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Nanomedicine and Drug Targeting Applications to Cross the Blood Brain Barrier (BBB)

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Abstract

A very effective blood-brain barrier (BBB) protects and isolates the brain from general circulation. Endothelial cells with tight connections, high enzymatic activity, and efflux transport systems characterize this barrier. As a result, the BBB is intended to allow the selective movement of chemicals required for brain function. Some small lipophilic drugs diffuse enough through the BBB to be therapeutically effective. Many potentially beneficial medications, on the other hand, cannot. This barrier poses a significant problem for treating central nervous system illnesses that need therapeutic medication levels entering the brain. This paper provides an overview of the BBB structure and several ways to overcome the difficulty of drug transport at the BBB and ensure efficient delivery of therapeutic substances to the brain, emphasizing nanoparticles (NPs) as drug carriers. To better understand the ideas of brain medicine administration, the architecture and physiology of the BBB and its transport mechanisms are discussed. The use of NPs targeting the transporter systems that already exist on the BBB to transport nutrients has been suggested as one way to overcome BBB impermeability and improve selectivity and medication delivery to the brain. Various methods of transport through the BBB and ways to target specific routes are discussed. Additionally, NP strategies to reduce circulatory clearance and lengthen the time a medicine interacts with the BBB are discussed.

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A. Introduction

A.1 Drug delivery to the brain through the blood-brain barrier (BBB)

Brain diseases, such as neurodegenerative diseases, infections, chronic pain, and psychiatric disorders, continue to be one of the world's major causes of disability, morbidity, and mortality, despite significant improvements in brain research. Neurological illnesses are anticipated to become the primary cause of morbidity among the world's 15-45-year-olds (Mathers and Loncar 2006).

The central nervous system (CNS) is a very sensitive milieu and maintaining neuronal signaling requires rigorous homeostatic control. The physical isolation of the CNS from the rest of the body via CNS barriers. These barriers restrict the transport of molecules to and from the brain. They are an essential part of regulating ions, neurotransmitters, macromolecules, nutrients, strictly control the entry and exit of xenobiotics. Three barriers surround the CNS:

- the BBB between the blood and brain interstitial fluid
- the choroid plexus (CP) in between the blood and the ventricular cerebrospinal fluid (CSF)
- the arachnoid membrane separating the blood from the subarachnoid CSF. These three cellular barriers regulate the transport and homeostasis of chemicals at the interfaces between the blood and the brain.

The BBB, which separates the blood from the extracellular fluid (ECF) of CNS neuronal tissue, has the biggest surface area for the passage of compounds from the blood into the brain and is considered one of the most impenetrable in vivo barriers. It isolates the brain from the circulating blood and prevents medication transport to the brain. Capillaries control cerebral blood flow in both health and sickness, strongly linked to brain metabolism and homeostasis. Brain capillaries have been developed to restrict the movement of macromolecules and cells between the blood and the brain to block toxins or infectious agents (Faria et al. 2012).

The BBB's impermeability is caused by the interaction of multiple transmembrane protein complexes (zonula occludens) that extend into and limit the flux between adjacent endothelial cells, resulting in tight junctions between capillary cells (Greene and Campbell 2016). The endothelium lacks fenestrations and exhibits a high transepithelial electrical resistance because of the tight junctions. BBB functions as an efficient ion barrier and further restricts transport along the paracellular channel due to this weak conductivity (AM, HC, and NJ 1990). The presence of intercellular tight connection complexes (tight junctions, adherence junctions, and junctional adhesion molecules that hold neighboring endothelial cells together) causes this high resistance (Ceña and Játiva 2018; Johnsen et al. 2019). These structures restrict chemicals from entering the CNS, limiting therapeutic drug absorption in the brain.

In contrast to other endothelia, on BBB, very few endocytotic vesicles are present in the cytoplasm. In fact, BBB cells only comprise 16–20% of the muscle (Claudio et al. 1989; NJ et al. 2010). Due to the BBB and tight junctions, most large molecules are already physically blocked from entering the brain. The BBB transport of molecules is further limited by the minimal number of endocytotic vehicles available for transit.

Drug transport over the BBB was once thought to occur by passive diffusion and was dependent on the drug's unique physicochemical qualities, such as lipophilicity. However, many lipophilic molecules exhibit low concentrations within the CNS (Zhang et al. 2015). The ATP-binding cassette (ABC) transporters present on BBB, such as P-glycoprotein (P-gp), multidrug resistance-related proteins (MRP), and breast cancer resistance protein (BCRP), can explain this phenomenon. They're all membrane transporters that transfer substrates across concentration gradients (Pinzon-Daza et al. 2013). The ABC transporters' main job in the BBB is to act as efflux pumps. They use ATP while moving a variety of lipid-soluble substances out of the brain. Many medications are substrates for these ABC efflux transporters, resulting in limited brain penetration.

