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DOI: 10.1002/anie.201006565

Drug Delivery

Shuttle-Mediated Drug Delivery to the Brain

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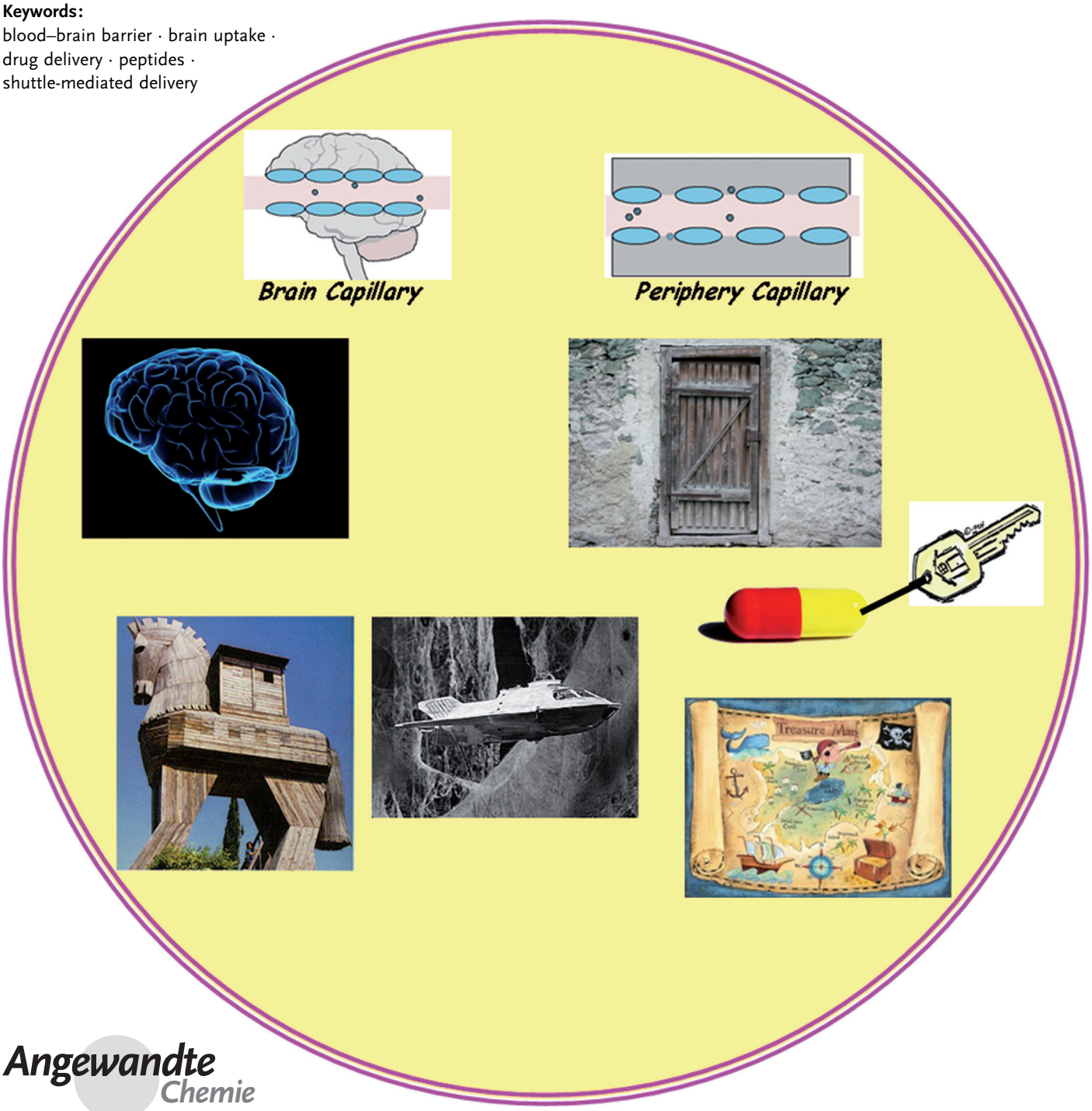
Keywords:
blood–brain barrier · brain uptake · drug delivery · peptides · shuttle-mediated delivery



Advances in the field of shuttle-mediated drug delivery have been made in the last decade; however, the treatment of brain disorders still remains a great challenge because of the presence of the blood–brain barrier (BBB), a structure that limits the access of drugs to their site of action in the central nervous system. Several strategies have been proposed to enhance the transport of drugs across the BBB. In this Review, we focus on the vector-mediated approach, in which a drug is coupled to a molecule (shuttle) that has the ability to cross the BBB and deliver the drug to the brain.

