). The density of TfR antibodies fused to NPs positively influences this internalization since the highest density promotes the highest uptake (Johnsen et al., 2019; Li et al., 2020). Fluorescent hydrophobic probes reached more the CNS when they were included into CDs conjugated to lactoferrin (Ye et al., 2013). Similar conclusions were obtained when PLGA-NPs were fused with targeting systems recognizing other receptors such as LRP1 or opioid receptors expressed by the BBB endothelial cells (Duskey et al., 2020; Hoyos-Ceballos et al., 2020).

TfR is also targeted in order to develop new treatments in LSDs. As mentioned above, the treatment of LSDs patients with neurodegenerative symptoms remains challenging because of the presence of the BBB, impeding the injected enzyme to reach neurons. Patients with mucopolysaccharidosis type II (MPSII), for example, show a deficiency of iduronate 2-sulfatase (IDS) enzyme. Recently, Ullman et al., fused this enzyme with a Fc domain that has been engineered to bind to TfR (Ullman et al., 2020). When injected into IDS deficient mice, the authors observed a wide brain distribution of IDS and an improvement of lipid metabolism and CNS inflammatory state. Similarly, as shown in Table 3, a clinical trial consisting in intravenously injections of IDS fused with an anti-TfR antibody (JR-141) to MPSII patients resulted in an improvement of their cognitive and motor symptoms (Okuyama et al., 2019, 2020).

Promising results were also obtained in MPSII mice treated by weekly injection of g7-PLGA-NPs containing IDS. After 6 weeks, the general improvement of mice's health and physiological markers were reported (Rigon et al., 2019). The authors reported that the internalization of these NPs and their neuronal delivery is mediated by clathrin. Optimization of this g7-PLGA-NP formulation should be considered to gain efficacy for this approach.

In mucopolysaccharidosis type I (MPSI), patients show deficiency in α -L-iduronidase (IDUA). This enzyme was fused to an antibody targeting the Insulin receptor expressed at the BBB level and the construct, named AGT-181, was administrated in children and older patients Table 3. Cognitive stabilization or improvement was observed in all patients demonstrating that AGT-181 is successfully reaching the brain (Giugliani et al., 2018).

Although extremely promising, the use of the RMT system as a delivery method faces several obstacles that need to be seriously considered. First, transferrin receptor sequences differ in human, mouse, and rat. Therefore, a TfR antibody designed in rat could display a different affinity for the TfR in other species and these cross-species differences must be considered while establishing the efficacy of the treatment *in vivo* before its use in human (Lee et al., 2000). Secondly, if the TfR antibody targets the transferrin binding site, it could compete with the high concentrations of endogenous plasmatic transferrin. Finally, it has

been clearly established that both the affinity and valency for the TfR could impact brain delivery of antibodies or their associated cargoes. Antibodies with high affinity for TfR, when used at low concentrations, showed an increased brain uptake compared to their lower-affinity counterparts. However, these antibodies were trapped within BBB endothelial cells and directed into the lysosomal compartment to be degraded (Bien-Ly et al., 2014). On the contrary, lower-affinity antibodies were uptaken by brain parenchyma when injected at higher concentrations (Yu et al., 2011). Additional *in vitro* and *in vivo* experiments demonstrated that decreasing the antibody-affinity for the receptor or that the use of a monovalent form instead of a bivalent structure increase their transcytosis rates (Niewoehner et al., 2014; Sade et al., 2014; Johnsen et al., 2018).

Similarly, the abundance of Tf molecules present at the surface of the NPs, is also important. If the Tf abundance is too high, NPs remain strongly attached to the BBB endothelial cells, whereas decreasing the Tf content at the NPs surface allows its transcytosis across the BBB (Wiley et al., 2013). Altogether, these data strongly suggest that a compromise needs to be found when the TfR is used as a Trojan system. Interaction with the receptor needs to be important enough to allow binding and transcytosis process, but not too high to avoid a limited release into brain parenchyma. It remains unknown whether other BBB receptors such as LDLR or insulin receptor also display these features, in particular when they are used as Trojan horse system for delivery of NPs (Portioli et al., 2017). At last, the level of receptors might be heterogeneous amongst the entire brain vasculature and might vary depending on the specific region of the CNS that is targeted.

3.4. Opening the BBB to promote CNS drug delivery

3.4.1. Targeting the TJ

Another approach to efficiently deliver drugs, macromolecules, and NPs to the CNS is to transiently disrupt the BBB with the use of physical or chemical methods. One of the commonly used approaches is the intracarotid injection of arabinose or mannitol in patients with malignant brain tumors or brain metastasis. It provokes an osmotic shock on the BBB endothelial cells, leading to a massive water efflux from cells, which in turn provokes a cell shrinkage. This triggers a physical BBB opening, with defective paracellular junctions, which is reversed in few minutes. During this time-lapse, the passage of molecules such as doxorubicin, methotrexate, or carboplatin, is facilitated by up to 90-fold (Williams et al., 1995). Even though the whole brain neural cells are unselectively exposed for 10–20 min to components and pathogens present in plasma, this technique performed by experienced surgeons is therapeutically beneficial in the treatment of high-grade malignant glioma. However, in some cases, the use of this method to disrupt BBB permeability can be highly traumatic and might result in serious side effects, such as seizures, permanent neurological disorders, and brain edema.

As an alternative to mannitol, Cereport (RMP-7), a selective bradykinin B₂ receptor agonist, has been used in clinic to trigger vasodilation of the capillaries around brain tumors, but this approach gave mitigated results in patients (Prados et al., 2003; D'Amico et al., 2020). Other chemical agents disrupting TJ between BBB endothelial cells have also been proposed such as Regadenoson, an activator of A_{2A} adenosine receptors. Despite its efficacy in animal models, Regadenoson failed to achieve convincing results in clinical trials involving healthy subjects or patients with glioblastoma (Jackson et al., 2017, 2018) and Table 3. A new clinical trial is currently in progress to determine the optimal Regadenoson concentrations to transiently achieve BBB opening (Table 3).

Peptides mimics can also be used as TJ modulators to transiently open the BBB and improve brain delivery of drugs or NPs. These synthetic peptides are designed from endogenous protein sequences, usually claudins. Because they interact with the cellular claudin as their endogenous counterparts, they impede the real interaction between two

intact proteins, thus rendering them inactive. As a consequence, the leakiness of the BBB is increased providing access of molecule to the brain parenchyma. The peptides C1C2 and C5C2, derived from claudin-1 and claudin-5 respectively were reported to trigger transient opening of the BBB both *in vitro* and *in vivo* (Dithmer et al., 2017; Staat et al., 2015; Sauer et al., 2014). Alternatively, the C terminal domain of the *Clostridium perfringens* enterotoxin's can be used. *In vitro*, variants synthesized from this domain have been shown to interact with claudin-5, thus leading to a transient TJ disruption promoting an increased passage of carboxyfluorescein across the BBB (Neuhauser et al., 2018). Further studies are necessary to demonstrate the efficiency of such approach in humans.