In summary, transport through BBB is complex for several important reasons. The brain capillary endothelial cells differ from the peripheral ones because they have fewer endocytic vesicles. The BBB also prevents free diffusional movement of solutes out of the CNS, preventing bidirectional free diffusion and constraining solute movement to transcellular transport via passive diffusion or carrier transport pathways. Limited paracellular passage is possible and BBB lacks fenestrations. Additionally, when small molecules permeate the cell membrane, they are pumped out by highly expressed efflux transport proteins, restricting access to the brain even further. All these mechanisms restrict the transport through BBB and form a very impenetrable and tight barrier.

Even though paracellular transport is restricted, the transcellular transport of chemicals to the brain is an alternative. Passive permeability or active transport are the two fundamental processes by which solutes penetrate the brain endothelial barrier. Targeting these routes may be the solution for drug delivery to the brain. Nanotechnology has emerged as a viable method for targeting these restricted channels and efficiently delivering medications to the brain.

A.2 NP strategies to overcome the BBB impermeability

"Nanotechnology" refers to one billionth of a meter in size technology or a nanometer (six carbon atoms wide). Biocompatible materials, surgical practice, biostructures, and biomolecular research, vaccine design, and chemical and biochemical production processes, as well as pharmacology, have all benefited from nanoscale materials technology (Coombs and Robinson 1996). New drugs are needed for many CNS diseases because the treatment available is only symptomatic rather than an actual cure. To fill this need, various approaches have been used to pass through BBB. Functionalizing NPs with ligands that bind target

proteins associated with the BBB is one of the most promising NP-related strategies to deliver substances to the brain. In recent years, several studies have been conducted on nanomedicine, the medical application of nanotechnology, to solve the problem with drug delivery to the brain (I, S, and V 2016). Nanomaterials can be given new functions by combining them with biological molecules. By modifying the design of polymer-based nanoparticles, the pharmacokinetics and biodistribution of the medication may be improved, or bioavailability can be increased both at particular locations in the body and over time.

This research provides an in-depth examination of medication delivery to the brain, focusing on NPs as drug carriers. The architecture and physiology of the BBB are briefly reviewed to grasp the concepts of brain medication delivery better. The objective is to give several transport options for the successful targeting of nanoparticles to the brain despite the roadblocks that have prevented this goal from being achieved thus far. The employment of transport systems has been considered one method of overcoming the BBB impermeability and improving the selectivity and drug delivery to the brain.

B. Transport through BBB

Diffusion, carrier-mediated uptake, and endocytosis are three ways to target and penetrate the BBB (Lalatsa, Schätzlein, and Uchegbu 2019).

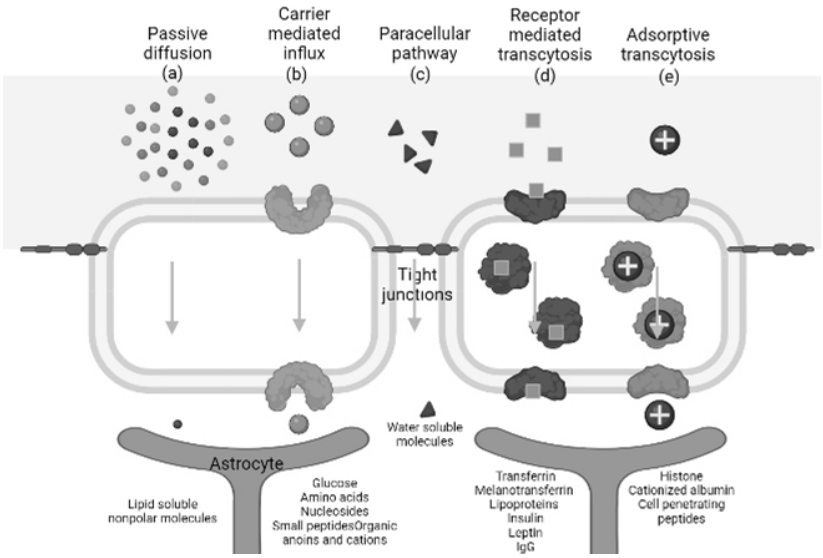

Figure 1 Transport routes across the BBB. (a) High lipid solubility and several physicochemical factors favor passive diffusion through the large surface of the lipid cell membrane and cross the endothelium. (b) Solute carriers (SLC) can transport essential polar molecules, such as glucose, amino acids, small peptides. (c) The existence of tight junctions restricts polar molecule transport via the paracellular route. Signals from brain endothelial cells can cause tight junction modulation, and molecular rearrangement of tight junction proteins causes the opening of the paracellular channel. (d) RMT can transport macromolecules such as peptides and proteins such as transferrin, insulin, leptin, and cytokines. (e) AMT induced by positively charged macromolecules. RMT and AMT both appear to be vesicular-based mechanisms that transport macromolecules through endothelial cells. Figure adapted from (NJ et al. 2010) and created with BioRender.com.