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1. Introduction

1.1. Blood–Brain Barrier (BBB)

Small molecules can readily cross the spaces between the endothelial cells of blood capillaries in the periphery (Figure 1A). However, tight junctions are formed between endothelial cells in brain capillaries, thus preventing paracellular transport of molecules into the brain (Figure 1B). Moreover, a basal membrane and brain cells, such as pericytes and astrocytes, surrounding the endothelial cells further form and maintain an enzymatic and physical barrier known as the blood–brain barrier (BBB). There are approximately 643 km of capillaries in the brain, and these cover 20 m^2. The BBB serves to prevent the entry of toxins from the blood into the brain and to maintain brain homeostasis. However, this barrier is also a formidable obstacle for the effective delivery of drugs to the central nervous system (CNS). Another barrier between the blood and the brain is the blood–cerebrospinal fluid barrier (BCSFB), which separates the blood from cerebrospinal fluid (CSF). However, this barrier is not considered a main route for the uptake of drugs since its surface area is 5000-fold smaller than that of the BBB.[1–8]

Brain penetration, that is, the availability of a drug at its site of action in the brain for sufficient time to exert its biological effect, is the ultimate aim of any CNS drug-development program. To this end, permeation of the BBB is a key issue, although there are other mechanistic aspects that should not be overlooked, such as drug distribution in brain compartments, metabolic clearance, nonspecific binding to proteins and lipids from the plasma or brain, and clearance from intercellular fluid (ICF) into the blood and CSF.[8,9]

1.2. BBB Permeation Mechanisms

Compounds cross the BBB by means of active or passive mechanisms (Figure 2). Small lipophilic molecules enter the brain by passive diffusion (Figure 2A). Specific carriers (carrier-mediated transport) present on endothelial membranes transport nutrients such as glucose and amino acids to this organ (Figure 2B). However, larger molecules (peptides and proteins) are transported either via a specific receptor (receptor-mediated endocytosis, Figure 2C) or by electrostatic interaction with endothelial membranes (adsorptive-mediated endocytosis, Figure 2D). In contrast, active efflux transporters, such as P-glycoprotein (P-gp), pump out their substrates from the brain and BBB endothelial cells into the blood (Figure 1).[3,4,10–13]

1.3. Strategies for Drug Delivery to the Brain

Although effective drugs are available for some brain disorders, their application is limited because of their low distribution in the brain or inability to cross the BBB. For example, most of the drugs systematically administered for chemotherapy do not gain access to the brain in the required concentration for tumors, which may explain the high mortality rates for brain cancer. It has been proposed that small molecules (molecular weight < 400 Da) with high lipophilicity that are not substrates for active efflux transporters cross the BBB; however, $> 98\,\%$ of small molecules do not meet these requirements to enter the brain. In addition, 100 % of large molecules are unable to gain entry to this organ. Moreover, considering the fact that about 1.5 billion people worldwide suffer from brain diseases and given the growing demand for CNS drugs for an aging population, there is a clear need for a suitable strategy to deliver nonpermeating therapeutic agents to the brain.[14–16] To overcome the BBB and improve uptake of a drug in the brain, the following approaches have been applied: temporarily opening the BBB, administration of very high doses of a drug, and direct injection of a drug into the spinal cord. However, these

