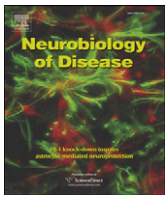
Neurobiology of Disease 37 (2010) 48–57

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases

Reinhard Gabathuler *

Angiochem Inc., 201 President Kennedy Ave., Suite PK-R220, Montreal, Quebec, Canada H2X3Y7

ARTICLE INFO

Article history:
Received 5 February 2009
Revised 6 July 2009
Accepted 25 July 2009
Available online 5 August 2009

ABSTRACT

The central nervous system is protected by barriers which control the entry of compounds into the brain, thereby regulating brain homeostasis. The blood–brain barrier, formed by the endothelial cells of the brain capillaries, restricts access to brain cells of blood–borne compounds and facilitates nutrients essential for normal metabolism to reach brain cells. This very tight regulation of the brain homeostasis results in the inability of some small and large therapeutic compounds to cross the blood–brain barrier (BBB). Therefore, various strategies are being developed to enhance the amount and concentration of therapeutic compounds in the brain. In this review, we will address the different approaches used to increase the transport of therapeutics from blood into the brain parenchyma. We will mainly concentrate on the physiologic approach which takes advantage of specific receptors already expressed on the capillary endothelial cells forming the BBB and necessary for the survival of brain cells.

Among all the approaches used for increasing brain delivery of therapeutics, the most accepted method is the use of the physiological approach which takes advantage of the transcytosis capacity of specific receptors expressed at the BBB. The low density lipoprotein receptor related protein (LRP) is the most adapted for such use with the engineered peptide compound (EPiC) platform incorporating the Angiopep peptide in new therapeutics the most advanced with promising data in the clinic.

Contents

* Fax: +1 514 788 7801.
E-mail address: rgabathuler@angiochem.com.
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2009.07.028

Introduction

The problem of drug transport to the brain

The idea of a BBB that segregates the blood and brain was developed 100 years ago, following the demonstration that most organs could be stained by dye injected intravenously, with the exception of the brain and spinal cord (Ehrlich, 1885). The capillaries of the brain have evolved to constrain the movement of molecules and cells between blood and brain, providing a natural defense against circulating toxic or infectious agents. The relative impermeability of the BBB results from tight junctions between capillary endothelial cells which are formed by cell adhesion molecules. Brain endothelial cells also possess few alternate transport pathways (e.g., fenestra, transendothelial channels, pinocytotic vesicles), and express high levels of active efflux transport proteins, including P-glycoprotein (P-gp), Multidrug Resistance Protein-1 (MRP-1), and Breast Cancer Resistance Protein (BCRP). The BBB also has additional enzymatic aspects which serve to protect the brain. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes, ecto- and endo-enzymes, present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles.

Some small molecules with appropriate lipophilicity, molecular weight (mw) and charge will diffuse from blood into the CNS. However, the overwhelming majority of small molecules (mw>500 daltons, D), proteins and peptides do not cross the BBB. It has been reported that approximately 98% of the small molecules and nearly all large molecules (mw>1 kD, kilodaltons), such as recombinant proteins or gene-based medicines do not cross the BBB (Pardridge, 1998, 2007c). Therefore, to reach the brain, most molecules must cross the BBB through interaction with specific transporters and/or receptors expressed at the luminal (blood) side of the endothelial cells.

In response to the insufficiency in conventional delivery mechanisms, aggressive research efforts have recently focused on the development of new strategies to more effectively deliver drug molecules to the CNS.

Crossing the BBB

Crossing the BBB remains a key obstacle in the development of drugs for brain diseases despite decades of research.

A schematic representation of different mechanisms used to cross the BBB is shown in Fig. 1.

- Small hydrophilic molecules such as amino acids, glucose, and other molecules necessary for the survival of brain cells use transporters expressed at the luminal (blood) and basolateral (brain) side of the endothelial cells.
- Larger and/or hydrophilic essential molecules such as hormones, transferrin for iron, insulin, and lipoproteins use specific receptors that are highly expressed on the luminal side of the endothelial cells. These receptors function in the endocytosis and transcytosis of compounds across the BBB.
- Small lipophilic molecules can diffuse passively across the BBB into the brain but will be exposed to efflux pumps (P-glycoprotein [P-gp], some Multidrug Resistance Proteins [MRP], Breast cancer Resistance Protein [BCRP] and others) expressed on the luminal side of the BBB and exposed to degrading enzymes (ecto- and endo-enzymes) localized in the cytoplasm of endothelial cells before brain penetration.

To bypass the BBB and to deliver therapeutics into the brain, three different approaches are currently used — invasive, pharmacological and physiological. These are considered below:

Invasive approach

These are physical based techniques include the use of: 1) Intracerebro-ventricular infusion, 2) Convection-enhanced delivery and 3) polymer or microchip systems which directly release therapeutics after implantation in the CNS.

Invasive approaches deliver drug to the brain by mechanically breaching the BBB and are summarized below:

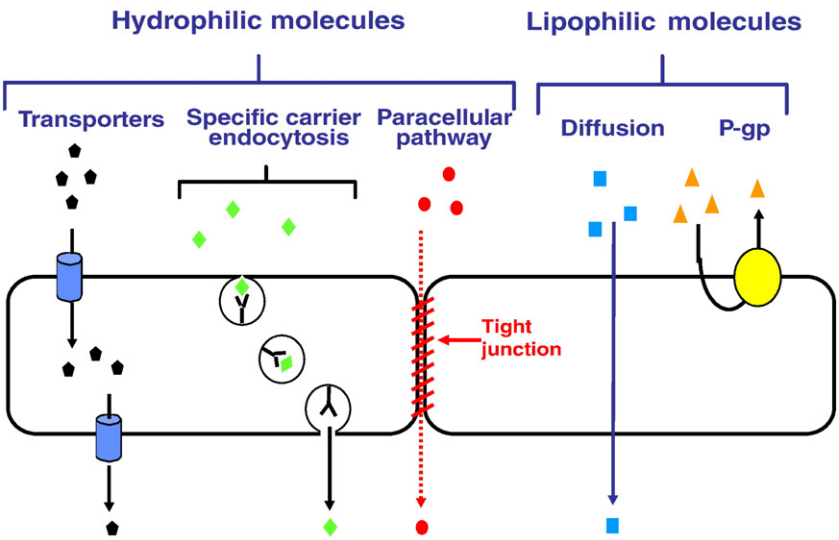


Fig. 1. Schematic representation of the transport of molecules across the BBB.

Intra-cerebro-ventricular (ICV) infusion

It has been reported that the concentration of a drug in the brain is only 1–2% of the CSF concentration at just 1–2 mm from the surface (Blasberg et al., 1975). The drug eventually distributes to the general circulation, where the drug then enters the brain parenchyma following transport across the BBB. This result is similar to a slow intravenous infusion rather than a direct administration of drugs into the brain (Pardridge, 2005). Pharmacologic effects can be seen after ICV administration, if the target receptors of the drug for example, opioid peptides) are located near the ependymal surface of the brain (Pardridge, 2007a).

Limitations: The diffusion of the drug in the brain parenchyma is very low. Unless the target is close to the ventricles it is not an efficient method of drug delivery.

Convection-enhanced delivery (CED)

The general principle of CED involves the stereotactically guided insertion of a small-caliber catheter into the brain parenchyma. Through this catheter, infusate is actively pumped into the brain parenchyma and penetrates in the interstitial space. The infusion is continued for several days and the catheters are removed at the bedside. In contrast to the mm distances obtained with simple diffusion, CED has been shown in laboratory experiments to deliver high molecular weight proteins 2 cm from the injection site in the brain parenchyma after as little as 2 h of continuous infusion (Bobo et al., 1994).

The success of CED relies on precise placement of the catheters and other infusion parameters for delivery into the correct location in the brain parenchyma.

Limitations: Some areas of the brain are difficult to saturate fully with infusate, particularly — infiltrated tissues surrounding a cavity. Proper drug delivery depends on the placement of catheters based on knowledge of these factors (Vandergrift et al., 2006).

Intra-cerebral injection or use of implants

Both the bolus injection of chemotherapy agents and the placement of a biodegradable, chemotherapeutic impregnated, wafer into a tumour resection cavity, rely on the principle of diffusion to drive the drug into the infiltrated brain. Fung et al. (1998) have demonstrated the presence of high drug concentrations (0.5–3.5 mM for carmustine, 0.2–1 mM for paclitaxel) within the first 3 mm from the polymer implants in monkeys; significant concentrations (0.4 μM for carmustine, 0.6 μM for paclitaxel) were measured up to approx. 5 cm from the implant as long as 30 days after implantation.