3.4.2. Focused ultrasound (FUS) with microbubbles

A new promising approach to transiently open the BBB has emerged recently with the use of ultrasound energy. Using focused ultrasound (FUS), specifically targeting certain brain regions, directly through the skull, might allow to transiently and safely opening the BBB. The low energy of FUS is transmitted to microbubbles that are injected into cerebral blood circulation, leading to their oscillation, and allowing them to interact with the BBB endothelial cells provoking (i) tight junction disruption, (ii) sonoporation of cell membranes, and (iii) stimulation of transcytosis (Han et al., 2017). Studies performed *in vivo* demonstrated that the TJ disruption is mainly due to the downregulation of Claudins, Occludin, and ZO-1 expression (Sheikov et al., 2008), thus enabling the passage of molecules of 3 kDa and 70 kDa (Pandit et al., 2020). Besides, the downregulation of P-gp was also reported in FUS-treated rats (Cho et al., 2016). Further studies performed in *Cav-1*^{-/-} mice have shown that the extravasation of large macromolecules (500 kDa and 2000 kDa) is controlled by the FUS-mediated increase of Cav-1 expression that promotes the unspecific transcytosis activity (Pandit et al., 2020). When used in disease animal models, coated PEG- and PS80 PLGA-NPs highly diffuse into FUS-treated brain regions thus showing an enhanced drug delivery, resulting in significantly stronger antitumour efficacy and longer survival time of the treated tumour-bearing mice (Nance et al., 2014; Li et al., 2018). Several other studies have demonstrated similar results with the use of NPs produced from other biomaterials, as well as liposomes (reviewed in (Fisher and Price, 2019)). Interestingly, the first trials performed in humans also gave promising and exciting results. When applied in patients with early AD, magnetic resonance imaging (MRI)-guided FUS shows a safe and focal opening of the BBB in the hippocampus and entorhinal cortex followed by a BBB closure within 24 h (Rezai et al., 2020). In patients with recurrent glioblastoma, repeated BBB opening did not show any deleterious effects and was well tolerated by patients (Carpentier et al., 2016).

Surprisingly, studies performed in animals suggested that the BBB opening is also capable of stimulating hippocampal neurogenesis (Mooney et al., 2016) and microglial activation (Bobola et al., 2020). These mechanisms remain unclear and need further studies.

Altogether these data demonstrate that low-intensity FUS used with microbubbles might be a very promising method to deliver molecules within specific areas of the CNS of patients with NDs and to promote neurogenesis or phagocytosis. In addition, it has been demonstrated that it is possible to control the size of the drug to be delivered by strictly modulating the FUS parameters. This possibility of a safe and localized brain delivery combined with the non-invasive nature of ultrasound confer to this approach several advantages over conventional technologies. However, current drawbacks need to be taken into account, such as the difficulty to monitor the process in real time using MRI or CT scanning, the cost, or the consequence of the CNS exposure to neurotoxic substances present in the bloodstream. Improvement of these issues remains necessary and several clinical trials are currently in progress (Table 3).

4. Conclusion and future perspectives

To date, no effective treatment exists to cure neurodegenerative diseases such as AD or PD, or to prevent the detrimental processes underlying the neurodegenerative symptoms observed in LSDs. Besides the complexity of these diseases, one of the main obstacles for such therapeutic improvement is the presence of the BBB, the real gatekeeper of the major part of the CNS. Among the different approaches developed since several decades, the use of NPs has received a lot of attention to improve the CNS delivery of small molecules but also of peptides, DNA, or neurotrophic factors such as BDNF, nerve growth factor (NGF) or glial derived neurotrophic factor (GDNF). A promising strategy to improve the brain delivery of NPs is the targeting of endogenous RMT systems that employ vesicular trafficking to transport ligands across the BBB endothelium. The recent successes obtained with this strategy, in particular with the enzyme replacement in LSDs (Okuyama et al., 2019, 2020; Giugliani et al., 2018), demonstrate that the RMT system remains an exciting and promising therapeutic approach to deliver CNS drug or molecule for other NDs. However, in the case of NPs delivery, more attention should be given to the corona that might influence NPs size, charge and could alter biodistribution and then BBB crossing via RMT system.

Further works are compulsory to better characterize BBB physiology and receptors expression patterns, especially in humans and in disease conditions. Indeed, possible alteration of its tightness and changes in the expression of receptors at the BBB in NDs should be better characterized. Recent development of new and relevant tools such as the human and non-human primate BBB models will help to reach these objectives (Cecchelli et al., 2014; Lippmann et al., 2012; Chaves et al., 2020). Information on receptors expression at the BBB in healthy (Shawahna et al., 2011) but also in disease situations could be used to improve NPs access to the brain via RMT by targeting these receptors with antibodies or an appropriate targeting ligand. Another possibility is to combine the use of NPs with a method to open the BBB and thereby facilitate drug delivery into the brain parenchyma. FUS has recently gained attention for its potential application as a method for locally and transiently opening the BBB and might be used to achieve sufficient exposure of the brain parenchyma for therapeutic agents or diagnostic molecules. Despite encouraging results obtained in humans several key aspects of the effects of FUS on BBB opening and the possible side effects of repeated FUS treatments remain unexplored and need to be addressed before this methodology could be used for NDs such as AD and PD.

CRedit authorship contribution statement

Fabien Gosselet: Supervision, Writing - original draft. **Rodrigo Azevedo Loiola:** Writing - review & editing. **Anna Roig:** Writing - review & editing. **Anna Rosell:** Writing - review & editing. **Maxime Culot:** Writing - review & editing.

Acknowledgments

We apologize to those researchers whose works have not been cited owing to space restrictions.

This work has been supported under the Euronomed 8th Joint Call-MAGGBRIS collaborative project by grants from the Spanish Ministry of Science and Innovation (PCIN-2017-090) and Instituto Salud Carlos III (AC17/00004 with FEDER funds) and from the French national agency (ANR-ANR-17-ENM3-0005-01) for FG and RAL. This work has also been supported by the SGR program (2017SGR1427 and 2017SGR765 grants) from the Generalitat de Catalunya. MC thanks the support of the ANR in the framework of the Diasyn project (ERA-Net Euronomed II JTC2015 grant number: ANR-15 -ENM2 -0002-06).