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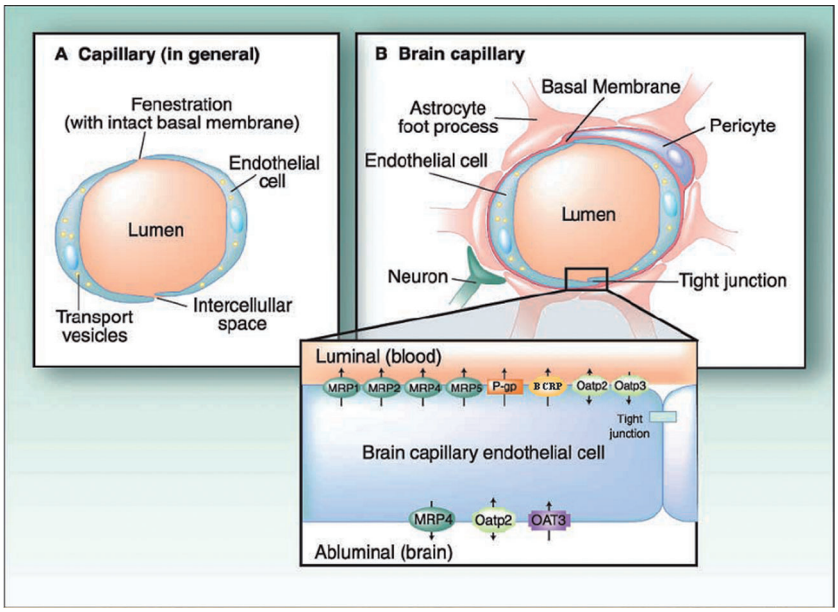

Figure 1. Schematic comparison of a capillary in the periphery (A) and a brain capillary (B). The BBB is formed by capillary endothelial cells, surrounded by a basal membrane and astrocytic perivascular end-feet. Tight junctions present between the cerebral endothelial cells form a diffusion barrier, which severely restricts the penetration of water-soluble compounds, including polar drugs, into the brain. Astrocytic end-feet appear to be critical for the induction and maintenance of the endothelial barrier. Furthermore, pericytes intimately embrace the brain capillaries and seem to contribute to the development, maintenance, and regulation of the BBB. The tightness of the endothelial barrier results in the paracellular transport of substances being negligible under physiological conditions. Reprinted from Ref. [4] with permission.

methods are invasive and imply a risk of infection and toxicity. Furthermore, they require highly skilled personnel. In addition, the following strategies have been developed: 1) co-administration of inhibitors of active efflux transporters together with drugs that are recognized by these transporters; however, these inhibitors may produce harmful side effects in the brain and peripheral tissues;[17–19] 2) focused ultrasound (FUS), which locally and reversibly disrupts the BBB;[20,21] however, FUS has been reported to damage brain tissues;[22] and 3) magnetic targeting (MT), which uses magnetic nanoparticles (MNPs) with external magnetic forces to increase the concentration of a drug in the brain while decreasing its concentration in other organs. However, this technique requires greater optimization of the properties of the MNPs and the magnetic field parameters to reduce the possible toxic effects on the BBB.[23,24] The combined use of FUS and MT showed more efficiency than the use of either approach alone.[25] Another option for overcoming the BBB is to modify the structure of a drug so that it ceases to be a substrate for active efflux transporters.[17,26] Other approaches would be to enhance the lipophilicity of a drug and consequently increase its passive permeation, or to have a structure that mimics a nutrient and thus facilitates transport by one of several specific carriers within the BBB.[3,10–12,27] However, modification of a drug may affect its affinity to its receptor once inside the brain or may cause the drug to exceed a molecular weight of 400 Da. Furthermore, improved drug lipophilicity can increase its uptake by other organs and, therefore, decrease the drug concentration in the blood.[16] In this context, the conjugation of therapeutic molecules to compounds (shuttles) that can cross the BBB and thus carry drugs into the brain emerges as an attractive alternative.[16,28,29] In some cases, conjugation can also improve the solubility of the drug or help bypass active efflux transporters without the need for drug

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Ernest Giralt received his PhD in 1974 from the University of Barcelona. After postdoctoral work at the University of Montpellier, he returned to the University of Barcelona as an Associate Professor, and in 1986 he was promoted to Full Professor. In 2005 he was appointed Director of the Chemistry and Molecular Pharmacology Programme at the Institute for Research in Biomedicine (IRB Barcelona). His major research interests lie in the fields of peptide synthesis, molecular recognition, and structure determination, in particular using NMR spectroscopy.