Limitations: Distribution in the brain by diffusion decreases exponentially with distance. The injection site has to be very precisely mapped to get efficacy and overcome the problem associated with diffusion of drugs in the brain parenchyma.

The direct injection of recombinant adeno-associated virus (rAAV) expressing neurotrophic factors is in development for the treatment of CNS disorders (Herzog et al., 2007; Marks et al., 2008). In animal models, expression of rAAV2 delivered vectors has been shown for brain derived neurotrophic factors (BDNF) in atrophy of spinal neurons, glial cell derived neurotrophic factors (GDNF) and other neurotrophic factors such as neurturin (NTN) in PD.

Limitations with rAAV are that no global brain transduction is achievable, immune responses are stimulated, and the packaging capacity of rAAV is small, at only 4.5 kilobase (kb).

Disruption of the BBB

Disruption of the BBB can open access of the brain to components in the blood by making the tight junction between the endothelial cells of the brain capillaries leaky. Different techniques are used to disrupt the tight junctions:

- Osmotic disruption: The osmotic shock causes endothelial cells to shrink, thereby disrupting the tight junctions. Intracarotid administration of a hypertonic mannitol solution with subsequent administration of drugs can increase drug concentration in brain and tumour tissue to reach therapeutic concentration (Kroll and Neuwelt, 1998; Doolittle et al., 2000; Fortin et al., 2007).
- MRI-guided focused ultrasound BBB disruption technique: Ultrasound has been shown to be capable of BBB disruption. The combination of microbubbles (preformed microbubbles of ultrasound contrast agent, optison, with a diameter of 2–6 μm which is injected into the blood stream before exposures to ultrasound). This technique has been shown to increase the distribution of Herceptin in brain tissue by 50% in a mice model (Hynynen et al., 2001, 2006; Kinoshita et al., 2006).
- Application of bradykinin-analogue (RMP-7, Cereport® from Alkermes Inc.): There is evidence of the opening of the tight junctions to occur by activation of bradykinin B2 receptors through a calcium-mediated mechanism (Dean et al., 1999; Borlongan and Emerich, 2003). This technique was abandoned due to lack of efficacy in Phase II and III studies when administered in combination with carboplatin.

Limitations: All these approaches are relatively costly, require anaesthesia and hospitalization, and are non-patient friendly. These techniques may enhance tumour dissemination after successful disruption of the BBB. Neurons may be damaged permanently from unwanted blood components entering the brain.

Pharmacological approach

The pharmacological approach to crossing the BBB is based on the observation that some molecules freely enter the brain, e.g. alcohol, nicotine and benzodiazepine. This ability to passively cross the BBB depends on the molecular size being less than 500 D), charge (low hydrogen bonding capabilities) and lipophilicity (the more lipophilic, the better the transport) (Lipinski et al., 2001). This approach consists of modifying, through medicinal chemistry, a molecule that is known to be active against a CNS target to enable it to penetrate the BBB. Modification of drugs through a reduction in the relative number of polar groups increases the transfer of a drug across the BBB. Lipid carriers have been used for transport, and there are successful examples of both these approaches (Pardridge, 1995). Modification of antioxidants with pyrrolopyrimidines increases their ability to access target cells within the CNS (Sawada et al., 1999). Enhanced delivery of ganciclovir to the brain was observed by covalently attaching 1-methyl-1,4-dihydronicotinate to an hydroxymethyl group (Bodor et al., 1982; Brewster et al., 1994). Fatty acid such as N-docosahexaenoyl (DHA) have been incorporated in small drugs to increase their brain uptake (Shashoua and Hesse, 1996; Bradley et al., 2001).

Limitations: The modifications necessary to cross the BBB often result in loss of the desired CNS activity. Increasing the lipophilicity of a molecule to improve transport can also result in making it a substrate for the efflux pump P-glycoprotein (P-gp).

Formulation of drugs facilitates brain delivery by increasing the drug solubility and stability in plasma

Incorporation of low molecular mass drugs into pluronic micelles can increase drug solubility and drug stability, and can improve drug pharmacokinetics and biodistribution (Batrakova and Kabanov, 2008). Polymeric micelles have been utilized for delivery of CNS drugs across the BBB, and for oral delivery of drugs and tumour-

specific delivery of antineoplastic agents (Kabanov et al., 2003). For example, in one early study, pluronic P85 micelles loaded with a neuroleptic drug were targeted to the brain by conjugating micelles with neurospecific antibodies, or using insulin as targeting moieties.

Amphiphilic chitosan-based polymers (mw<20 kD) self-assemble in aqueous media at low micromolar concentrations to give previously unknown micellar clusters of 100–300 nm of size (Qu et al., 2006). Intravenous anaesthetic propofol was used as a model drug. It is known that the sleep times obtained with the carbohydrate propofol formulations are up to 10 times those obtained when using either the commercial Fresenius or Diprivan formulations. A loss of righting reflex time could not be recorded as animals were asleep by the end of the injection period; evidence that delivery of the centrally active drug across the blood–brain barrier is rapid and efficient (Qu et al., 2006). Mechanisms involved in the increase CNS efficacy of these formulations are not known.

Limitations: The exact mechanism by which these specific nanoparticle-based formulations cross the BBB is not known, and may involve the induction of increased leakiness of the BBB and inhibition of efflux pumps. In addition the application of nanoparticles to large hydrophilic therapeutic molecules has not been proven.

Physiological approaches

The brain requires essential substances for metabolism and survival, such as glucose, insulin, growth hormone, low density lipoprotein (LDL), etc. These substances are recognized by specific receptors or transport mechanisms, resulting in specific transport into the brain. Since almost every neuron in the brain is perfused by its own capillary as a result of the small distance separating capillaries (on average 40 μm) and the brain's very high perfusion rate (Pardridge, 2005 and 2007c). Therefore, the most effective way of delivering neuroactive drugs is via transporters or internalizing receptors on these capillaries.

Drugs can be modified to take advantage of native BBB nutrient transport systems or by conjugation to ligands that recognize receptors expressed at the BBB. This will result in their being carried across the BBB after receptor-mediated transcytosis. This physiological approach is recognized by the scientific community as the one with the most likely chance of success.

Transporter-mediated delivery

Peptides and small molecules may use specific transporters expressed on the luminal and basolateral side of the endothelial cells forming the BBB to cross into the brain.

At least 8 different nutrient transport systems have been identified, with each transporting a group of nutrients of similar structure. Only drugs that closely mimic the endogenous carrier substrates will be taken up and transported into the brain. Drugs may be modified such that their transport is increased by using a carrier-mediated transporter expressed on the endothelial cells forming the BBB. Use of small molecules that directly target transporters to overcome BBB restrictions eliminate the need for the drug to be transformed for example by conjugation to antibodies (Allen et al., 2003) and to deliver the metabolic precursor of dopamine. Use of BBB transport proteins such as the choline transporter and the amino acid transporter has been achieved successfully for a few drugs, for example, the large neutral amino acid carrier has been used to deliver dopamine's metabolic precursor, L-Dopa, to patients with Parkinson's disease, resulting in clear clinical benefit; dopamine itself is non-brain penetrant.

Limitations: To use a BBB transporter protein as a CNS drug delivery vector, multiple factors must be considered. These include: 1) the kinetics available to transport physiologic molecules, 2) the structural binding requirements of the transporter 3) therapeutic compound manipulation so that the compound binds but also remains active in-vivo and 4) actual transport of the molecule into the brain, as opposed to just binding to the transporter.

Receptor-mediated transcytosis

Receptors at the blood–brain barrier

Large molecules which are necessary for the normal function of the brain are delivered to the brain by specific receptors. These receptors are highly expressed on the endothelial cells forming the BBB. These include the insulin receptor, transferrin receptor, LDL receptor and its related protein, and others. Research is still on-going to identify new receptors.

The receptor-mediated transcytosis occurs in 3 steps:

1. Receptor-mediated endocytosis of the compound at the luminal (blood) side.
2. Movement through the cytoplasm of the endothelial cell.
3. Exocytosis of the drug at the abluminal (brain) side of the brain capillary endothelium.

The precise mechanism of transcytosis across polarized endothelial cells has not been determined. Additional molecules may be involved in the transcytosis across the BBB and bypassing of lysosomes in the cytoplasm which could degrade the molecules being transported.