References

- Abbott, N.J., Patabendige, A.A., Dolman, D.E., Yusof, S.R., Begley, D.J., 2010. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* 37, 13–25.
- Alyautdin, R.N., Petrov, V.E., Langer, K., Berthold, A., Kharkevich, D.A., Kreuter, J., 1997. Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharm. Res. (N. Y.)* 14, 325–328.
- Alyautdin, R.N., Tezikov, E.B., Range, P., Kharkevich, D.A., Begley, D.J., Kreuter, J., 1998. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study. *J. Microencapsul.* 15, 67–74.
- Ando, Y., Okada, H., Takemura, G., Suzuki, K., Takada, C., Tomita, H., Zaikokuji, R., Hotta, Y., Miyazaki, N., Yano, H., Muraki, I., Kuroda, A., Fukuda, H., Kawasaki, Y., Okamoto, H., Kawaguchi, T., Watanabe, T., Doi, T., Yoshida, T., Ushikoshi, H., Yoshida, S., Ogura, S., 2018. Brain-specific ultrastructure of capillary endothelial glycocalyx and its possible contribution for blood brain barrier. *Sci. Rep.* 8, 17523.
- Anselmo, A.C., Mitragotri, S., 2019. Nanoparticles in the clinic: an update. *Bioengineering & translational medicine* 4, e10143.
- Association, A.s., 2019. Alzheimer's disease facts and figures Alzheimer's dementia 2019. *Annu. Rep.* 15, 321–387.
- Banks, W.A., 2009. Characteristics of compounds that cross the blood-brain barrier. *BMC Neurol.* 9 (Suppl. 1), S3.
- Banks, W.A., Engelke, K., Hansen, K.M., Bullock, K.M., Calias, P., 2019. Modest blood-brain barrier permeability of the cyclodextrin kleptose: modification by efflux and luminal surface binding. *J. Pharmacol. Exp. Therapeut.* 371, 121–129.
- Barton, N.W., Brady, R.O., Dambrosia, J.M., Di Bisceglie, A.M., Doppelt, S.H., Hill, S.C., Mankin, H.J., Murray, G.J., Parker, R.L., Argoff, C.E., et al., 1991. Replacement therapy for inherited enzyme deficiency—macrophage-targeted glucocerebrosidase for Gaucher's disease. *N. Engl. J. Med.* 324, 1464–1470.
- Berezowski, V., Landry, C., Dehouck, M.P., Cecchelli, R., Fenart, L., 2004. Contribution of glial cells and pericytes to the mRNA profiles of P-glycoprotein and multidrug resistance-associated proteins in an in vitro model of the blood-brain barrier. *Brain Res.* 1018, 1–9.
- Bien-Ly, N., Yu, Y.J., Bumbaca, D., Elstrott, J., Boswell, C.A., Zhang, Y., Luk, W., Lu, Y., Dennis, M.S., Weimer, R.M., Chung, I., Watts, R.J., 2014. Transferrin receptor (TfR) trafficking determines brain uptake of TfR antibody affinity variants. *J. Exp. Med.* 211, 233–244.
- Bobola, M.S., Chen, L., Ezeokeke, C.K., Olmstead, T.A., Nguyen, C., Sahota, A., Williams, R.G., Mourad, P.D., 2020. Transcranial focused ultrasound, pulsed at 40 Hz, activates microglia acutely and reduces A β load chronically, as demonstrated in vivo. *Brain stimulation* 13, 1014–1023.
- Borchard, G., Audus, K.L., Shi, F., Kreuter, J., 1994. Uptake of surfactant-coated poly (methyl methacrylate) nanoparticles by bovine brain microvessel endothelial cell monolayers. *Int. J. Pharm.* 110, 29–35.
- Braga, S.S., 2019. Cyclodextrins: emerging medicines of the new millennium. *Biomolecules* 9.
- Candela, P., Gosselet, F., Miller, F., Buee-Scherrer, V., Torpier, G., Cecchelli, R., Fenart, L., 2008. Physiological pathway for low-density lipoproteins across the blood-brain barrier: transcytosis through brain capillary endothelial cells in vitro. *Endothelium* 15, 254–264.
- Carpentier, A., Canney, M., Vignot, A., Reina, V., Beccaria, K., Horodyckid, C., Karachi, C., Leclercq, D., Lafon, C., Chapelon, J.Y., Capelle, L., Cornu, P., Sanson, M., Hoang-Xuan, K., Delattre, J.Y., Idhah, A., 2016. Clinical trial of blood-brain barrier disruption by pulsed ultrasound. *Sci. Transl. Med.* 8, 343re342.
- Cecchelli, R., Berezowski, V., Lundquist, S., Culot, M., Renftel, M., Dehouck, M.P., Fenart, L., 2007. Modelling of the blood-brain barrier in drug discovery and development. *Nat. Rev. Drug Discov.* 6, 650–661.
- Cecchelli, R., Aday, S., Sevin, E., Almeida, C., Culot, M., Dehouck, L., Coisne, C., Engelhardt, B., Dehouck, M.P., Ferreira, L., 2014. A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells. *PLoS One* 9, e99733.
- Chaves, C., Do, T.M., Cegarra, C., Roudières, V., Tolou, S., Thill, G., Rocher, C., Didier, M., Lesuisse, D., 2020. Non-human primate blood-brain barrier and in vitro brain endothelium: from transcriptome to the establishment of a new model. *Pharmaceutics* 12.
- Cho, H., Lee, H.Y., Han, M., Choi, J.R., Ahn, S., Lee, T., Chang, Y., Park, J., 2016. Localized down-regulation of P-glycoprotein by focused ultrasound and microbubbles induced blood-brain barrier disruption in rat brain. *Sci. Rep.* 6, 31201.
- Coisne, C., Hallier-Vanuxeem, D., Boucau, M.C., Hachani, J., Tilloy, S., Bricout, H., Monflier, E., Wils, D., Serpelloni, M., Parissaux, X., Fenart, L., Gosselet, F., 2016a. Beta-cyclodextrins decrease cholesterol release and ABC-associated transporter expression in smooth muscle cells and aortic endothelial cells. *Front. Physiol.* 7, 185.
- Coisne, C., Tilloy, S., Monflier, E., Wils, D., Fenart, L., Gosselet, F., 2016b. Cyclodextrins as emerging therapeutic tools in the treatment of cholesterol-associated vascular and neurodegenerative diseases. *Molecules* 21.
- D'Amico, R.S., Khatri, D., Reichman, N., Patel, N.V., Wong, T., Fralin, S.R., Li, M., Ellis, J. A., Ortiz, R., Langer, D.J., Boockvar, J.A., 2020. Super selective intra-arterial cerebral infusion of modern chemotherapeutics after blood-brain barrier disruption: where are we now, and where we are going. *Journal of neuro-oncology* 147, 261–278.
- Daneman, R., Zhou, L., Kebede, A.A., Barres, B.A., 2010. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 468, 562–566.
- Davidson, C.D., Ali, N.F., Micsenyi, M.C., Stephney, G., Renault, S., Dobrenis, K., Ory, D. S., Vanier, M.T., Walkley, S.U., 2009. Chronic cyclodextrin treatment of murine Niemann-Pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression. *PLoS One* 4, e6951.