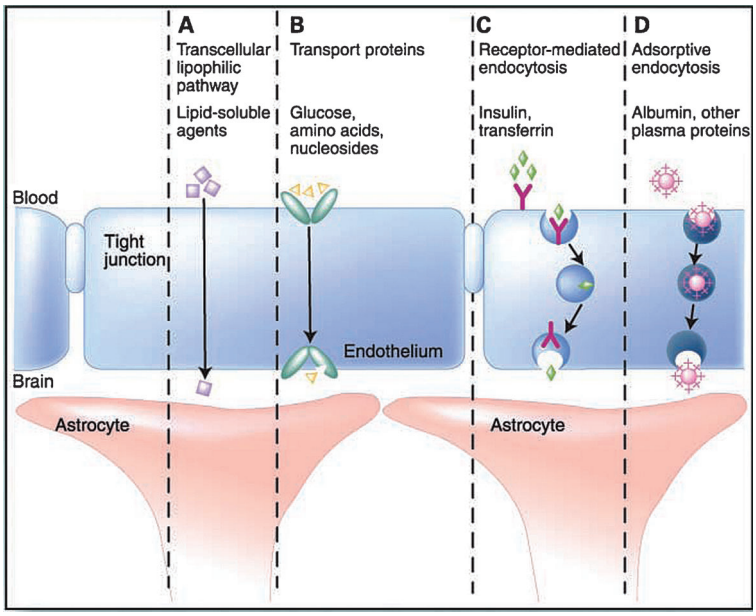

Figure 2. Schematic representation of the mechanisms available to transport endogenous molecules across the BBB. A) Small, lipid-soluble substrates diffuse across the membrane, although they are subject to efflux back into the circulation through transporters. B) Small endogenous molecules, including amino acids, nucleosides, and glucose, are transported across the BBB by transport proteins. C) Endogenous larger molecules, including insulin and transferrin, are recognized by receptors on the luminal side of the endothelium and transported across the cell for release into the brain parenchyma. D) Endogenous large plasma proteins including albumin are transported across the BBB by adsorptive-mediated endocytosis. Reprinted from Ref. [4] with permission.

solubilizers or P-gp inhibitors.[29,30] Here we discuss a number of the recent drug-transport shuttles used for this purpose (Table 1).

Table 1: Distinctive shuttle-mediated strategies for drug delivery to the brain.

Strategy	Mechanism	Drug	Application	Refs.
chemical delivery system	passive diffusion	zidovudine (AZT) dopamine	AIDA encephalopathy Parkinson's disease	[38] [32,46]
carrier-mediated transport	carrier-mediated transport	nipecotic acid (Nip) ketoprofen	anticonvulsant anti-inflammatory	[48,49] [50,51]
molecular trojan horses	receptor-mediated endocytosis	anti-Aβ antibody α-L-iduronidase (IDUA)	Alzheimer's disease Hurler's syndrome	[75,79] [78]
colloidal carriers	receptor-mediated endocytosis adsorptive-mediated endocytosis	NC-1900 daunorubicin	enhancement in memory impairment anticancer	[102] [107]
peptide vector-mediated	adsorptive-mediated endocytosis receptor-mediated endocytosis	dalargin paclitaxel (PAX)	antinociceptive anticancer	[117] [30]

2. Chemical Delivery System (CDS)

The chemical delivery system (CDS) was one of the first approaches to achieve specific drug delivery to the brain (Figure 3). The system is based on a dihydropyridine (T)↔pyridinium salt (T^+) redox carrier, in which a drug is chemically attached to the dihydropyridine (drug-T). Following intravenous (i.v.) injection, drug-T, as a result of its enhanced lipophilicity, is distributed in body tissues, including the brain. Enzymatic oxidation of drug-T then produces drug-T^+, which, because of its charge, is rapidly eliminated from the periphery by the kidney and the liver. However, the charge of drug-T^+ causes it to be "locked in" in the brain, where its enzymatic cleavage releases the drug in a slow and sustained manner.[31–33]