The physiologic approach comprises targeting these receptors at the BBB by specific ligands, modified ligands and antibodies. Therapeutic compounds are able to cross the BBB after association/conjugation to these specific ligands forming molecular Trojan horses (MTH) (Pardridge, 2003). To delivery larger amounts of therapeutics liposomes decorated with specific ligand have also been developed

Transferrin receptor (TR)

The function of the TR is to provide iron to cells. Drug targeting to the TR can be achieved by using the endogenous ligand transferrin, or by using antibodies directed against the TR.

- For transferrin (Tf) the in-vivo application is limited due to high endogenous concentrations of Tf in plasma. Transferrin is an essential protein needed for iron delivery to cells and is found at mg/ml amounts in plasma.
- Using the antibody approach against TR, the receptor specific mAb binds to the receptor on the endothelial cells, and allows the associated therapeutic agent to cross the BBB via receptor-mediated transcytosis (Pardridge, 2003; Zhang and Pardridge, 2005). For antibodies against the TR, proof of concept studies in rats have demonstrated that a mAb that binds to a distinct epitope from Tf (OX26) can be used as a brain delivery agent (Zhang and Pardridge, 2006).

Insulin receptor

Pardridge et al. have extensively documented the use of the insulin receptor for the targeted delivery of drugs to the brain using specific antibodies directed against the IR (Coloma et al., 2000). For example, using the 83-14 mouse MAb against the human insulin receptor (HIR) in rhesus monkey, shows that total uptake of the MAb is 4%, which corresponds to 0.04%/g brain tissue 3 h after iv injection (Jones and Shusta, 2007). Both the chimeric antibody and a fully humanized form of the 83-14 antibody against HIR have been created (Boado et al., 2007) and shown to be able to transport an associated/conjugated molecule across the BBB.

Antibodies against TR and HIR for brain drug targeting

Applications of these antibodies to molecular Trojan horses (MTH) for the delivery of therapeutics have been documented by Pardridge's

group. Different forms of fusion and conjugated proteins have been generated (Pardridge, 2003):

- VIP-TR mAb (vasoactive intestinal peptide conjugated to transferrin receptor monoclonal antibodies [TR mAb])
- BDNF-HIR mAb (brain derived neurotrophic factors conjugated to the human insulin receptor monoclonal antibodies [HIR mAb])
- FGF2-HIR mAb (fibroblast growth factor-2 conjugated to the HIR mAb)
- EGF-TR mAb (epidermal growth factor conjugated to TR mAb)
- A_β1-40 — TR mAb (amyloid β1–40 peptide conjugated to TR mAb)
- Peptide nucleic acid (siRNA) — HIR mAb
- β-galactosidase — TR mAb, IDUA- HIR fusion
- Neurotrophin — HIR fusion.

Liposomes coated with targeting molecules such as antibodies, Trojan Horses Liposomes (THL)

THL have been constructed by Pardridge et al. for the delivery of non-viral plasmid DNA across the BBB for expression of interfering RNA (shRNA). The plasmid DNA was encapsulated in the interior of a 100 nm liposome, and the surface of the liposome was coated with 2000 D PEG. The ends of 1–2% of the PEG strands were conjugated with a receptor (R) — specific mAb (HIR or TR). TR mAbs-targeted THL with expression plasmid for tyrosine hydrolase (TH) has been shown effective in treating Parkinson's disease (PD) in rat model. This approach has been used to deliver shRNA against EGFR and resulted in the knock-down of EGFR expression and increased survival of mice implanted intracranially with brain tumours (Pardridge, 2007b).

Nanoparticles coated with transferrin or transferrin receptor antibodies

Human serum albumin (HSA) nanoparticles covalently coupled to transferrin or transferrin receptor monoclonal antibodies have been used to transport loperamide across the BBB. Loperamide which adsorbs to the nanoparticles and does not cross the BBB by itself is used as a model drug. Significant anti-nociceptive effects were observed, thereby demonstrating the value of this approach to increase BBB transport of these small drugs (Ulbrich et al., 2008). The application of this technology for the transport of large and other small molecules across the BBB still remains to be proven.

Limitations: Some open questions still remain on the efficiency of mAb against specific receptors to transport compounds across the BBB as the results obtained by Pardridge's group have not been confirmed by others (Moos and Morgan, 2001; Gosk et al., 2004). Moos and Morgan (2001) *have shown that OX26 mainly accumulates in the brain capillary endothelial cells and not in the parenchymal compartment. It has been demonstrated using anti-TR mAb in animal model that although the total amount of drug delivery after IV injection of fusion molecules is high, most of it stays associated with brain capillary endothelial cells* (Gosk et al., 2004). *Dissociation from their specific receptor may be challenging due to the high affinity of the antibodies.*

Additionally, widespread expression of these receptors on peripheral organs limits its capabilities for specific brain delivery and, especially for HIR antibodies, may add additional toxicity.

Low-density lipoprotein receptor related proteins 1 and 2 (LRP-1 and 2)

LRP is a multifunctional endocytic receptor that mediates the internalization and degradation of multiple ligands involved in diverse metabolic pathways (Kounnas et al., 1995; Hertz and Strickland, 2001). LRP is a multiligand lipoprotein receptor which interacts with a broad range of secreted proteins and resident cell surface molecules (eq. apoE (apolipoprotein E), α2 M (α2 macroglobulin), tPA (tissue Plasminogen Activator), PAI-1 (Plasminogen Activator Inhibitor 1), APP (Amyloid Precursor Protein), Factor VIII, Lactoferrin,…), mediating their endocytosis or activating signalling pathways through multiple cytosolic adaptor and scaffold proteins. LRP contains four putative-ligand binding domains (LBD) labeled with numerals I, II, III and IV.

LRP, a type I transmembrane protein, is synthesized as a 600 kD precursor protein cleaved in the trans Golgi compartment by furin, to generate a large 515 kD subunit and a smaller 86 kD that remain non-covalently linked. The shorter cytoplasmic tail of LRP contains NPxY motifs and two dileucine-based motifs, and interacts with a number of cytoplasmic adaptor and scaffold proteins (Yoon et al., 2007).

LRP is expressed in many tissues and in the CNS (Rebeck et al., 1993). In the cerebellum, LRP expression was observed in neurons diffusely scattered throughout the granular cell layer. LRP expressed on neuronal cells functions similar to that of other cell types (i.e. hepatocytes) in both binding and endocytosis of ligand. Expression of LRP in astrocytes is detectable with moderate expression (Wolf et al., 1992; Moestrup et al., 1992; Bu et al., 1994). LRP is over-expressed in malignant astrocytomas, especially in glioblastomas (Yamamoto et al., 1997).

LRP 1 and 2 have been exploited to target drugs to the brain in a similar fashion as TR and IR.

- Nanoparticles have been used for drug delivery to the brain (Kreuter et al., 2003; Steiniger et al., 2004; Kreuter, 2005). The precise mechanism of transcytosis has not been clarified (Olivier, 2005) and is still debated. A possible mechanism is that polysorbate 80 (tween-80) coated polybutyl-cyanoacrylate nanoparticles adsorb apolipoprotein E and B from the bloodstream after IV injection (Kreuter et al., 2002) and therefore use LRP for transcytosis across the BBB.
- Melanotransferrin, also known as human melanoma-associated antigen p97, has been characterized previously as a glycosyl-phosphatidyl-inositol (GPI) anchored, membrane-bound form, and as a soluble secreted form (Food et al., 1994; Jefferies et al., 1996a,b). Melanotransferrin, like transferrin, is a syaloglycoprotein with a molecular weight between 95–97 kD, and has been shown to be actively transcytosed across the BBB (Demeule et al., 2002). Melanotransferrin has been further developed for the transport of anti-cancer agents such as doxorubicin across the BBB. Therapeutic amounts can be delivered into the brain parenchyma after chemical conjugation to melanotransferrin, resulting in a significant increase of survival of mice implanted intracranially with tumour cells (Gabathuler et al., 2005; Karkan et al., 2008).
- Receptor Associated Protein (RAP) has been shown to be efficiently transported across the BBB into the brain parenchyma (Pan et al., 2004). RAP is found in the endoplasmic reticulum (ER) where it plays the role of a chaperone for the LDL receptor family, including LRP-1 and 2, facilitating their transport to the cell surface avoiding interaction with endogenous ligands (Migliorini et al., 2003). The application of RAP as a drug carrier to the brain is being developed and for chemically conjugated anti-cancer agents and fusion proteins (Prince et al., 2004).
- The use of the lentivirus vector system to deliver the lysosomal enzyme, glucocerebrosidase, and a secreted form of Green Fluorescent Protein (GFP) to the neurons and astrocytes in the CNS has been demonstrated (Spencer and Verma, 2007). This approach, which used the Low-density lipoprotein receptor-binding domain of the apolipoprotein B fused to the targeted protein, proved to be feasible for delivery of the protein and could possibly be used as a general method for delivery of therapeutic proteins to the CNS.
- This transport was specific to the protein with the ApoB LDL receptor (LDLR) binding domain, as the control protein did not cross the BBB. Although the ApoB LDLR sequence is 38 aa long, the

length did not appear to greatly affect either the delivery or function of the recombinant protein.