B.1 Transcellular diffusion

BBB penetration via passive transport or diffusion through endothelial plasma membranes is aided by a low hydrogen-bonding potential, lipophilicity, low molecular weight, and small size (preferably less than 300 Da), as well as the absence of free rotatable bonds (Gleeson 2008). The lipid solubility of a molecule and its rate to reach the brain have a well-established connection. However, lipophilic compounds must have a molecular weight of less than 600 Da to enter the brain passively since as molecular weight (MW) increases, CNS penetration decreases. The ionization state of a molecule also affects CNS penetration. While basic compounds penetrate the CNS more than neutral molecules, acidic molecules are hard to pass through.

Plasma protein binding and brain tissue binding of drugs have widespread pharmacokinetic implications. Only the free fraction of the drug can pass the BBB to elicit a pharmacological response. This percentage highly depends on protein binding since it affects the clearance, volume of distribution, and drug efficacy. Medicines that are highly protein-bound, acidic, or have a large molecular weight are less likely to pass across the BBB since most central nervous system-related drugs are big in size and have many different characteristics limiting the effectivity and specificity of this route.

B.2 Saturable carrier-mediated influx

Transport proteins are used by brain capillary endothelial cells to allow the entrance of essential nutrients, including glucose and amino acids, and small peptides, hexoses, monocarboxylic acids, organic anions, organic cations, neurotransmitters that cannot diffuse through the BBB due to their polarity (Figure 1). These transport proteins can be found on either the luminal or abluminal membranes of endothelial cells or both membranes (NJ et al. 2010). Utilizing these BBB-expressed carrier systems for therapeutic medication or peptide delivery to the CNS might be beneficial.

One of the most well-known examples is the precursor levodopa, which is used to treat Parkinson's. Dopamine cannot cross the BBB on its own, but when converted to levodopa, it can be transported via amino acid transporters. Some of the most often utilized endogenous carrier-mediated BBB transporters for BBB transport include the glucose transporter type 1 (GLUT1), the large neutral amino acid transporter type 1 (LAT1), and the cationic amino-acid transporter type 1 (CAT1).

All these transporters are a potential target, but not much research is done on NPs targeting solute carriers. The main explanation for this might be that this technique is limited to only transporting compounds structurally similar to endogenous ligands since only endogenous carriers can transport them. Furthermore, the drug molecules' size should be comparable to that of endogenous ligands, which is not the case with most drug molecules making this approach ineffective for targeting the brain.

Table 1
Some solute carriers of the BBB

Transporter	Receptor/subtype	Location	Direction	Example of endogenous substrates/mechanism
Glucose	GLUT1	Luminal & Abluminal	Blood to brain	Glucose
Sodium-dependent glucose transporter	SGLT1	Abluminal	Brain to endothelium	Glucose
Cationic L-amino acid transporter	CAT	Luminal	Brain to endothelium	Basic L-amino acids
	CAT3	Luminal	Brain to endothelium	Lysine, arginine (Sodium-independent)

B.3 Endocytosis

Endocytosis is the primary pathway for bigger MW molecules like peptides and proteins to enter the body. Endocytosis can be triggered by receptor-mediated and nonspecific adsorptive mechanisms, leading to transcytosis. When a ligand binds to a receptor or a chemical interacts with the cell membrane, endocytosis begins. The binding on one side of the cell triggers endocytosis; the ligand or chemical then moves through vesicular transport across, and exocytosis of the vesicle contents occurs on the other side, known as transcytosis. In both circumstances, the lysosomal compartment must be avoided by diverting the endosome and its contents away from degradation.

B.3.1 Receptor-mediated transcytosis (RMT)

RMT is a transport mechanism that uses the endothelial cells' vesicular transport system to move substrates over the brain-blood barrier. The interaction of molecules with its membrane receptor (insulin receptor, transferrin receptor, LDL receptor seen on **Table 2**.) abundantly expressed on the endothelial cell surface causes modifications on receptor protein. This modification leads to endocytotic events occurring in the luminal membrane, leading to endocytotic vesicles (Broadwell, Balin, and Salcman 1988). These endocytotic ligand-containing vesicles fuse with an endosome, releasing the ligand from the receptor and crossing the BBB (Lalatsa, Schätzlein, and Uchegbu 2019). Many of the receptors implicated in RMT are multifunctional and multiligand in nature, making it difficult to identify them. As a result, many systems may carry specific ligands meaning some receptors may eventually be recognized as the same for multiple ligands (CC et al. 2004; H. J and P 2003; NJ et al. 2010). More research needs to be done to thoroughly understand these receptors and transport system and use it as a potential delivery route for the brain.