The human immunodeficiency virus (HIV), which enters the brain through adsorptive-mediated endocytosis,[34,35] can infect this organ and cause acquired immune deficiency syndrome (AIDS) encephalopathy. However, drugs for the treatment of AIDS do not have the capacity to cross the BBB.[36,37] Consequently, despite the availability of pharmacological agents, treatment is complicated. Moreover, the brain can act as an HIV-1 reservoir. The CDS was used to the improve delivery of zidovudine (AZT, Figure 4A), the first drug approved for treating AIDS. Administration of AZT-CDS (Figure 4B) in dogs increased the concentration of AZT in the brain 1.8- to 3.3-fold compared to free AZT. The concentration of AZT in the brain was not significantly improved; however, since AZT-CDS also decreased the concentration of AZT in the blood, it led to an increased ratio of the concentration of AZT in the brain to that in blood, compared with the administration of AZT alone.[38] The CDS method has also been used to deliver several neuropeptides, such as enkephalin, TRH (thyrotro-

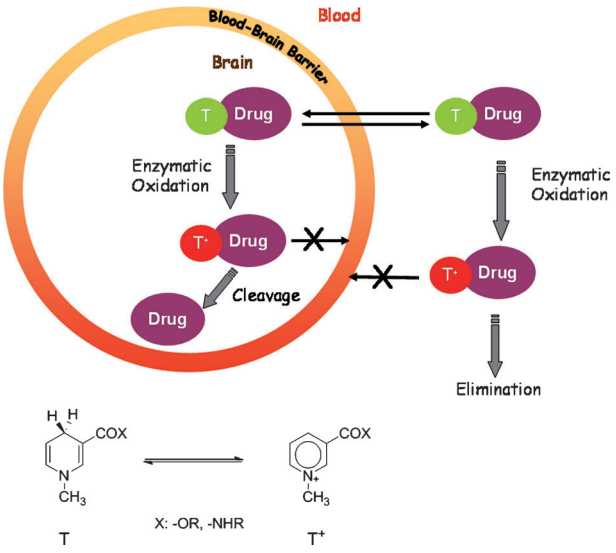

Figure 3. Brain targeting by the CDS approach.