- De Bock, M., Van Haver, V., Vandenbroucke, R.E., Decroock, E., Wang, N., Leybaert, L., 2016. Into rather unexplored terrain-transcellular transport across the blood-brain barrier. *Glia* 64, 1097–1123.
- Decuzzi, P., Godin, B., Tanaka, T., Lee, S.Y., Chiappini, C., Liu, X., Ferrari, M., 2010. Size and shape effects in the biodistribution of intravascularly injected particles. *J. Contr. Release* 141, 320–327.
- Dhanasekaran, M., Polt, R., 2005. New prospects for glycopeptide based analgesia: glycoside-induced penetration of the blood-brain barrier. *Curr. Drug Deliv.* 2, 59–73.
- Dithmer, S., Staat, C., Müller, C., Ku, M.C., Pohlmann, A., Niendorf, T., Gehne, N., Fallier-Becker, P., Kittel, A., Walter, F.R., Veszelka, S., Deli, M.A., Blasig, R., Haseloff, R.F., Blasig, I.E., Winkler, L., 2017. Claudin peptidomimetics modulate tissue barriers for enhanced drug delivery. *Ann. N. Y. Acad. Sci.* 1397, 169–184.
- Dorsey, E.R., Sherer, T., Okun, M.S., Bloem, B.R., 2018. The emerging evidence of the Parkinson pandemic. *J. Parkinsons Dis.* 8, S3–S8.
- Doshi, N., Prabhakarandian, B., Rea-Ramsey, A., Pant, K., Sundaram, S., Mitragotri, S., 2010. Flow and adhesion of drug carriers in blood vessels depend on their shape: a study using model synthetic microvascular networks. *J. Contr. Release* 146, 196–200.
- Duskey, J.T., Ottonelli, L., Da Ros, F., Vilella, A., Zoli, M., Kovachka, S., Spyraakis, F., Vandelli, M.A., Tosi, G., Ruozi, B., 2020. Novel peptide-conjugated nanomedicines for brain targeting: in vivo evidence. *Nanomed. Nanotechnol. Biol. Med.* 28, 102226.
- Fenart, L., Casanova, A., Dehouck, B., Duhem, C., Slupek, S., Cecchelli, R., Betbeder, D., 1999. Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an in vitro model of the blood-brain barrier. *J. Pharmacol. Exp. Therapeut.* 291, 1017–1022.
- Fisher, D.G., Price, R.J., 2019. Recent advances in the use of focused ultrasound for magnetic resonance image-guided therapeutic nanoparticle delivery to the central nervous system. *Front. Pharmacol.* 10, 1348.
- Francisco, D.M.F., Marchetti, L., Rodríguez-Lorenzo, S., Frías-Anaya, E., Figueiredo, R. M., Winter, P., Romero, I.A., de Vries, H.E., Engelhardt, B., Bruggmann, R., 2020. Advancing brain barriers RNA sequencing: guidelines from experimental design to publication. *Fluids Barriers CNS* 17, 51.
- Freskgård, P.O., Ulrich, E., 2017. Antibody therapies in CNS diseases. *Neuropharmacology* 120, 38–55.
- Gao, X., Qian, J., Zheng, S., Changyi, Y., Zhang, J., Ju, S., Zhu, J., Li, C., 2014. Overcoming the blood-brain barrier for delivering drugs into the brain by using adenosine receptor nanoagonist. *ACS Nano* 8, 3678–3689.
- Ge, S., Song, L., Pachter, J.S., 2005. Where is the blood-brain barrier really? *J. Neurosci. Res.* 79, 421–427.
- Gil, E.S., Li, J., Xiao, H., Lowe, T.L., 2009. Quaternary ammonium beta-cyclodextrin nanoparticles for enhancing doxorubicin permeability across the in vitro blood-brain barrier. *Biomacromolecules* 10, 505–516.
- Giugliani, R., Giugliani, L., de Oliveira Poswar, F., Donis, K.C., Corte, A.D., Schmidt, M., Boado, R.J., Nestrail, I., Nguyen, C., Chen, S., Pardridge, W.M., 2018. Neurocognitive and somatic stabilization in pediatric patients with severe Mucopolysaccharidosis Type I after 52 weeks of intravenous brain-penetrating insulin receptor antibody-iduronidase fusion protein (valanafusp alpha): an open label phase 1-2 trial. *Orphanet J. Rare Dis.* 13, 110.
- Greig, N.H., Daly, E.M., Sweeney, D.J., Rapoport, S.I., 1990. Pharmacokinetics of chlorambucil-tertiary butyl ester, a lipophilic chlorambucil derivative that achieves and maintains high concentrations in brain. *Canc. Chemother. Pharmacol.* 25, 320–325.
- Gulyaev, A.E., Gelperina, S.E., Skidan, I.N., Antropov, A.S., Kivman, G.Y., Kreuter, J., 1999. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharm. Res. (N. Y.)* 16, 1564–1569.
- Ham, J.H., Yi, H., Sunwoo, M.K., Hong, J.Y., Sohn, Y.H., Lee, P.H., 2014. Cerebral microbleeds in patients with Parkinson's disease. *J. Neurol.* 261, 1628–1635.
- Han, M., Hur, Y., Hwang, J., Park, J., 2017. Biological effects of blood-brain barrier disruption using a focused ultrasound. *Biomedical engineering letters* 7, 115–120.
- Hansen, C.G., Nichols, B.J., 2009. Molecular mechanisms of clathrin-independent endocytosis. *J. Cell Sci.* 122, 1713–1721.
- Hoyos-Ceballos, G.P., Ruozi, B., Ottonelli, L., Da Ros, F., Vandelli, M.A., Forni, F., Daini, E., Vilella, A., Zoli, M., Tosi, G., Duskey, J.T., López-Osorio, B.L., 2020. PLGA-PEG-ANG-2 nanoparticles for blood-brain barrier crossing: proof-of-concept study. *Pharmaceutics* 12.
- Irudayanathan, F.J., Wang, N., Wang, X., Nangia, S., 2017. Architecture of the paracellular channels formed by claudins of the blood-brain barrier tight junctions. *Ann. N. Y. Acad. Sci.* 1405, 131–146.
- Jackson, S., George, R.T., Lodge, M.A., Piotrowski, A., Wahl, R.L., Gujar, S.K., Grossman, S.A., 2017. The effect of regadenoson on the integrity of the human blood-brain barrier, a pilot study. *Journal of neuro-oncology* 132, 513–519.
- Jackson, S., Weingart, J., Nduom, E.K., Harfi, T.T., George, R.T., McCreavey, D., Ye, X., Anders, N.M., Peer, C., Figg, W.D., Gilbert, M., Rudek, M.A., Grossman, S.A., 2018. The effect of an adenosine A_{2A} agonist on intra-tumoral concentrations of temozolomide in patients with recurrent glioblastoma. *Fluids Barriers CNS* 15, 2.
- Jallouli, Y., Paillard, A., Chang, J., Sevin, E., Betbeder, D., 2007. Influence of surface charge and inner composition of porous nanoparticles to cross blood-brain barrier in vitro. *Int. J. Pharm.* 344, 103–109.
- Jefferies, W.A., Brandon, M.R., Hunt, S.V., Williams, A.F., Gatter, K.C., Mason, D.Y., 1984. Transferrin receptor on endothelium of brain capillaries. *Nature* 312, 162–163.