- A new family of peptides derived from proteins (Fig. 2) that efficiently cross the BBB using LRP has been designed as a new peptide technology for the transport of therapeutics (Demeule et al., 2008a). Angiopeps, a family of 19 amino acid peptides derived from the kunitz domain, demonstrate a high transcytosis rate (Demeule et al., 2008a) using LRP-1 (Demeule et al., 2008b).

This new platform technology is applicable to small and large molecules, ranging in size from 500 D to 150 kD (including antibodies).

Using a CyDye fluorophore, cy5.5 labeled Angiopep-2; we have demonstrated that the labeled construct is very rapidly transported into the brain parenchyma, as measured by in-vivo imaging followed by fluorescence analysis of brain slices. In Fig. 3, Angiopep-2-cy5.5 (red) is clearly localized in the brain parenchyma 1 h post injection, and in close proximity to brain cell nuclei (Gabathuler et al., 2008a,b). The insert on the right confirms that Angiopep-7-cy5.5 a peptide with Lys residues replaced by Arg residues does not interact with LRP-1 is not transported into the brain. Other markers are used, brain capillaries are labeled green, brain cell nuclei are labeled blue.

Additional data demonstrate that Angiopep-2 is transported very efficiently in rat brain parenchyma after in-situ brain perfusion (Thomas et al., 2009 submitted) and confirm previous results (Demeule et al., 2008a).

Angiochem Inc. has developed these Angiopep peptides for the transport of therapeutic agents to the brain parenchyma, and has, as proof of concept, created several entities (NCEs), the most advanced of which is ANG1005. ANG1005 is a novel engineered peptide (EPiC) compound designed using Angiochem's novel platform technology incorporating the Angiopep amino acid sequence in the design of new therapeutics for the treatment of brain cancers. ANG1005 is formed by chemical conjugation of the peptide (Angiopep-2) with 3 molecules of paclitaxel, and brain uptake measured by in-situ rat brain perfusion assay (Takasato et al., 1984; Smith, 2003) shows 10 and 100 fold greater uptake into brain than the peptide Angiopep-2 or the free drug paclitaxel, respectively (Thomas et al., 2009 submitted). Therapeutic amounts of ANG1005 are delivered to the brain, as evidenced by the increased survival of mice implanted intracranially with tumour cells (Regina et al, 2008). These data were confirmed in rat brains measured by MRI, demonstrating regression of intracranial tumours following IV administration of ANG1005 (Bichat et al., 2008; Fig. 4).

The MRI images from representative animals (Fig. 4) show that treatment with ANG1005 results in tumour reduction while Taxol

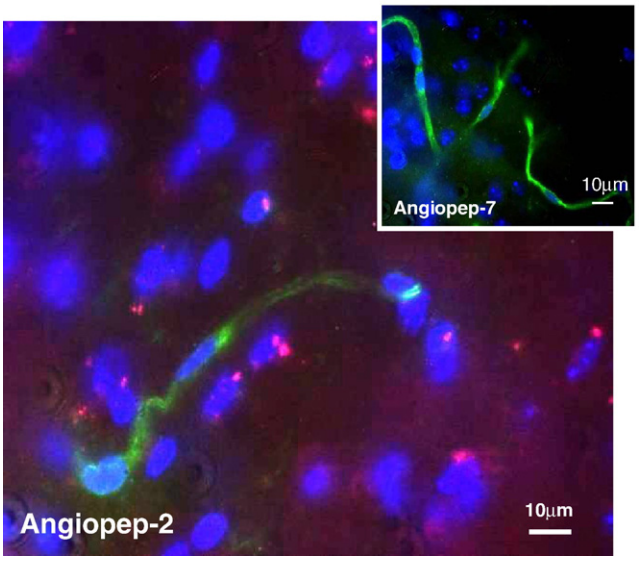


Fig. 3. Fluorescence microscopy 1 h post injection — distribution of Angiopep-2 and Angiopep-7.

treatment does not. In addition, when the animals were sacrificed and examined on day 24, tumours were undetectable in 5/8 animals treated with ANG1005. Additional experience with administration of ANG1005 by IV infusion demonstrates a similar effect on IC tumour growth.

The Angiopep technology developed by Angiochem Inc. is to date the most advanced technology for the brain delivery of drugs. The company has started clinical development with its most advanced product ANG1005 which is now in two Phase I clinical trial for recurrent malignant gliomas and for brain metastases of advanced cancer and has shown so far very promising data and validated the preclinical data.

Diphteria toxin receptor

Gaillard et al. (2005) have examined the utility of CRM197, a non toxic mutant of diphtheria toxin, as a targeting vector, for drug delivery to the brain. CRM197 has been shown to endocytose after binding the membrane-bound precursor of heparin binding epidermal growth factor-like growth factor (HB-EGF) (Raab and Klagsbrun, 1997), also known as the diphtheria toxin receptor (DTR). Conjugates

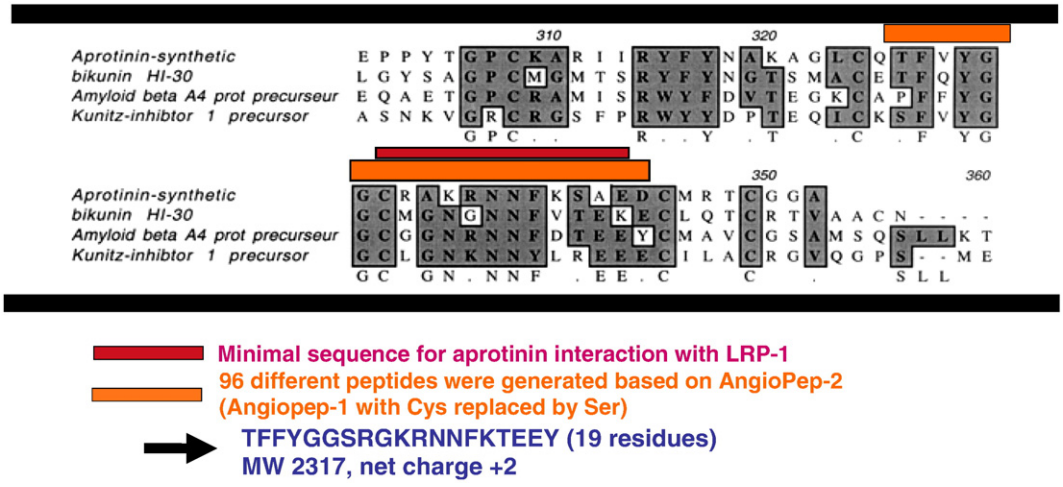


Fig. 2. Schematic description of the design of the new Angiopep family of peptides.