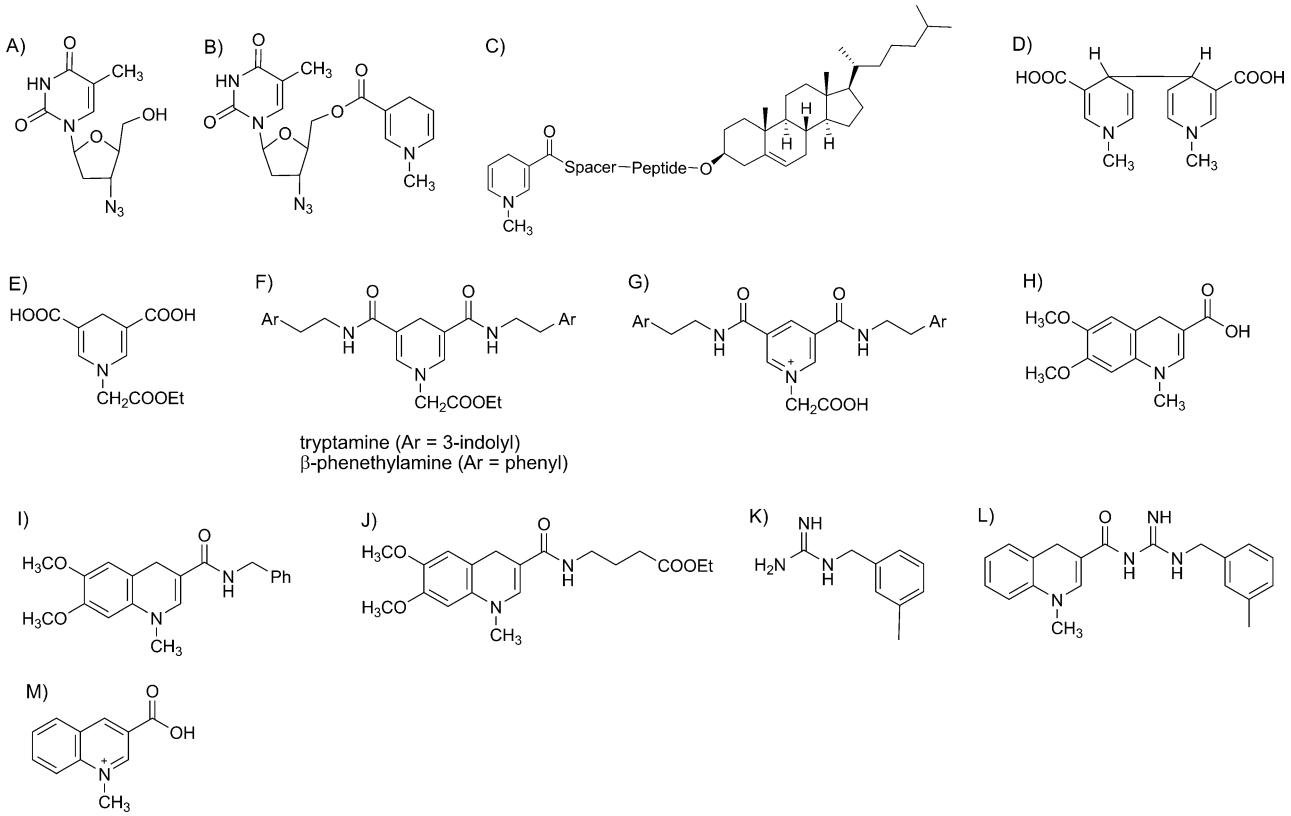

Figure 4. Chemical structures of drugs or shuttle-drug constructs for a chemical delivery system.

pin-releasing hormone), and kyotorphin analogues (Tyr-D-Ala-Gly-Phe-D-Leu, Gln-Leu-Pro-Gly and Tyr-Lys, respectively), to the brain. In this approach, a dihydropyridine (T) is conjugated to the N terminus of the peptide through a spacer amino acid (Pro or/and Ala) and a lipophilic cholesteryl ester is anchored to the C terminus (Figure 4C). The cholesteryl group is applied to increase the lipophilicity and to prevent degradation of the peptide by peptidases. The spacer also facilitates or controls the enzymatic cleavage of the peptides once inside the brain.[39–41] Dihydropyridines were also successfully used for the brain-specific delivery of several compounds, such as dopamine,[32] various estrogens,[42] and 4-aminobutanoic acid (GABA).[43]

The major disadvantage of the dihydropyridine, which seriously limits its further development, is its short shelf-life stability, caused by the susceptibility of the 5,6-double bond to undergo air oxidation and/or hydration. This oxidation/hydration reaction produces 6-hydroxy-1,4,5,6-tetrahydropyridine, which does not undergo enzymatic oxidation in vivo to give the corresponding quaternary pyridinium salt.[44,45]