B.3.2 Adsorptive-mediated transcytosis (AMT)

The brain can take up cationized proteins, cell-penetrating proteins, and cationic drugs at physiological pH by adsorptive-mediated mechanisms instead of receptor-mediated transport, which requires specialized plasma membrane receptors. AMT needs an excess positive charge on the molecule, making it cationic, followed by contact with cell surface binding sites, which causes endocytosis and transcytosis. Endocytosis and following transcytosis are triggered when these chemicals contact electrostatically with the anionic sites of acidic glycoproteins on the cell surface.

In adsorptive transcytosis, the surface features of NPs make it easier for the NP and its payload to attach to endothelial cells' luminal plasma membrane. The plasma membrane of endothelial cells is negatively charged, which makes it more possible that the positively charged NPs will undergo this process than neutral or negatively charged ones.

Table 2
Examples of transcytosis/transport of large molecules and complexes across the BBB

Transport system	Receptor	Ligand	Type	Direction
Insulin	Insulin	Insulin	RMT	Blood to brain
Transferrin	TfR	Transferrin	RMT	Blood to brain
Melanotransferrin	MTfR	Melanotransferrin	RMT	Blood to brain
Leptin		Leptin	RMT	Blood to brain
Tumour necrosis factor		TNFa	RMT	Blood to brain
Epidermal growth factor		EGF	RMT	Blood to brain
Immunoglobulin G	Fcγ-R	IgG	RMT	Blood to brain
Apolipoprotein E receptor 2	ApoER2	Lipoproteins and ApoE bound molecules	RMT	Blood to brain
LDL-receptor-related protein 1 and 2	LRP1 and LRP2	Lipoproteins, Amyloid-β, lactoferrin, α 2-macroglobulin, ApoE, melanotransferrin	RMT	Bi-directional
Cationised proteins		Cationised albumin	AMT	Blood to brain
Cell penetrating peptides		SynB5/pAnt-(43–58)	AMT	Blood to brain

B.4 Efflux transporters expressed at the BBB

Another essential transport process in the BBB is carrier-mediated efflux (efflux transporters). ABC transporters have a broad affinity for various solutes, particularly big, lipid-soluble compounds containing a significant number of nitrogen and oxygen atoms. These transporters drive the efflux of solutes against a concentration gradient by pumping molecules across the membrane via ATP hydrolysis. The most known ABC efflux transporters in the BBB are P-glycoprotein (Pgp) and breast cancer-related protein (BCRP). The treatment of brain tumors and metastases is complicated because many cytotoxic medicines are substrates for efflux transporters (Begley 2004).

More than 90% of small and almost all large therapeutics are anticipated to fail to cross the BBB (Pardridge 2005). As a result, extensive research is being conducted to find novel ways to bridge the BBB and deliver materials to the CNS. Many studies are being undertaken to improve medication physicochemical qualities to increase their associated permeability across the BBB, enabling CNS brain targeting.

Recent advancements in science and technological tools have led to better and more profound knowledge regarding the several receptors targeted to deliver drugs across the BBB. It is feasible to enhance the attachment of NPs to the endothelial cell surface to improve the specificity and targeting of drugs to the brain through the transcytosis route. The upcoming sections overview some strategic therapeutic approaches for brain targeting and transport through the BBB.

C. NP applications

For drug release in the brain, the NP method primarily focuses on utilizing nanosized technology. This approach employs several nanoscale drug delivery platforms, the most common lipid- and polymer-based nanoparticles (NPs), which ensure a regulated and enhanced cargo release by preventing loaded pharmaceuticals from being metabolized. Today's efforts focus on improving NPs' capacity to efficiently target the therapeutic site, reducing medication dosages released to unwanted areas.

NPs have been shown to improve transport across the BBB by passive targeting (modifying the NP for the uptake within the BBB cells) or active targeting (endocytosis followed by transcytosis) in several studies. Confocal microscopy analysis and cell fractioning experiments revealed that NPs were primarily associated with the plasma membrane and vesicular compartments (Pinzon-Daza et al. 2013; Smith and Gumbleton 2006).

C.1 Passive targeting

The surface's chemical structure and physical features are crucial in passive targeting to make NPs appropriate for absorption into BBB cells. The synthesized NPs must be of a specific size. Transcytosis allows NPs smaller than 20 nm to pass through BBB endothelial cells (Smith and Gumbleton 2006). Surface characteristics of NPs are well understood to be necessary for delivery via the BBB; alterations in surface charge and coating may affect the capacity to pass biological barriers.