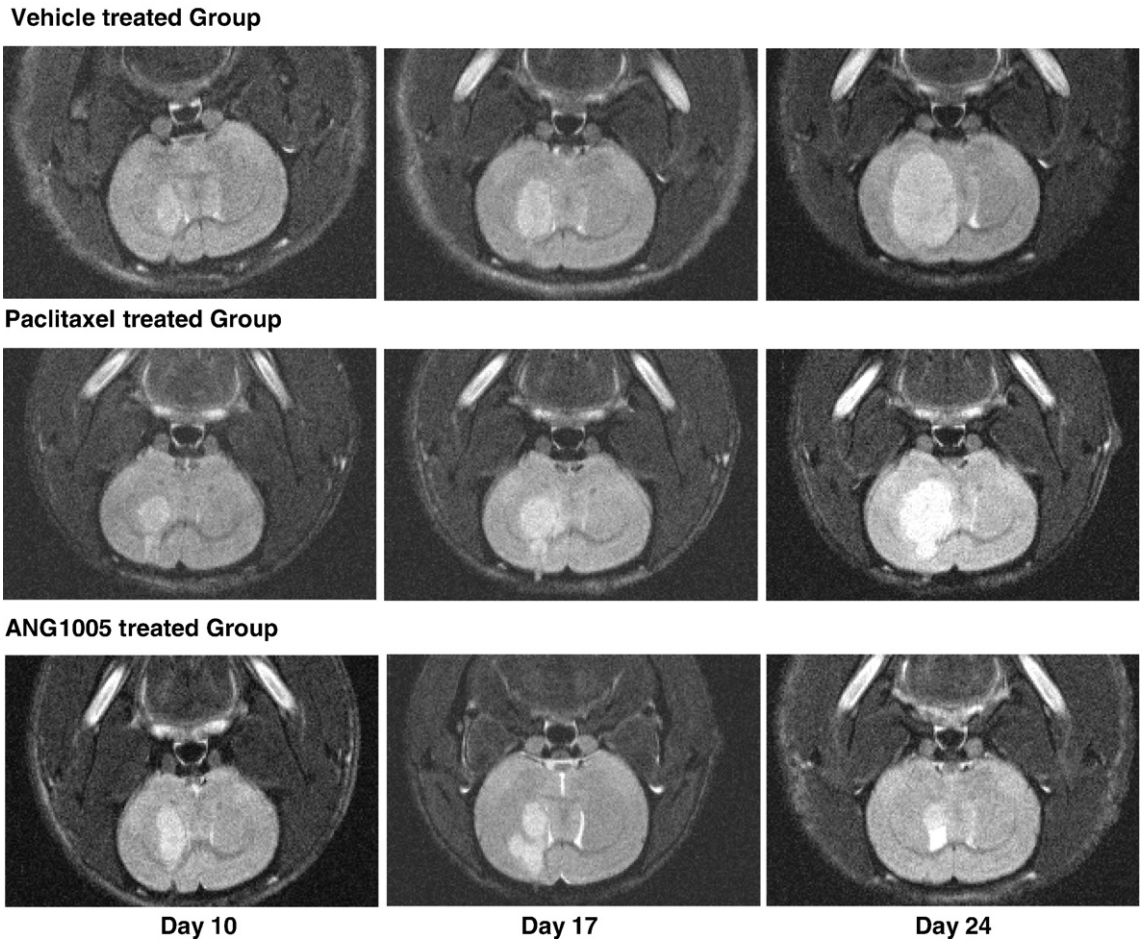


Fig. 4. MRI images of rat intracerebral glioblastoma model. Details on day of picture are indicated under the picture. Treatment started at day 10 twice a week. ANG1005 (75 mg/kg) and vehicle were administered IP and paclitaxel IV (5 mg/kg, maximum tolerated dose).

between CRM197 and horse radish peroxidase (HRP) were transported across the in-vitro model of the BBB using bovine brain capillary endothelial cells in co-culture with newborn rat astrocytes (Fenart and Cecchelli, 2003), and a fraction of intravenously injected CRM197-HRP was found in the brain parenchyma in guinea pigs (Gaillard et al., 2005). Using this vector brain delivery system, Gaillard et al. are developing a product for the treatment of Japanese Encephalitis Virus (JEV).

CRM197 usage presents some problems as it has been used for vaccination against diphtheria, and the presence of antibodies may hinder the efficacy of this vector. In addition, it has been recently reported that CRM197 has a weak toxicity and that specific care has to be taken in the use of CRM197 at high dosage, although the toxicity of CRM197 is about 100 times less than that of the wild-type diphtheria toxin (Kageyama et al., 2007).

The BBB transmigrating Llama single domain antibodies (sdAb)

Transport across the BBB in human brain endothelial cells has been reported for Llama single domain antibodies (Abulrob et al., 2005). Using a Llama single domain antibody (sdAb) phage display library (Tanha et al., 2002), a new antigen–ligand system was identified for transvascular brain delivery (Muruganandam et al., 2002; Tanha et al., 2003). sdAbs are half-size (13kD) fragments of the heavy chain IgGs which occur naturally (Tanha et al., 2002). The transport of two sdAbs, FC5 and FC44 across the human brain endothelial cells is polarized, charge independent and temperature-dependent, suggesting a receptor-mediated process. FC5 is detected in clathrin-enriched fractions following internalization. FC5 is targeted to early endosomes, bypasses late endosomes/lysosomes and remains intact after transcytosis. The receptor has been identified, and is related to a novel isoform of the transmembrane domain protein 30A (TMEM 30A). TMEM30A is also known as CDC50A, which is responsible for the cell surface expression of ATP8B1 (hypothesized to be a flippase for phosphatidylserine). CDC50A may be the potential β-subunit or chaperone for ATP8B1 (Paulusma et al., 2008). FC5 is now being developed to deliver therapeutic amounts of doxorubicin to the brain after pentamerization and association to liposomes.

Adsorptive mediated transcytosis

Adsorptive mediated endocytosis (AME) and transcytosis involve endocytosis in vesicles of charged substances, similarly to a receptor-mediated mechanism, but not by a specific mechanism. Peptides and proteins with a basic isoelectric point ("cationic" proteins) bind initially to the luminal plasma membrane (mediated by electrostatic interactions with anionic sites), which triggers adsorptive endocytosis.

Uptake of basic peptides can be followed experimentally by using primary cultured bovine brain capillary endothelial cells. The steady state uptake of the peptides was temperature-dependent and significantly decreased in the presence of dansylcadaverine, protamine and poly-L-Lysine which neutralize the charges at the plasma membrane inhibiting binding of these peptides. The C-terminal structure and the basicity of the molecule were the most important determinants of uptake by AME system in cultured bovine brain capillary endothelial cells (Temsamani et al., 2001), and not the number of constituent amino acids of the peptide.

- Protein transduction domains (PTDs) are typically amino acids sequences located on transcription factors allowing transport of larger molecules across the cell membranes, examples are TAT, homeodomain of Antennapedia, Syn-B, penetratin and others. This approach has been used by coupling of doxorubicin to either SynB1 (18aa) or SynB3 (10aa) vectors significantly enhances its brain uptake and bypasses the P-gp. Brain uptake of an enkephalin analogue (dalargin) was enhanced several 100-fold after vectorisation (Rousselle et al., 2003). Poly-arginines (9 mer of L-Arg, R9) showed a very efficient cellular uptake 20× superior than TAT49-57 and D-Arg oligomers (r9)>100 fold superior (Wender et al., 2000).
- Biologically active polymer core/shell nanoparticles, the surface of which is anchored with Tat molecules, have been successfully synthesized for drug delivery across the BBB (Liu et al., 2008). Ciprofloxacin as a model antibiotic was loaded into the nanoparticles. The presence of Tat on the surfaces of the nanoparticles promoted their uptake by human microvascular endothelial cells. The Tat-PEG-b-chol nanoparticles crossed the BBB, and entered the cytoplasm of neurons.
- Formulation of chitosan nanoparticles coated with polyamine (putrescine) modified F(ab′) portion of an anti-amyloid antibody to form smart nano-vehicle (SNV) was transported to the brain (Agyare et al., 2008). Efficiency of this approach to deliver therapeutic amounts of drugs to the brain remains to be demonstrated.
- For in-vivo imaging applications, the delivery module was conjugated to the NIR (near infrared) cy5.5 dye (Pham et al., 2005). To this end, a cysteine was deployed on a Myristoylated polyarginine peptide (MPAP) carboxyl-terminal where the thiol moiety reacted with the commercially available cy5.5 maleimide via a Michael addition reaction. To confirm that the observed fluorescence signal was truly from the brain, ex-vivo imaging was performed on excised mouse brains.
- Kumar et al. (2007) demonstrated the transvascular delivery of siRNA to the CNS using a mixture of a peptide composed of 29 aa

Table 1
Summary of the physiological approaches to deliver therapeutic molecules in the brain parenchyma.