- Johnsen, K.B., Bak, M., Kempen, P.J., Melander, F., Burkhart, A., Thomsen, M.S., Nielsen, M.S., Moos, T., Andresen, T.L., 2018. Antibody affinity and valency impact brain uptake of transferrin receptor-targeted gold nanoparticles. *Theranostics* 8, 3416–3436.
- Johnsen, K.B., Bak, M., Melander, F., Thomsen, M.S., Burkhart, A., Kempen, P.J., Andresen, T.L., Moos, T., 2019. Modulating the antibody density changes the uptake and transport at the blood-brain barrier of both transferrin receptor-targeted gold nanoparticles and liposomal cargo. *J. Contr. Release* 295, 237–249.
- Kaksonen, M., Roux, A., 2018. Mechanisms of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* 19, 313–326.
- Karatas, H., Aktas, Y., Gursoy-Ozdemir, Y., Bodur, E., Yemisci, M., Caban, S., Vural, A., Pinarbasli, O., Capan, Y., Fernandez-Megia, E., Novoa-Carballal, R., Riguera, R., Andrieux, K., Couvreur, P., Dalkara, T., 2009. A nanomedicine transports a peptide caspase-3 inhibitor across the blood-brain barrier and provides neuroprotection. *J. Neurosci.* 29, 13761–13769.
- Khalin, I., Alyautdin, R., Wong, T.W., Gnanou, J., Kocherga, G., Kreuter, J., 2016. Brain-derived neurotrophic factor delivered to the brain using poly(lactide-co-glycolide) nanoparticles improves neurological and cognitive outcome in mice with traumatic brain injury. *Drug Deliv.* 23, 3520–3528.
- Kolhar, P., Anselmo, A.C., Gupta, V., Pant, K., Prabhakarandian, B., Ruoslahti, E., Mitragotri, S., 2013. Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10753–10758.
- Kreuter, J., Alyautdin, R.N., Kharkevich, D.A., Ivanov, A.A., 1995. Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles). *Brain Res.* 674, 171–174.
- Kreuter, J., Shamenkov, D., Petrov, V., Ramge, P., Cychutek, K., Koch-Brandt, C., Alyautdin, R., 2002. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *J. Drug Target.* 10, 317–325.
- Kreuter, J., Hekmatara, T., Dreis, S., Vogel, T., Gelperina, S., Langer, K., 2007. Covalent attachment of apolipoprotein A-I and apolipoprotein B-100 to albumin nanoparticles enables drug transport into the brain. *J. Contr. Release* 118, 54–58.
- Kurakhmaeva, K.B., Djindjikhshvili, I.A., Petrov, V.E., Balabanyan, V.U., Voronina, T. A., Trofimov, S.S., Kreuter, J., Gelperina, S., Begley, D., Alyautdin, R.N., 2009. Brain targeting of nerve growth factor using poly(butyl cyanoacrylate) nanoparticles. *J. Drug Target.* 17, 564–574.
- Lachmann, R.H., 2020. Treating lysosomal storage disorders: what have we learnt? *J. Inher. Metab. Dis.* 43, 125–132.
- Lee, H.J., Engelhardt, B., Lesley, J., Bickel, U., Pardridge, W.M., 2000. Targeting rat anti-mouse transferrin receptor monoclonal antibodies through blood-brain barrier in mouse. *J. Pharmacol. Exp. Therapeut.* 292, 1048–1052.
- Li, Y., Wu, M., Zhang, N., Tang, C., Jiang, P., Liu, X., Yan, F., Zheng, H., 2018. Mechanisms of enhanced antitumor efficacy of polysorbate 80-modified paclitaxel-loaded PLGA nanoparticles by focused ultrasound. *J. Cell Mol. Med.* 22, 4171–4182.
- Li, G., Sun, X., Wan, X., Wang, D., 2020. Lactoferrin-loaded PEG/PLA block copolymer targeted with anti-transferrin receptor antibodies for Alzheimer disease. Dose-response: a publication of International Hormesis Society 18, 1559325820917836.
- Lim, J.P., Gleeson, P.A., 2011. Macropinocytosis: an endocytic pathway for internalising large gulps. *Immunol. Cell Biol.* 89, 836–843.
- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 46, 3–26.
- Lippmann, E.S., Azarin, S.M., Kay, J.E., Nessler, R.A., Wilson, H.K., Al-Ahmad, A., Palecek, S.P., Shusta, E.V., 2012. Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. *Nat. Biotechnol.* 30, 783–791.
- Loureiro, J.A., Andrade, S., Duarte, A., Neves, A.R., Queiroz, J.F., Nunes, C., Sevin, E., Fenart, L., Gosselet, F., Coelho, M.A., Pereira, M.C., 2017. Resveratrol and grape extract-loaded solid lipid nanoparticles for the treatment of Alzheimer's disease. *Molecules* 22.
- Lu, W., Zhang, Y., Tan, Y.Z., Hu, K.L., Jiang, X.G., Fu, S.K., 2005. Cationic albumin-conjugated pegylated nanoparticles as novel drug carrier for brain delivery. *J. Contr. Release* 107, 428–448.
- McMahon, H.T., Boucrot, E., 2011. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* 12, 517–533.
- Monge, M., Fornaguera, C., Quero, C., Dols-Perez, A., Calderó, G., Grijalvo, S., García-Celma, M.J., Rodríguez-Abreu, C., Solans, C., 2020. Functionalized PLGA nanoparticles prepared by nano-emulsion templating interact selectively with proteins involved in the transport through the blood-brain barrier. *Eur. J. Pharm. Biopharm.*
- Monnaert, V., Tilloy, S., Bricout, H., Fenart, L., Cecchelli, R., Monflier, E., 2004a. Behavior of alpha-, beta-, and gamma-cyclodextrins and their derivatives on an in vitro model of blood-brain barrier. *J. Pharmacol. Exp. Therapeut.* 310, 745–751.
- Monnaert, V., Betbeder, D., Fenart, L., Bricout, H., Lenfant, A.M., Landry, C., Cecchelli, R., Monflier, E., Tilloy, S., 2004b. Effects of gamma- and hydroxypropyl-gamma-cyclodextrins on the transport of doxorubicin across an in vitro model of blood-brain barrier. *J. Pharmacol. Exp. Therapeut.* 311, 1115–1120.
- Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., Harrington, M.G., Chui, H.C., Law, M., Zlokovic, B.V., 2015. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 85, 296–302.
- Mooney, S.J., Shah, K., Yeung, S., Burgess, A., Aubert, I., Hynynen, K., 2016. Focused ultrasound-induced neurogenesis requires an increase in blood-brain barrier permeability. *PLoS One* 11, e0159892.
- Nance, E.A., Woodworth, G.F., Sailor, K.A., Shih, T.Y., Xu, Q., Swaminathan, G., Xiang, D., Eberhart, C., Hanes, J., 2012. A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Sci. Transl. Med.* 4, 149ra119.
- Nance, E., Timbie, K., Miller, G.W., Song, J., Louttit, C., Klibanov, A.L., Shih, T.Y., Swaminathan, G., Tamargo, R.J., Woodworth, G.F., Hanes, J., Price, R.J., 2014. Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood-brain barrier using MRI-guided focused ultrasound. *J. Contr. Release* 189, 123–132.