Fenart and colleagues created cross-linked maltodextrin NPs that were derivatized with various ligands to produce anionic, cationic, and non-modified neutral NPs. After that, all of the NPs were coated with cholesterol and dipalmitoylphosphatidylcholine. Coating neutral NPs did not enhance their transport through the BBB much, whereas coating charged NPs boosted their absorption by fourfold *in vitro* model of the blood-brain barrier. At the same concentrations, anionic lipid NPs absorption was more significant than neutral or cationic lipid NPs, and only neutral and anionic NPs had undergone endocytosis (Fenart et al. 1999).

Coating the surface of NPs with polymers, such as polyethylene glycol (PEG), increases the hydration and solubility of the NP core while also protecting it against enzymatic breakdown and phagocytosis by circulating cells (Owens and Peppas 2006). Because the solubility of drugs carried by PEG-coated NPs is proportional to the NP concentration rather than the drug concentration, even hydrophobic medications can penetrate the BBB at clinically relevant amounts following systemic injection when transported by pegylated NPs. Still, with this approach, the size of the molecules stays as a problem since most drugs are too big to use target this way.

C.2 Mimicking lipoproteins for apolipoprotein-mediated transport

Over the last two decades, cyanoacrylate NPs have been extensively explored to use as controlled-release medication delivery vehicles. Drugs that ordinarily cannot cross the BBB can be delivered into the brain and have a pharmacological impact following intravenous administration by binding to poly butyl cyanoacrylate (PBCA) NPs coated with polysorbate 80, according to several studies. The hexapeptide dalargin, the dipeptide kytorphin, loperamide, and doxorubicin have been effectively delivered into the brain utilizing this carrier.

Table 3 Adapted from Jörg Kreuter et al. 2002

After an i.v. injection of dalargin-loaded (7.5 mg/kg), the mean percentage of maximally potential effect (percent MPE) and standard deviation (SD) of nociceptive threshold were calculated. The tail-flick test was used to evaluate the presence of apolipoprotein-coated PBCA nanoparticles in mice.

Preparation	15 min	45 min	90 min
Empty PBCA nanoparticles	3.8 ^ 3.3	0.75 ^ 3.2	22.0 ^ 9.8
Dalargin	2.3 ^ 4.6	9.3 ^ 2.8	2.0 ^ 6.1
Dalargin + polysorbate 80	4.8 ^ 1.7	7.8 ^ 2.3	6.6 ^ 2.6
Dalargin + PBCA	5.7 ^ 5.1	4.7 ^ 9.1	3.8 ^ 9.1
Dalargin + PBCA + APOX			
ApoAII	5.29 ^ 2.00	5.98 ^ 7.64	9.44 ^ 12.5
ApoB	6.76 ^ 5.26	37.74 ^ 6.61*	17.54 ^ 8.17*
ApoCII	8.39 ^ 2.19	3.65 ^ 5.67	1.14 ^ 7.67
ApoE	38.8 ^ 13.7*	29.7 ^ 5.57*	2.03 ^ 6.49
ApoJ	3.32 ^ 2.58	10.89 ^ 8.64	5.00 ^ 5.00
Dalargin + PBCA + polysorbate 80	35.2 ^ 5.8	49.5 ^ 4.5	7.1 ^ 6.3
Dalargin + PBCA + polysorbate 80 +APOX			
ApoAII	1.98 ^ 9.56	12.81 ^ 16.8	48.8 ^ 13.24†
ApoB	30.87 ^ 19.43	58.71 ^ 8.03†	25.51 ^ 16.44
ApoCII	7.76 ^ 2.56	49.48 ^ 10.88	3.72 ^ 8.58
ApoE	61.39 ^ 8.59†	64.52 ^ 13.98	51.73 ^ 12.9†
ApoJ	18.49 ^ 27.2	51.51 ^ 16.68	19.39 ^ 19.1

*Statistically significant difference ð2*p* . 0:05Þ compared to dalargin-loaded PBCA uncoated nanoparticles.
† Statistically significant difference ð2*p* . 0:05Þ compared to dalargin-loaded polysorbate 80-coated PBCA nanoparticles.