Method	Molecules used	Stage of development	Potential problems
Use of specific transporters	Large amino acid carrier has been used by L-Dopa	Clinic for PD	Dosage and side effects
Receptor-mediated			
Transferrin receptor	Small and large molecules conjugated to mAbs or expressed as fusion proteins	Preclinical	To get therapeutical concentration in the brain parenchyma Toxicity
	Liposomes and nanoparticles coated with mAbs	Preclinical	Mechanism not known, high quantities needed Toxicity
	Nanoparticles coated with transferrin	Preclinical	Mechanism not known Toxicity
Insulin receptor	Small and large molecules conjugated to mAbs or expressed as fusion proteins	Preclinical	To get therapeutical concentration in the brain parenchyma Toxicity
	Liposomes and nanoparticles coated with mAbs	Preclinical	To get therapeutical concentration in the brain parenchyma
Low-density lipoprotein receptor related protein (LRP)	Receptor Associated Protein (RAP) (fragment): ligand to LRP conjugated to small and large molecules or expressed as fusion protein	Preclinical	Potential toxicity Potential diminution of the affinity for LRP after modification Immunogenicity
	Melanotransferrin (p97): ligand of LRP conjugated to small molecules or expressed as a fusion protein	Preclinical	Large protein High cost of production High quantities needed Immunogenicity
	ApoE or ApoB a LRP ligand binding to nanoparticles coated with polysorbate-80 loaded with small anti-cancer agents	Preclinical	Mechanism not known Potential toxicity Potential disruption of the BBB
	LRP binding domain of the apolipoprotein B (peptide of 38 amino acids) LRP ligand expressed with proteins	Preclinical	Not tested on active molecules (anti-cancer agents or others) or in human
	LRP binding Angiopep (19 amino acids peptide) modified small and large molecules conjugated or expressed as fusion protein	Phase I/II for brain cancers	Potential immunogenicity for application to large molecules
		Shows efficacy and confirmed proof of concept of the platform technology in the clinic for anti-cancer agents ANG1005	No Neurotoxicity detected for ANG1005 High tolerability for ANG1005
Diphteria toxin receptor	CRM197, a non toxic mutant of diphtheria toxin, as a targeting vector for small and large drug delivery to the brain using liposomes	Preclinical	Toxicity of CRM197 Immunogenicity
TMEM 30A (Flippase)	Llama single domain antibody (FC5) is now being developed to deliver small anti-cancer agents in the brain after pentamerization and association to liposomes	Preclinical	Affinity for the receptor Therapeutical concentration of agents need to be delivered Immunogenicity
Adsorptive mediated			
Protein transduction domains (PTD)	TAT, Syn-B, penetratin positively charged conjugated to small or large drugs or nanoparticles	Phase II for analgesic peptide dalargine and preclinical for small anti-cancer agents	Toxicity in the periphery To get therapeutical concentration in the brain parenchyma
Poly-Arg peptides	Positively charged peptides	Preclinical	Toxicity Therapeutical concentration in the brain

from RVG (Rabies Virus Glycoprotein) which allow specific binding to the acetylcholine receptor expressed on neuronal cells and of a 9 aa peptide poly-Arg (9R) allowing binding to siRNA and contributing to its CNS delivery, probably by adsorptive endocytosis with the specific siRNA.

*Limitations: Lack of tissue selectivity, possible disruption of the BBB resulting in increased toxicity are important factors limiting efficiency of these peptides for brain delivery after iv administrations. Binding of polycationic substances to negative charges on plasma membranes (PM) followed by endocytosis. This process is not efficient and may disrupt the BBB as has been shown using nanoparticles (*Lockman et al., 2004).

Conclusions

In this review the current techniques and new approaches in development to deliver small and large molecules such as biologics to the brain are described. The different methods described in the physiological approach are summarized in the Table 1. The techniques used to this day involve direct injection or infusion of therapeutic compounds into the brain, the cerebro-ventricles or the CSF, but all these approaches are severely limited by poor distribution into brain parenchyma. Only the use of technologies able to transport molecules through the endothelial cells of the BBB will allow a homogenous distribution of therapeutics in the brain and thus provide a uniform and rapid exposure to brain cells.

The most promising new technology uses a physiological approach to take advantage of endogenous receptors highly expressed at the BBB. These receptors provide brain cells with nutrients, and belong to the LDL receptor family, transferrin and insulin receptors among others. Monoclonal antibodies and ligands of these receptors can be used as Trojan horses for transcytosis of therapeutic compounds to the CNS. A new technology using the peptide Angiopep as the ligand for the LRP-1 receptor demonstrates a high transport rate across the BBB and the ability to transport both small and large drugs to the brain parenchyma. This technology is the most advanced of the technologies targeting receptor-mediated endocytosis, and it is currently in Phase I for the treatment of recurrent gliomas and brain metastasis.

References

Abulrob, A., Sprong, H., Bergen en Henegouwen, P.V., Stanimirovic, D., 2005. The blood–brain barrier transmigrating single domain antibody: mechanisms of transport and antigenic epitopes in human brain endothelial cells. J. Neurochem. 95, 1201–1214.

Agyare, E.K., Curran, G.L., Ramakrisham, M., Yu, C.C., Poduslo, J.F., Kandimalla, K.K., 2008. Development of a smart nano-vehicle to target cerebrovascular amyloid deposits and brain parenchymal plaques observed in Alzheimer's disease and cerebral amyloid angiopathy. Pharm. Res. 25, 2674–2684.

Allen, D.D., Lockman, P.R., Roder, K.E., Dwoskin, L.P., Crooks, P.A., 2003. Active transport of high-affinity choline and nicotine analogs into the central nervous system by the blood–brain barrier choline transporter. J. Pharmacol. Exp. Ther. 304, 1268–1274.

Batrakova, E.V., Kabanov, A.V., 2008. Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers. J. Controlled Res. 130, 98–106.

Bichat, F., Demeule, M., Lawrence, B., Raguin, O., Sourzat, B., Gabathuler, R., Castaigne, J.-P., Genne, P., 2008. Enhanced drug delivery to brain tumours with a new paclitaxel-peptide conjugate. Eur. J. Cancer Suppl. 6, 45 (139).

Blasberg, R.G., Patlak, C., Fenstermacher, J.D., 1975. Intrathecal chemotherapy: brain tissue profiles after ventriculo-cisternal perfusion. J. Pharmacol. Exp. Ther. 195, 73–83.

Boado, R.J., Zhang, Y., Zhang, Y., Wang, Y., Pardridge, W.M., 2007. GDNF fusion protein for targeted-drug delivery across the human blood–brain barrier. Biotech. Bioeng. 100, 387–396.

Bobo, R.H., Douglas, W., Akbasak, A., Morrison, P.F., Dedrick, R.L., Oldfield, E.H., 1994. Convection-enhanced delivery of macromolecules in the brain. Proc. Natl. Acad. Sci. U. S. A. 91, 2076–2080.

Bodor, N., Farag, H., Brewster, M.E., 1982. Site-specific, sustained release of drugs to the brain. Science 214, 1370–1372.

Borlongan, C.V., Emerich, D.F., 2003. Facilitation of drug entry into the CNS via transient permeation of blood–brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist, Cereport. Brain Res. Bull. 60, 2970306.

Bradley, M.O., Webb, N.L., Anthony, F.H., Devanesan, P., Witman, P.A., Hemamalini, S., Chander, M.C., Baker, S.D., He, L., Horwitz, S.B., swindell, C.S., 2001. Tumor targeting by covalent conjugation of a natural fatty acid to paclitaxel. Clin. Cancer Res. 7, 3229–3238.

Brewster, M.E., Raghavan, K., Pop, E., Bodor, N., 1994. Enhanced delivery of ganciclovir to the brain through the use of redox targeting. Antimicrob. Agents Chemo. 38, 817–823.

Bu, G., Maksymovitch, E.A., Nerbonne, J.M., Schwartz, A.L., 1994. Expression and function of the low density lipoprotein receptor-related protein (LRP) in mammalian central neurons. J. Biol. Chem. 269, 18521–18528.

Coloma, M.J., Lee, H.J., Kurihara, A., Landaw, E.M., Boado, R.J., et al., 2000. Transport across the primate blood–brain barrier of a genetically engineered chimeric monoclonal antibody to the human insulin receptor. Pharm. Res. 17, 266–274.

Dean, R.L., Emerich, D.F., Hasler, B.P., Bartus, R.T., 1999. Cereport (RMP-7) increases carboplatin levels in brain tumors after pretreatment with dexamethasone. Neuro-Oncol. 1, 268–274.

Demeule, M., Poirier, J., Jodoin, J., Bertrand, Y., Desrosiers, R.R., Dagenais, C., Nguyen, T., Lanthier, J., Gabathuler, R., Kennard, M., Jefferies, W.A., Karkan, D., Tsai, S., Fenart, L., Cecchelli, R., Beliveau, R., 2002. High transcytosis of melanotransferrin (p97) across the blood–brain barrier. J. Neurochem. 83, 924–933.

Demeule, M., Regina, A., Che, C., Poirier, J., Nguyen, T., Gabathuler, R., Castaigne, J.P., Beliveau, R., 2008a. Identification and design of peptides as a new drug delivery system for the brain. J. Pharmacol. Exp. Ther. 324, 1064–1072.