- Naqvi, S., Panghal, A., Flora, S.J.S., 2020. Nanotechnology: a promising approach for delivery of neuroprotective drugs. *Front. Neurosci.* 14, 494.
- Neuhauss, W., Piontek, A., Protze, J., Eichner, M., Mahringer, A., Subileau, E.A., Lee, I.M., Schulzke, J.D., Krause, G., Piontek, J., 2018. Reversible opening of the blood-brain barrier by claudin-5-binding variants of Clostridium perfringens enterotoxin's claudin-binding domain. *Biomaterials* 161, 129–143.
- Neuwelt, E.A., 2004. Mechanisms of disease: the blood-brain barrier. *Neurosurgery* 54, 131–140. ; discussion 141–132.
- Neuwelt, E.A., Howieson, J., Frenkel, E.P., Specht, H.D., Weigel, R., Buchan, C.G., Hill, S. A., 1986. Therapeutic efficacy of multiagent chemotherapy with drug delivery enhancement by blood-brain barrier modification in glioblastoma. *Neurosurgery* 19, 573–582.
- Niewoehner, J., Bohrmann, B., Collin, L., Urich, E., Sade, H., Maier, P., Rueger, P., Stracke, J.O., Lau, W., Tissot, A.C., Loetscher, H., Ghosh, A., Freskgård, P.O., 2014. Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron* 81, 49–60.
- Nolay, F., Sevin, E., Létévé, M., Bil, A., Gosselet, F., El Kirat, K., Djedaini-Pilard, F., Morandat, S., Fenart, L., Przybylski, C., Bonnet, V., 2020. First step to the improvement of the blood brain barrier passage of atazanavir encapsulated in sustainable bioorganic vesicles. *Int. J. Pharm.* 587, 119604.
- Nowak, M., Brown, T.D., Graham, A., Helgeson, M.E., Mitragotri, S., 2020. Size, shape, and flexibility influence nanoparticle transport across brain endothelium under flow. *Bioengineering & translational medicine* 5, e10153.
- Okuyama, T., Eto, Y., Sakai, N., Minami, K., Yamamoto, T., Sonoda, H., Yamaoka, M., Tachibana, K., Hirato, T., Sato, Y., 2019. Iduronate-2-Sulfatase with anti-human transferrin receptor antibody for neuropathic mucopolysaccharidosis II: a phase 1/2 trial. *Mol. Ther.* 27, 456–464.
- Okuyama, T., Eto, Y., Sakai, N., Nakamura, K., Yamamoto, T., Yamaoka, M., Ikeda, T., So, S., Tanizawa, K., Sonoda, H., Sato, Y., 2020. A phase 2/3 trial of pabinafusp alfa, IDS fused with anti-human transferrin receptor antibody, targeting neurodegeneration in MPS-II. *Mol. Ther.*
- Omori, K., Tachikawa, M., Hirose, S., Taii, A., Akanuma, S.I., Hosoya, K.I., Terasaki, T., 2020. Developmental changes in transporter and receptor protein expression levels at the rat blood-brain barrier based on quantitative targeted absolute proteomics. *Drug Metabol. Pharmacokin.* 35, 117–123.
- Pahnke, J., Wolkenhauer, O., Krohn, M., Walker, L.C., 2008. Clinico-pathologic function of cerebral ABC transporters - implications for the pathogenesis of Alzheimer's disease. *Curr. Alzheimer Res.* 5, 396–405.
- Pandit, R., Koh, W.K., Sullivan, R.K.P., Palliyaguru, T., Parton, R.G., Götz, J., 2020. Role for caveolin-mediated transcytosis in facilitating transport of large cargoes into the brain via ultrasound. *J. Contr. Release* 327, 667–675.
- Park, K., Skidmore, S., Hadar, J., Garner, J., Park, H., Otte, A., Soh, B.K., Yoon, G., Yu, D., Yun, Y., Lee, B.K., Jiang, X., Wang, Y., 2019. Injectable, long-acting PLGA formulations: analyzing PLGA and understanding microparticle formation. *J. Contr. Release* 304, 125–134.
- Portioli, C., Bovi, M., Benati, D., Donini, M., Perduca, M., Romeo, A., Dusi, S., Monaco, H. L., Bentivoglio, M., 2017. Novel functionalization strategies of polymeric nanoparticles as carriers for brain medications. *J. Biomed. Mater. Res.* 105, 847–858.
- Praca, C., Rai, A., Santos, T., Cristovao, A.C., Pinho, S.L., Cecchelli, R., Dehouck, M.P., Bernardino, L., Ferreira, L.S., 2018. A nanoformulation for the preferential accumulation in adult neurogenic niches. *J. Contr. Release* 284, 57–72.
- Prados, M.D., Schold Jr., S.C., Fine, H.A., Jaecle, K., Hochberg, F., Mechtler, L., Fetell, M.R., Phuphanich, S., Feun, L., Janus, T.J., Ford, K., Graney, W., 2003. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro Oncol.* 5, 96–103.
- Ramalho, M.J., Sevin, E., Gosselet, F., Lima, J., Coelho, M.A.N., Loureiro, J.A., Pereira, M.C., 2018. Receptor-mediated PLGA nanoparticles for glioblastoma multimodal treatment. *Int. J. Pharm.* 545, 84–92.
- Ramge, P., Unger, R.E., Oltrogge, J.B., Zenker, D., Begley, D., Kreuter, J., Von Briesen, H., 2000. Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells. *Eur. J. Neurosci.* 12, 1931–1940.
- Reichel, A., 2009. Addressing central nervous system (CNS) penetration in drug discovery: basics and implications of the evolving new concept. *Chem. Biodivers.* 6, 2030–2049.
- Rezaei, A.R., Ranjan, M., D'Haese, P.F., Haut, M.W., Carpenter, J., Najib, U., Mehta, R.I., Chazen, J.L., Zibly, Z., Yates, J.R., Hodder, S.L., Kaplitt, M., 2020. Noninvasive hippocampal blood-brain barrier opening in Alzheimer's disease with focused ultrasound. *Proc. Natl. Acad. Sci. U. S. A.* 117, 9180–9182.
- Rigon, L., Salvaliaio, M., Pederzoli, F., Legnini, E., Duskey, J.T., D'Avanzo, F., De Filippis, C., Ruozzi, B., Marin, O., Vandelli, M.A., Ottonelli, L., Scarpa, M., Tosi, G., Tomanin, R., 2019. Targeting brain disease in MPSII: preclinical evaluation of IDS-loaded PLGA nanoparticles. *Int. J. Mol. Sci.* 20.
- Sade, H., Baumgartner, C., Hugenmatter, A., Moessner, E., Freskgård, P.O., Niewoehner, J., 2014. A human blood-brain barrier transcytosis assay reveals antibody transcytosis influenced by pH-dependent receptor binding. *PLoS One* 9, e96340.
- Saint-Pol, J., Gosselet, F., Duban-Deweer, S., Pottiez, G., Karamanos, Y., 2020. Targeting and crossing the blood-brain barrier with extracellular vesicles. *Cells* 9.
- Saubamea, B., Cochois-Guegan, V., Cisternino, S., Scherrmann, J.M., 2012. Heterogeneity in the rat brain vasculature revealed by quantitative confocal analysis of endothelial barrier antigen and P-glycoprotein expression. *J. Cerebr. Blood Flow Metabol.* 32, 81–92.
- Sauer, R.S., Krug, S.M., Hackel, D., Staat, C., Konasin, N., Yang, S., Niedermirtl, B., Bosten, J., Günther, R., Dabrowski, S., Doppler, K., Sommer, C., Blasig, I.E., Brack, A., Rittner, H.L., 2014. Safety, efficacy, and molecular mechanism of claudin-1-specific peptides to enhance blood-nerve-barrier permeability. *J. Contr. Release* 185, 88–98.