Dalargin, an antinociceptive peptide that does not cross the BBB, has been experimented with extremely. To enlighten the path through BBB, the apolipoproteins AII, B, CII, E, or J were coated on PBCA NPs loaded with dalargin (Jörg Kreuter et al. 2002). ApoE was also coated on loperamide-loaded NPs. Both experiments are conducted without or after polysorbate 80 precoating. The pain-killing responses of the mice after i.v injection using apolipoprotein-coated NPs or control preparations are listed in **Table 3**. According to the findings, only NPs coated with polysorbate 80 or ApoB or E could provide an antinociceptive effect in mice. All apolipoproteins showed a statistically significant antinociceptive response after being precoated with polysorbate 80. Polysorbate precoating coupled with ApoB or E overcoat seems to increase the effect dramatically and have a faster beginning of the antinociceptive response than polysorbate 80 alone. With ApoE, the effect remained high for the entire observation period (90 min). In ApoE-deficient mice, the effect was significantly decreased. Research demonstrates that ApoB and E mediate drug-bound PBCA NP transport across the BBB (Jörg Kreuter et al. 2002). Because other apolipoproteins, such as AII, CII, or J, could not induce effects with NPs without precoating with

polysorbate 80, the antinociceptive effects found after precoating with polysorbate 80 can be attributed to polysorbate 80's presence. The late beginning of antinociceptive effects seen with these apolipoproteins, particularly ApoA-II, since the effect started after 90 mins (as seen in **Table 3**), might indicate partial desorption and change of the apolipoproteins from the polysorbate 80-precoated NPs was required to allow dalargin trafficking into the brain.

Kreuter did a polyacrylamide gel electrophoresis and injected polysorbate 80-coated NPs into the bloodstream. They revealed that apoE was adsorbed onto the surface of NPs. Further research revealed a strong link between ApoE adsorption and BBB passage (K. J 2001; Wagner et al. 2012). Particles containing ApoE and ApoA have been found in human cerebrospinal fluid (Pitas et al. 1987). The polysorbate-coated nanoparticles may resemble LDL particles after ApoE adsorption when injected. They might behave as Trojan horses by engaging with the LDL receptor and causing their absorption by endocytic mechanisms. Polysorbates would play the role of an ApoE anchor, and their cargo would be transported to the brain.

Surfactants like polysorbate 80 have previously been found to change the distribution of nanoparticles in rats and rabbits following coating and intravenous administration. So, the effect of 12 different surfactants to facilitate delivery to the brain was studied. Surfactants were coated onto the surface of PBCA NPs with the model drug dalargin and were injected intravenously into mice. Only NPs coated with polysorbate 20, 40, 60, and 80 produced a noticeable impact, while polysorbate 80 had the most pronounced effect (J. Kreuter et al. 1997a).

Gulyaev et al. studied the pharmacokinetics of doxorubicin (5 mg/kg) bound to NPs following intravenous administration into rats. The researchers utilized four different preparations: doxorubicin in saline, doxorubicin in saline + polysorbate 80, doxorubicin bound to PBCA NPs, and doxorubicin bound to PBCA NPs and coated with polysorbate 80. The most important observation was that only polysorbate 80-coated NPs produced high doxorubicin concentrations in the brain. In contrast, quantities in the three other preparations were below the detection limit of 0.1 g/g (**Figure 2**). Because the study did not distinguish between drugs that have crossed the BBB and those that stay in the blood vessels, it is impossible to say if the medication reached the brain. The total brain concentrations were determined using the experimental approach used in this study. As a result, it's possible that the high quantities of nanoparticles in the brain were attributable to particles lingering in the brain's blood compartment or the brain's blood vessel endothelial cells. Given that blood capillaries account for just 1% of brain volume and brain blood vessel endothelial cells for only 0.1, doxorubicin concentrations in these compartments would have been 600 or 6000 g/g, respectively, and the animals would have died immediately. As a result, the medication likely made its way into the brain (J. Kreuter et al. 1997b).

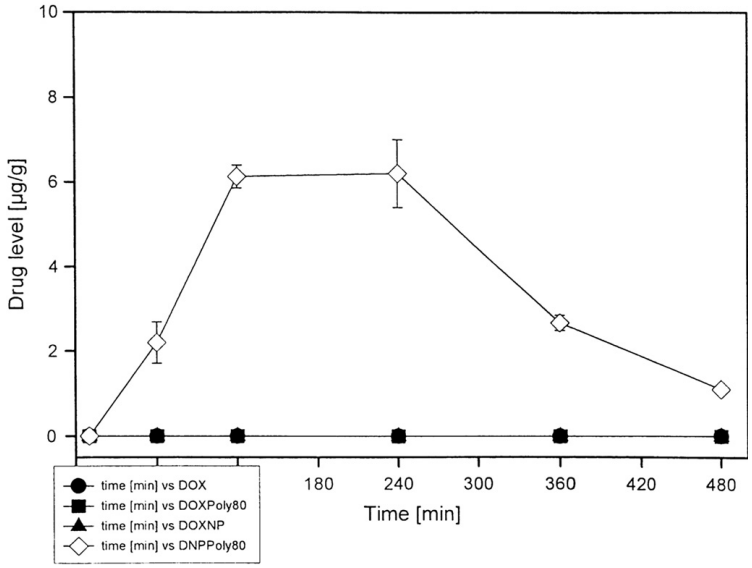

Figure 2 Uptake of doxorubicin (DOX) by rat brain. DOX brain [C] after i.v of: ●, DOX [5 mg/kg] solution in saline; ■, DOX [5 mg/kg] solution in saline plus 1% polysorbate 80; ▲, DOX [5 mg/kg] bound to PBCA NPs; ◊, DOX [5 mg/kg] bound to PBCA NPs plus 1% polysorbate 80. Reprinted from AE et al. 1999.