Demeule, M., Currie, J.C., Bertrand, Y., Che, C., Nguyen, T., Regina, A., Gabathuler, R., Castaigne, J.P., Beliveau, R., 2008b. Involvement of the low-density lipoprotein receptor-related protein in the transcytosis of the brain delivery vector Angiopep-2. J. Neurochem. 106, 1534–1544.

Doolittle, N.D., Miner, M.E., Hall, W.A., Siegal, T., Hanson, E.J., Osztie, E., McAllister, L.D., Bubalo, J.S., Kraemer, D.F., Fortin, D., Nixon, R., Muldoon, L.L., Neuwelt, E.A., 2000. Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood–brain barrier for the treatment of patients with malignant brain tumors. Cancer 88, 637–647.

Ehrlich, P., 1885. Das Sauerstoff-Bedurfnis des Organismus: Eine Farbenanalytische Studie. Hirschwald, Berlin.

Fenart, L., Cecchelli, R., 2003. Protein transport in cerebral endothelium. In vitro transcytosis of transferrin. Methods Mol. Med. 89, 277–290.

Food, M., Rothenberger, S., Gabathuler, R., Haidl, I.D., Reid, G., Jefferies, W.A., 1994. Transport and expression in human melanomas of a transferrin like glycosylphosphatidylinositol-anchored proteins. J. Biol. Chem. 269, 3034–3040.

Fortin, D., Gendron, C., Boudrias, M., Garant, M.P., 2007. Enhanced chemotherapy delivery by intraarterial infusion and blood–brain barrier disruption in the treatment of cerebral metastasis. Cancer 109, 751–760.

Fung, L.K., Ewend, M.G., sills, A., Sipos, E.P., Thompson, R., Watts, M., Colvin, O.M., Brem, H., Saltzman, W.M., 1998. Pharmacokinetics of interstitial delivery of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. Cancer Res. 58, 672–684.

Gabathuler, R., Arthur, G., Kennard, M.L., Chen, Q., Tsai, S., Yang, J., schoorl, W., Vitalis, T.Z., Jefferies, W.A., 2005. Development of a potential protein vector (NeuroTrans) to deliver drugs across the blood–brain barrier. Int. Congr. Ser. 1277, 171–184.

Gabathuler, R., Demeule, M., Regina, A., Che, C., Thomas, F., Abulrob, A., Smith, Q.R., Stanimirovic, D., Beliveau, R., Castaigne, J.P., 2008a. ANG1005, paclitaxel conjugated to the Angiopep brain transport vector for the treatment of brain cancer: preclinical studies. Eur. J. Cancer Suppl. 6, 39 (117).

Gabathuler, R., Demeule, M., Regina, A., Che, C., Lockman, P., Thomas, F., Gaasch, J., Thorsheim, H., Abulrob, A., Smith, Q.R., Stanimirovic, D., Beliveau, R., Castaigne, J.P., 2008b. A new drug, ANG1005, a conjugate of paclitaxel and Angiopep peptide vector, is able to cross the blood–brain barrier for the treatment of brain cancers. Neuro-Onco. 10, 784 (ET-09).

Gaillard, P.J., Brink, A., de Boer, A.G., 2005. Diphteria toxin receptor-targeted brain drug delivery. Int. Congr. Ser. 1277, 185–195.

Gosk, S., Vermehren, C., Storm, G., Moos, T., 2004. Targeting anti-transferrin receptor antibody (OX26) and OX26-conjugated liposomes to brain capillary endothelial cells using in situ perfusion. J. Cereb. Blood Flow Metab. 24, 1193–1204.

Hertz, J., Strickland, D.K., 2001. LRP: a multifunctional scavenger and signalling receptor. J. Clin. Invest. 108, 779–784.

Herzog, C.D., Dass, B., Holden, J.E., Stansell, J., Gasmi, M., Tuszynski, M.H., Bartus, R.T., Kordower, J.H., 2007. Striatal delivery of CERE-120, an AAV2 vector encoding human neurturin, enhances activity of dopaminergic nigrostriatal system in aged monkeys. Mov. Disord. 22, 1124–1132.

Hynynen, K., McDannold, N., Vykhodtseva, N., Jolesz, F.A., 2001. Noninvasive MR imaging-guided focal opening of the blood–brain barrier in rabbits. Radiology 220, 640–646.

Hynynen, K., McDannold, N., vykhodtseva, N., Raymond, S., Weissleder, R., Jolesz, F.A., Sheikov, N., 2006. J. Neurosurg. 105, 445–454.

Jefferies, W.A., Food, M., Gabathuler, R., Rothenberger, S., Yamada, T., McGeer, P.L., 1996a. Reactive microglia specifically associated with amyloid plaques in Alzheimer's disease brain tissue express melanotransferrin. Brain Res. 712, 122–126.

Jefferies, W.A., Gabathuler, R., Rothenberger, S., Food, M., Kennard, M.L., 1996b. Pumping iron in the '90s. Trends Cell Biol. 6, 223–228.

Jones, A.R., Shusta, E.V., 2007. Blood–brain barrier transport of therapeutics via receptor mediation. Pharma. Res. 24, 1759–1771.

Kabanov, A.V., Batrakova, E.V., Miller, D.W., 2003. Pluronic block copolymers as modulators of drug efflux transporter activity in the blood–brain barrier. Adv. Drug Deliv. Rev. 55, 151–164.

Kageyama, T., Ohishi, M., Miyamoto, S., Mizushima, H., Iwamoto, R., Mekada, E., 2007. Diphteria toxin mutant CRM197 possesses weak EF2-ADP-ribosyl activity that potentiates its anti-tumorigenic activity. J. Biochem. 142, 95–104.

Karkan, D., Pfeifer, C., Vitals, T.Z., Arthur, G., Ujiie, M., Chen, Q., Tsai, S., Kolaitis, G., Jefferies, WA, 2008. A unique carrier for delivery of therapeutical compounds beyond the blood–brain barrier. Plos One 3, e2469.

Kinoshita, M., McDannold, N., Jolesz, F.A., Hynynen, K., 2006. Targeted delivery of antibodies through the blood–brain barrier by MRI-guided focused ultrasound. Biochem. Biophys. Res. Commun. 340, 1085–1090.

Kounnas, M.Z., Moir, R.D., Rebeck, G.W., Bush, A.I., Argraves, W.S., Tanzi, R.E., Hyman, B.T., Strickland, D.K., 1995. LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted β-amyloid precursor protein and mediates its degradation. Cell 82, 331–340.

Kreuter, J., Ramge, P., Petrov, V., Hamm, S., Gelperina, S.E., engelhardt, B., Alyautdin, R., von Briesen, H., Begley, D.J., 2003. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharma Res. 20, 409–416.

Kreuter, J., 2005. Application of nanoparticles for the delivery of drugs to the brain. Int. Congr. Ser. 1277, 85–94.

Kreuter, J., Shamenkov, D., Petrov, V., Ramge, P., Cychutek, K., Koch-Brandt, C., Alyautdin, R., 2002. Apolipoprotein-mediated transport of nanoparticles-bound drugs across the blood–brain barrier. J. Drug Target 10, 317–325.

Kroll, R.A., Neuwelt, E.A., 1998. Outwitting the blood–brain barrier for therapeutic purposes: osmotic opening and other means. Congr. Neurol. Sur. 42, 1083–1099.

Kumar, P., Wu, H., McBride, J.L., Jung, K.E., Kim, M.H., Davidson, B.L., Lee, S.K., Shankar, P., Manjunath, N., 2007. Transvascular delivery of small interfering RNA to the central nervous system. Nature 448, 33–40.

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.

Liu, L., K Guo, J.L.u., Venkatraman, S.S., Luo, D., Ng, K.C., Ling, E.A., Moochhala, S., Yang, Y.Y., 2008. Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood–brain barrier. Biomaterials 29, 1509–1517.

Lockman, P.R., Koziara, J.M., Mumper, R.J., Allen, D.D., 2004. Nanoparticle surface charges alter blood–brain barrier integrity and permeability. J. Drug Target. 12, 635–641.

Marks, W.J., Ostrem, J.L., Verhagen, L., Starr, P.A., Larson, P.S., Bakay, R.A.E., Taylor, R., Cahn-Weiner, D.A., Stoessel, A.J., Olanow, C.W., Bartus, R.T., 2008. Safety and tolerability of intraputaminal delivery of CERE-120 (adenovirus-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. Lancet Neurol. 7, 400–408.