- Schröder, U., Sabel, B.A., 1996. Nanoparticles, a drug carrier system to pass the blood-brain barrier, permit central analgesic effects of i.v. dalargin injections. *Brain Res.* 710, 121–124.
- Schroeder, U., Sommerfeld, P., Ulrich, S., Sabel, B.A., 1998. Nanoparticle technology for delivery of drugs across the blood-brain barrier. *J. Pharmacol. Sci.* 87, 1305–1307.
- Schroeder, U., Schroeder, H., Sabel, B.A., 2000. Body distribution of 3H-labelled dalargin bound to poly(butyl cyanoacrylate) nanoparticles after i.v. injections to mice. *Life Sci.* 66, 495–502.
- Shawahna, R., Uchida, Y., Declèves, X., Ohtsuki, S., Younis, S., Dauchy, S., Jacob, A., Chassoux, F., Daumas-Duport, C., Couraud, P.O., Terasaki, T., Scherrmann, J.M., 2011. Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol. Pharm.* 8, 1332–1341.
- Shawahna, R., Declèves, X., Scherrmann, J.M., 2013. Hurdles with using in vitro models to predict human blood-brain barrier drug permeability: a special focus on transporters and metabolizing enzymes. *Curr. Drug Metabol.* 14, 120–136.
- Sheikov, N., McDannold, N., Sharma, S., Hynynen, K., 2008. Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound Med. Biol.* 34, 1093–1104.
- Shityakov, S., Salmas, R.E., Durdagi, S., Salvador, E., Pápai, K., Yáñez-Gascón, M.J., Pérez-Sánchez, H., Puskás, I., Roewer, N., Förster, C., Broscheit, J.A., 2016. Characterization, in vivo evaluation, and molecular modeling of different propofol-cyclodextrin complexes to assess their drug delivery potential at the blood-brain barrier level. *J. Chem. Inf. Model.* 56, 1914–1922.
- Singh, N., Ecker, G.F., 2018. Insights into the structure, function, and ligand discovery of the large neutral amino acid transporter 1, LAT1. *Int. J. Mol. Sci.* 19.
- Smith, M.W., Gumbleton, M., 2006. Endocytosis at the blood-brain barrier: from basic understanding to drug delivery strategies. *J. Drug Target.* 14, 191–214.
- Soontornmalai, A., Vlaming, M.L., Fritschy, J.M., 2006. Differential, strain-specific cellular and subcellular distribution of multidrug transporters in murine choroid plexus and blood-brain barrier. *Neuroscience* 138, 159–169.
- Staat, C., Coisne, C., Dabrowski, S., Stamatovic, S.M., Andjelkovic, A.V., Wolburg, H., Engelhardt, B., Blasig, I.E., 2015. Mode of action of claudin peptidomimetics in the transient opening of cellular tight junction barriers. *Biomaterials* 54, 9–20.
- Strazielle, N., Ghersi-Egea, J.F., 2013. Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules. *Mol. Pharm.* 10, 1473–1491.
- Sweeney, M.D., Zhao, Z., Montagne, A., Nelson, A.R., Zlokovic, B.V., 2019. Blood-brain barrier: from physiology to disease and back. *Physiol. Rev.* 99, 21–78.
- Tilloy, S., Monnaert, V., Fenart, L., Bricout, H., Cecchelli, R., Monflier, E., 2006. Methylated beta-cyclodextrin as P-gp modulators for deliverance of doxorubicin across an in vitro model of blood-brain barrier. *Bioorg. Med. Chem. Lett* 16, 2154–2157.
- Tosi, G., Costantino, L., Rivasi, F., Ruozzi, B., Leo, E., Vergoni, A.V., Tacchi, R., Bertolini, A., Vandelli, M.A., Forni, F., 2007. Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123. *J. Contr. Release* 122, 1–9.
- Tosi, G., Bondioli, L., Ruozzi, B., Badiali, L., Severini, G.M., Biffi, S., De Vita, A., Bortot, B., Dolcetta, D., Forni, F., Vandelli, M.A., 2011a. NIR-labeled nanoparticles engineered for brain targeting: in vivo optical imaging application and fluorescent microscopy evidences. *Vienna, Austria J. Neural. Transm.* 118, 145–153, 1996.
- Tosi, G., Fano, R.A., Bondioli, L., Badiali, L., Benassi, R., Rivasi, F., Ruozzi, B., Forni, F., Vandelli, M.A., 2011b. Investigation on mechanisms of glycopeptide nanoparticles for drug delivery across the blood-brain barrier. *Nanomedicine* 6, 423–436.
- Tosi, G., Duskey, J.T., Kreuter, J., 2020. Nanoparticles as carriers for drug delivery of macromolecules across the blood-brain barrier. *Expet Opin. Drug Deliv.* 17, 23–32.
- Uchida, Y., Ohtsuki, S., Katsukura, Y., Ikeda, C., Suzuki, T., Kamiie, J., Terasaki, T., 2011. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J. Neurochem.* 117, 333–345.
- Ullman, J.C., Arguello, A., Getz, J.A., Bhalla, A., Mahon, C.S., Wang, J., Giese, T., Bedard, C., Kim, D.J., Blumenfeld, J.R., Liang, N., Ravi, R., Nugent, A.A., Davis, S.S., Ha, C., Duque, J., Tran, H.L., Wells, R.C., Lianoglou, S., Daryani, V.M., Kwan, W., Solanoy, H., Nguyen, H., Earr, T., Dugas, J.C., Tuck, M.D., Harvey, J.L., Rezyer, M.L., Caprioli, R.M., Hall, S., Poda, S., Sanchez, P.E., Dennis, M.S., Gunasekaran, K., Srivastava, A., Sandmann, T., Henne, K.R., Thorne, R.G., Di Paolo, G., Astarita, G., Diaz, D., Silverman, A.P., Watts, R.J., Sweeney, Z.K., Kariolis, M.S., Henry, A.G., 2020. Brain delivery and activity of a lysosomal enzyme using a blood-brain barrier transport vehicle in mice. *Sci. Transl. Med.* 12.
- van de Haar, H.J., Burgmans, S., Jansen, J.F., van Osch, M.J., van Buchem, M.A., Muller, M., Hofman, P.A., Verhey, F.R., Backes, W.H., 2016. Blood-brain barrier leakage in patients with early Alzheimer disease. *Radiology* 281, 527–535.
- Verselle, R., Corsi, M., Fusco, A., Sevin, E., Businaro, R., Gosselet, F., Fenart, L., Candela, P., 2020. Ketone bodies promote amyloid-beta1-40 clearance in a human in vitro blood-brain barrier model. *Int. J. Mol. Sci.* 21.
- Vilella, A., Belletti, D., Sauer, A.K., Hagnmeyer, S., Sarowar, T., Masoni, M., Stasiak, N., Mulvihill, J.J.E., Ruozzi, B., Forni, F., Vandelli, M.A., Tosi, G., Zoli, M., Grabrucker, A. M., 2018. Reduced plaque size and inflammation in the APP23 mouse model for Alzheimer's disease after chronic application of polymeric nanoparticles for CNS targeted zinc delivery. *J. Trace Elem. Med. Biol. : organ of the Society for Minerals and Trace Elements (GMS)* 49, 210–221.
- Wager, T.T., Chandrasekaran, R.Y., Hou, X., Troutman, M.D., Verhoest, P.R., Villalobos, A., Will, Y., 2010. Defining desirable central nervous system drug space