ApoE is involved in the transfer of lipoproteins into the brain through endocytosis through receptors such as the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein (KR et al. 2002). Several publications have established LDL receptors in the BBB, and ApoE and ApoA-I-containing particles have been found in human cerebrospinal fluid (S et al. 1989). This link may mean that, following injection, polysorbate 80-coated NPs adsorb apolipoproteins from the blood. By adsorbing them, they mimic lipoprotein particles that might be taken up by brain capillary endothelial cells via endocytosis. Following release inside the cells, the bound drug may be carried farther into the brain by diffusion or transcytosis.

Only ApoB and E-coated nanoparticles produced a substantial analgesic effect after treatment with dalargin and loperamide preparations created with various surfactants and/or Apo coatings, which indicated that the preparations without polysorbate 80 coating did not. However, this effect was less pronounced than when the polysorbate 80 coating was applied alone. All apolipoproteins showed a statistically significant antinociceptive response after polysorbate 80-recoating as compared to the dalargin solution. After these results, we can say coating the NPs with polysorbate 80 yields better results than coating with any apolipoproteins. Targeting the LDL proteins expressed on the BBB has a great potential to become the principal solution for brain targeting.

C.3 Targeting the transferrin receptor for brain drug delivery

Targeting the LDL receptors is not the only way to take advantage of the RMT; various NPs with various ligands on their surfaces can bind to specific receptors and stimulate endocytosis. GLUT1, lactoferrin (Lf), and others seen in **Table 2** can all be potential targets. The transferrin receptor (TfR) is

another common target exploited to ensure adequate drug transport to the brain among the several possible targets at the BBB (Johnsen et al. 2019).

The iron-binding protein transferrin (Tf) binds to iron and transports it to the brain via intracellular trafficking. Tests to reveal the TfR dynamics on BBB show that neither the TfR nor the Tf transcytoses into the brain parenchyma from the vessel's luminal side. Instead, the iron is freed from its Tf binding and pushed out of the endosome, then out of the cell (Duck and Connor 2016).

Numerous *in vitro* and *in vivo* research aimed at delivering medicines to the brain have focused on the Tf receptor (TfR). Liposomes coated with Tf have been utilized to transport DNA, and an iron-mimetic peptide has been employed as a ligand. Alternative approaches using anti-TfR antibodies have been developed since high blood levels of Tf necessitate competing with the endogenous ligand. When gold nanoparticles associated with 8D3 anti-TfR antibodies were given intravenously to animals, RMT was seen. However, most vesicles were degraded by endosomes, and just a few made it to the abluminal membrane (Cabezón et al. 2015). Modifying the antibody-binding strength can be a strategy to overcome this limitation, allowing the ligand to imitate natural transferrin and allowing antibodies to be taken into the brain. Clark and Davis produced gold nanoparticles with a transferrin ligand, and Kang encapsulated dopamine into PEGylated liposomes, which were then coupled to the anti-TfR monoclonal antibodies (mAb) OX26. They discovered an eightfold rise in dopamine content in the rat model of Parkinson's disease (Clark and Davis 2015; Kang et al. 2016). Liposomes with OX26 functionalization were administered to immortal rat brain capillary endothelial cell monolayers they were observed to transcytose in these tests (Cerletti et al. 2000). This observed transcytosis can be predicted to happen *in vivo* and is the possible mechanism to cross BBB via the TfR.

MYBE/4C1 mAb coated liposomes encapsulating the anticancer medication doxorubicin are one example of such studies. By comparing six murine mAb specific for various epitopes of the human TfR, the MYBE/ 4C1 mAb was discovered. When the highest performing one (MYBE/4C1) was employed to functionalize liposome particles, it facilitated doxorubicin in vitro passage 3.9 times more than liposomes functionalized with a nonspecific IgG (Gregori et al., 2016).

The transferrin modification on various nanoparticle formulations improves selective cellular absorption via transferrin-mediated mechanisms and therapeutic effect by allowing large quantities of appropriate medication to be encapsulated and delivered to the brain. In its free form, doxorubicin is inefficient at penetrating the blood-brain barrier. However, it can be loaded into transferrin-conjugated nanocarriers and cause cytotoxicity in glioma cells *in vitro* and *vivo*.