Migliorini, M.M., Behre, E.H., Brew, S., Ingham, K.C., Strickland, D.K., 2003. Allosteric modulation of ligand binding to low density lipoprotein receptor-related protein by the receptor-associated protein requires critical lysine residues within the carboxy-terminal domain. J. Biol. Chem. 278, 17986–17992.

Moestrup, S.K., Gliemann, J., Pallesen, G., 1992. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. Cell Tissue Res. 269, 342–375.

Moos, T., Morgan, E.H., 2001. Transferrin and transferrin receptor function in brain barrier systems. Cell Mol. Neurobiol. 20, 77–95.

Muruganandam, A., Tanha, J., Narang, S., Stanimirovic, D., 2002. Selection of phage-displayed llama single-domain antibodies that transmigrate across human blood–brain barrier endothelium. FASEB J. 16, 240–242.

Olivier, J.C., 2005. Drug transport to brain with targeted nanoparticles. NeuroRx 2, 108–119.

Pan, W., Kastin, A.J., Zankel, T.C., van Kerkhof, P., Terasaki, T., Bu, G., 2004. Efficient transfer of receptor-associated protein (RAP) across the blood–brain barrier. J. Cell Sci. 117, 5071–5078.

Pardridge, W.M., 1995. Transport of small molecules through the blood–brain barrier: biology and methodology. Adv. Drug Deliv. Rev. 15, 5–36.

Pardridge, W.M., 1998. CNS drug design based on principles of blood–brain barrier transport. J. Neurochem. 70, 1781–1792.

Pardridge, W.M., 2003. BBB drug targeting: the future of brain drug development. Mol. Interv. 3, 90–105.

Pardridge, W.M., 2005. The blood–brain barrier: bottleneck in brain drug development. NeuroRx 2, 3–14.

Pardridge, W.M., 2007a. Drug targeting to the brain. Pharma. Res. 24, 1733–1744.

Pardridge, W.M., 2007b. shRNA and siRNA delivery to the brain. Adv. Drug Deliv. Rev. 59, 141–152.

Pardridge, W.M., 2007c. Blood–brain barrier delivery. Drug Discov. Today 12, 54–61.

Paulusma, C.C., Folmer, D.E., Ho-Mok, K.S., de Wart, D.R., Hilarius, P.M., Verhoeven, A.J., Elferink, R.P.J., 2008. ATP8B1 requires an accessory protein for endoplasmic reticulum exit and plasma membrane lipid flippase activity. Hepatology 47, 268–278.

Pham, W., Zhao, B.Q., Lo, E.H., Medarova, Z., Rosen, B., Moore, A., 2005. Crossing the blood–brain barrier: a potential application of myristoylated polyarginine for in vivo neuroimaging. NeuroImage 28, 287–292.

Prince W.S., McCormick L.M., Wendt D.J., Fitzpatrick P.A., Schwartz K.L., Aguilera A.I., Koppaka V., Christianson T.M., Vellard M.C., Pavloff N., Lemontt J.F., Qin M., Starr C.M., Buaaaa G., Zankel T.C. 2004. Lipoprotein receptor binding, cellular uptake, and lysosomal delivery of fusions between the receptor-associated protein (RAP) and α-L-iduronidase or acid α-glucosidase.

Qu X., Khutoryanskiy V.V., Stewart A., Rahman A., Papahadjopoulos-Sternberg B., Dufes C., McCarthy D., Wilson C.G., Lyons R., Carter K.C., Schatzlein A. and Uchegbu I.F. 2006. Carbohydrate-based micelle clusters which enhance hydrophobic drug availability by up to 1 order of magnitude.

Raab, G., Klagsbrun, M., 1997. Heparin-binding EGF like growth factor. Bioch. Bioph. Acta 1333, F179–F199.

Rebeck, G.W., Reiter, J.S., Strickland, D.K., Hyman, B.T., 1993. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron 11, 575–580.

Regina, A., Demeule, M., Che, C., Lavallee, I., Poirier, J., Gabathuler, R., Beliveau, R., Castaigne, J.P., 2008. Antitumor activity of ANG1005, a conjugate between paclitaxel and the new brain delivery vector Angiopep-2. Br. J. Pharmacol. 155, 185–197.

Rousselle, C., Clair, P., Smirnova, M., Kolesnikov, Y., Pasternak, G.W., Gac-Breton, S., Rees, A.R., Scherrmann, J.M., Temsamani, J., 2003. Improved brain uptake and pharmacological activity of dalargin using a peptide-vector-mediated strategy. JPET 306, 371–376.

Thomas, F.C., Taskar, K., Rudraraju, V., Goda, S., Thorsheim, H., Gaasch, J., Palmieri, D., Lockman, P., Steeg, P.S., Smith, Q.R., 2009. Brain uptake of ANG1005 — a novel paclitaxel-peptide vector conjugate with improved blood–brain barrier transport. Submitted.

Sawada, G.A., Williams, L.R., Lutzke, B.S., Raub, T.J., 1999. Novel, highly lipophilic antioxidants readily diffuse across the blood–brain barrier and access intracellular sites. J. Pharm. Exp. Ther. 288, 1327–1333.

Shashoua, V.E., Hesse, G.W., 1996. N-Docosahexaenoyl, 3 hydroxytyramine: a dopaminergic compound that penetrates the blood–brain barrier and suppresses appetite. Life Sci. 58, 1347–1357.

Smith, Q.R., 2003. A review of blood–brain barrier transport techniques. Methods Mol. Med. 89, 193–208.

Spencer, B.J., Verma, I.M., 2007. Targeted delivery of proteins across the blood–brain barrier. PNAS USA 104, 7594–7599.

Steiniger, S.C.J., Kreuter, J., Khalansky, A.S., Skidan, I.N., Bobruskin, A.I., Smirnova, Z.S., Severin, S.E., Uhl, R., Kock, M., Geigerand, K.D., Gelperina, S.E., 2004. Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. Int. J. Cancer 109, 759–767.

Takasato, Y., Rapoport, S.I., Smith, Q.R., 1984. An in situ brain perfusion techniques to study cerebrovascular transport in the rat. Am. J. Physiol. 247, H484–H493.

Tanha, J., Dubuc, G., Hirama, T., Narang, S.A., MacKenzie, C.R., 2002. Selection by phage display of llama conventional V_H fragments with heavy chain antibody V_HH pproperties. J. Immunol. Meth. 263, 97–109.

Tanha, J., Muruganandam, A., Stanimirovic, D., 2003. Phage display technology for identifying specific antigens on brain endothelial cells. Meth. Mol. Med. 89, 435–449.

Temsamani, J., Rousselle, C., Rees, A.R., Scherrmann, J.M., 2001. Vector-mediated drug delivery to the brain. Exp. Opin. Biol. Ther. 1, 773–782.

Ulbrich, K., Herbert, E., Kreuter, J., 2008. Transferrin- and transferrin-receptor monoclonal antibody-modified nanoparticles enable drug delivery across the blood–brain barrier (BBB). Eur. J. Pharm. Biopharm. 10415.

Vandergrift, W.A., Patel, S.J., Nicholas, J.S., Varma, A.K., 2006. Convection-enhanced delivery of immunotoxins and radioisotopes for treatment of malignant gliomas. Neurosurg. Focus 20 (4), E10.

Wender, P.A., Mitchell, D.J., Pattabiraman, K., Pelkey, E.T., Steinman, L., Rothbard, J.B., 2000. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. PNAS USA 97, 13003–13008.

Wolf, G.G., Lopes, M.B.S., VandenBerg, S.R., Gonias, S.L., 1992. Characterization and immunohistochemical localization of a2-macroglobulin receptor (low-density lipoprotein receptor-related protein) in human brain. Am. J. Pathol. 141, 37–42.

Yamamoto, M., Ikeda, K., Ohshima, K., Tsugu, H., Kimura, H., Tomonaga, M., 1997. Increased expression of low density lipoprotein receptor-related protein/α2-macroglobulin receptor in human malignant astrocytomas. Cancer Res. 57, 2799–2805.

Yoon, I.S., Chen, E., Busse, T., Repetto, E., Lakshmana, M.K., Koo, E.H., Kang, D.E., 2007. Low-density lipoprotein receptor-related protein promotes amyloid precursor protein trafficking to lipid rafts in the endocytic pathway. FASEB J. 21, 2742–2752.

Zhang, Y., Pardridge, W.M., 2005. Delivery of β-galactosidase to mouse brain via the blood–brain barrier transferrin receptor. J. Pharmaco. Exp. Therap. 313, 1075–1081.

Zhang, Y., Pardridge, W.M., 2006. Blood–brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion. Brain Res. 1111, 227–229.