- through the alignment of molecular properties, in vitro ADME, and safety attributes. *ACS Chem. Neurosci.* 1, 420–434.
- Walkey, C.D., Olsen, J.B., Guo, H., Emili, A., Chan, W.C., 2012. Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. *J. Am. Chem. Soc.* 134, 2139–2147.
- Wiley, D.T., Webster, P., Gale, A., Davis, M.E., 2013. Transcytosis and brain uptake of transferrin-containing nanoparticles by tuning avidity to transferrin receptor. *Proc. Natl. Acad. Sci. U. S. A.* 110, 8662–8667.
- Williams, P.C., Henner, W.D., Roman-Goldstein, S., Dahlborg, S.A., Brummett, R.E., Tableman, M., Dana, B.W., Neuwelt, E.A., 1995. Toxicity and efficacy of carboplatin and etoposide in conjunction with disruption of the blood-brain tumor barrier in the treatment of intracranial neoplasms. *Neurosurgery* 37, 17–27. ; discussion 27-18.
- Yang, S., Zhu, J., Lu, Y., Liang, B., Yang, C., 1999. Body distribution of camptothecin solid lipid nanoparticles after oral administration. *Pharm. Res. (N. Y.)* 16, 751–757.
- Ye, Y., Sun, Y., Zhao, H., Lan, M., Gao, F., Song, C., Lou, K., Li, H., Wang, W., 2013. A novel lactoferrin-modified β -cyclodextrin nanocarrier for brain-targeting drug delivery. *Int. J. Pharm.* 458, 110–117.
- Yu, Y.J., Zhang, Y., Kenrick, M., Hoyte, K., Luk, W., Lu, Y., Atwal, J., Elliott, J.M., Prabhu, S., Watts, R.J., Dennis, M.S., 2011. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. *Sci. Transl. Med.* 3, 84ra44.
- Zhang, Y., Liu, A.T., Cornejo, Y.R., Van Haute, D., Berlin, J.M., 2020a. A Systematic comparison of in vitro cell uptake and in vivo biodistribution for three classes of gold nanoparticles with saturated PEG coatings. *PLoS One* 15, e0234916.
- Zhang, Y., Garcia-Gabilondo, M., Grayston, A., Feiner, I.V.J., Anton-Sales, I., Lloila, R.A., Llop, J., Ramos-Cabrer, P., Barba, I., Garcia-Dorado, D., Gosselet, F., Rosell, A., Roig, A., 2020b. PLGA protein nanocarriers with tailor-made fluorescence/MRI/PET imaging modalities. *Nanoscale* 12, 4988–5002.