These results raise the idea that liposomes can carry drug payload to brain epithelial cells and be transported deeper into the brain parenchyma. Utilizing them with ligands or antibodies specific to the transferrin receptors of the BBB may be another promising approach to target the brain with NPs. Despite

all current breakthroughs in science, the influence of different anti-TfR antibody variants on the ensuing liposome transport needs to be still investigated in depth.

C.4 Targeting folic acid receptors on the BBB

Apart from transferrin and LDL receptors, folic acid receptor-mediated endocytosis is another approach to target the brain. Cellulose nanocrystals are not cytotoxic and promising agents to use as a carrier. Coating these NPs with folic acid facilitates the folic acid receptor-mediated endocytosis and delivery to the brain.

Dong and colleagues used elongated cellulose nanocrystals coated with folic acid for targeted delivery of chemotherapeutic drugs to the brain. On human and rat brain tumor cells, folate receptor-mediated transport of the conjugates was observed. To reveal the mechanism, they preincubated the cells with chlorpromazine or genistein, which are used as uptake inhibitors (Dong et al., 2014).

Docetaxel is used to treat a variety of cancers, although it has a limited ability to enter the brain. For brain targeting, docetaxel and ketoconazole were loaded into solid lipid nanoparticles, and the surface of these NPs was coated with folic acid to facilitate the uptake. Surface-modified dual drug-loaded solid lipid nanoparticles showed enhanced brain absorption of docetaxel in plasma and brain pharmacokinetics. As a result, these NPs might be used to transport lipophilic anticancer medicines to the brain (Venishetty et al. 2013). This approach remains to be one of the least researched ways to deliver therapeutics. This might be because, while the FR is expressed on the BBB, it is also located in a few other tissues such as the choroid plexus, thyroid, and kidneys, reducing its brain selectivity (Ross, Chaudhuri, and Ratnam 1994).

C.5 Targeting the efflux pumps present on the BBB

Polysorbate 80, which was used to deliver drugs via the LDL receptors of the BBB, also was shown to inhibit the Pgp (Woodcock et al. 1992). This glycoprotein is found in brain endothelial cells and is responsible for multidrug resistance, a significant barrier to medication delivery to the brain. Inhibition of this efflux pump may thus be a way to increase the amount of drug that reaches the brain through RMT. The relevance of polysorbate 80 inhibiting efflux pumps during nanoparticle-mediated medication transport to the brain is unknown at this time. Nonetheless, the author of this paper believes that blocking the efflux system with polysorbate 80 may aid in distributing nanoparticles to the brain. Still, that other mechanisms, including endocytosis, are more critical. The fundamental rationale for this assumption is that substantial brain concentrations were only attained after 2–4 hours in the pharmacokinetic trial with doxorubicin. Such a slow reaction appears to be due to time-consuming processes, such as endocytosis or transcytosis.

D. Conclusion and future scope

Because of the tight junctions between endothelial cells, decreased endocytosis at the cell membrane, and a mix of transporters that allow nutrient absorption while limiting xenobiotic uptake into the brain, the BBB offers excellent protection against circulating materials. The utilization of nanosized technology for drug release employs a range of nanoscale drug delivery platforms, mostly lipid- and polymer-based NPs, to ensure that the payload reaches the therapeutic location successfully. Given that NPs have been proved to be promising drug transporters, the feasibility of utilizing them to improve medication delivery to organs has been thoroughly examined.

This study examines pharmaceutical delivery to the brain in-depth, with an emphasis on NPs as drug transporters. Despite the barriers that have prevented this aim from being realized thus far, the goal is to provide numerous transport alternatives for the effective targeting of nanoparticles to the brain. Using carrier systems to overcome BBB impermeability and improve selectivity and medication delivery to the brain, targeting the efflux pumps to increase the amount of drug reached to the brain, and modifying the surface characteristics with NPs to be taken up passively haves been suggested as possible solutions.

One way of brain targeting is to optimize the charge, surface characteristics, and physicochemical properties of the NPs to take them up by passive mechanisms. NPs can be specially modified to protect the cargo, target BBB, and control the release characteristics. Still, since most molecules are too large to be taken up this way and this route lacks specificity, targeting passive uptake has not become the golden rule of targeting BBB.

RMT is one of the most focused strategies to target the BBB to deliver large molecules. Since these transporters allow larger molecules to pass, transferrin receptors, LDL, and folic acid receptors have been studied for targeted brain delivery. NPs used to target RMT showed some effect against the efflux pumps on the BBB. The author believes that the answer for successful brain targeting lies in the NPs that can carry their load through the BBB with RMT and have the characteristics to inhibit efflux pumps. This way, with a small administration, large concentrations can be achieved in the brain. NPs, which can be ligands for RMT proteins, need to be more studied to achieve this goal.

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