To overcome this problem, several dihydropyridine derivatives with higher stability were developed. Carelli et al.[46] reported that tetrahydrobipyridine (Figure 4D) is more potent than dihydropyridine for the brain-specific delivery of dopamine. *N*-ethoxycarbonylmethyl-1,4-dihydropyridine-3,5-dicarboxylic acid (Figure 4E) coupled to β-phenethylamine and tryptamine as model drugs (Figure 4F) entered the brain following i.v. administration in rats. The corresponding quaternary acids (Figure 4G) were eliminated from the blood 2 h after injection; however, these compounds were locked in the brain for 5 h.[45] Foucout et al.[44] recently proposed a carboxylic 1,4-dihydroquinoline derivative (Figure 4H) as a novel CDS. They found that the amido 1,4-dihydroquinoline derivative (Figure 4I) did not produce the corresponding quaternary salt or any by-products in rat blood in vitro for up to 20 min; however, following i.v. injection, the amido derivative (Figure 4I) was detected in the brain and was fully converted into the corresponding quaternary salt after 5 min. In addition, the 1,4-dihydroquinoline derivative coupled to GABA (Figure 4J) demonstrated significant central GABAergic activity following intraperitoneal (i.p.) administration in mice, whereas GABA alone did not have this effect. These results confirm the capacity of the carboxylic 1,4-dihydroquinoline derivative (Figure 4H) to deliver drugs to the brain. MIBG (Figure 4K) is a guanidine derivative used as a tumor marker; however, it does not penetrate the BBB. In another study, the authors found that the conjugate of MIBG and a 1,4-dihydroquinoline moiety (Figure 4L) showed moderate penetration of the brain after i.v. injection in rats. While the concentration of the conjugate in the brain decreased within 60 min, the amount of the quaternary salt (Figure 4M) in the brain increased over the same period of time. This finding indicates the oxidation of the conjugate in the brain followed by enzymatic release of MIBG.[47]

3. Carrier-Mediated Transport

Substrates for endogenous BBB carriers can be used as shuttles to deliver small drugs to the brain. It was proposed that ascorbic acid (AA) enters the brain through sodium-dependent transporters SVCT2. A cell-based assay showed that nipecotic acid (Nip, Figure 5A) conjugated to AA (Figure 5B) inhibits [^{14}C]AA uptake in a concentration-dependent manner. In addition, i.p. injection of Nip-AA in mice produced an anticonvulsant effect, while Nip alone did not.[48]

Tyrosine, which enters the brain through the large natural amino acid transporter (LAT1) system, can act as a vector to deliver Nip to the brain. Nip was conjugated to tyrosine (Figure 5C) and systematic administration of this conjugate to mice caused significant dose-dependent anticonvulsant activity; however, Nip alone did not have this effect.[49] These observations suggest that Nip-AA and Nip-tyrosine conjugates can be actively transported across the BBB. Furthermore, Gynther et al.[50] found that ketoprofen conjugated to L-tyrosine (Figure 5D) is a substrate for LAT1 and that it crosses the BBB in rats by means of this transporter.

Glucose, which penetrates the brain through its transporter (GLUT-1) in the BBB, was used as a vector for the delivery of drugs to the brain. By means of in situ rat brain perfusion, Gynther et al.[51] showed that ketoprofen and indomethacin conjugated to glucose (Figure 5E and 5F) inhibited the uptake of glucose by the brain. This observation indicates that the two conjugates had a higher binding affinity to GLUT-1 than glucose. In addition, the conjugates crossed the BBB in a temperature-dependent manner, thus confirming that the uptake of these compounds in the brain is mediated by GLUT-1. Egleton et al.[52] demonstrated that glycosylation of Ser^6 of the linear opioid peptide amide Tyr-D-Thr-Gly-Phe-Leu-Ser-NH_2 produced greater analgesia with a longer duration after i.v. administration in rats compared to the nonglycosylated peptide. The improved analgesic activity of the glycosylated peptide was attributed to several factors, such as a 2-fold higher uptake in the brain (measured by in situ rat brain perfusion), and a 1.8-fold and a 1.5-fold higher stability in the plasma and brain, respectively. However, the authors concluded that the glycosylated peptide does not enter the brain through GLUT-1, and proposed that it may cross the BBB by the organic anion transporting peptide transporter. In another study, the authors found that the glycosylation of the cyclic opioid peptide [D-Cys^{2,5},Ser^6,Gly^7] enkephalin (Figure 5G) produced a greater analgesic response when the glucose was axial or equatorial to the peptide backbone. They also observed greater uptake in the brain, as shown by in situ perfusion in rats. However, the improvement in analgesia was due not only to the enhancement of BBB permeation but may in part be related to the binding affinity of the peptide to the μ-opioid receptor (the primary receptor for analgesia) in the brain, since one of the glycosylated peptides with lower uptake in the brain but higher binding affinity exhibited a greater analgesic effect.[53]

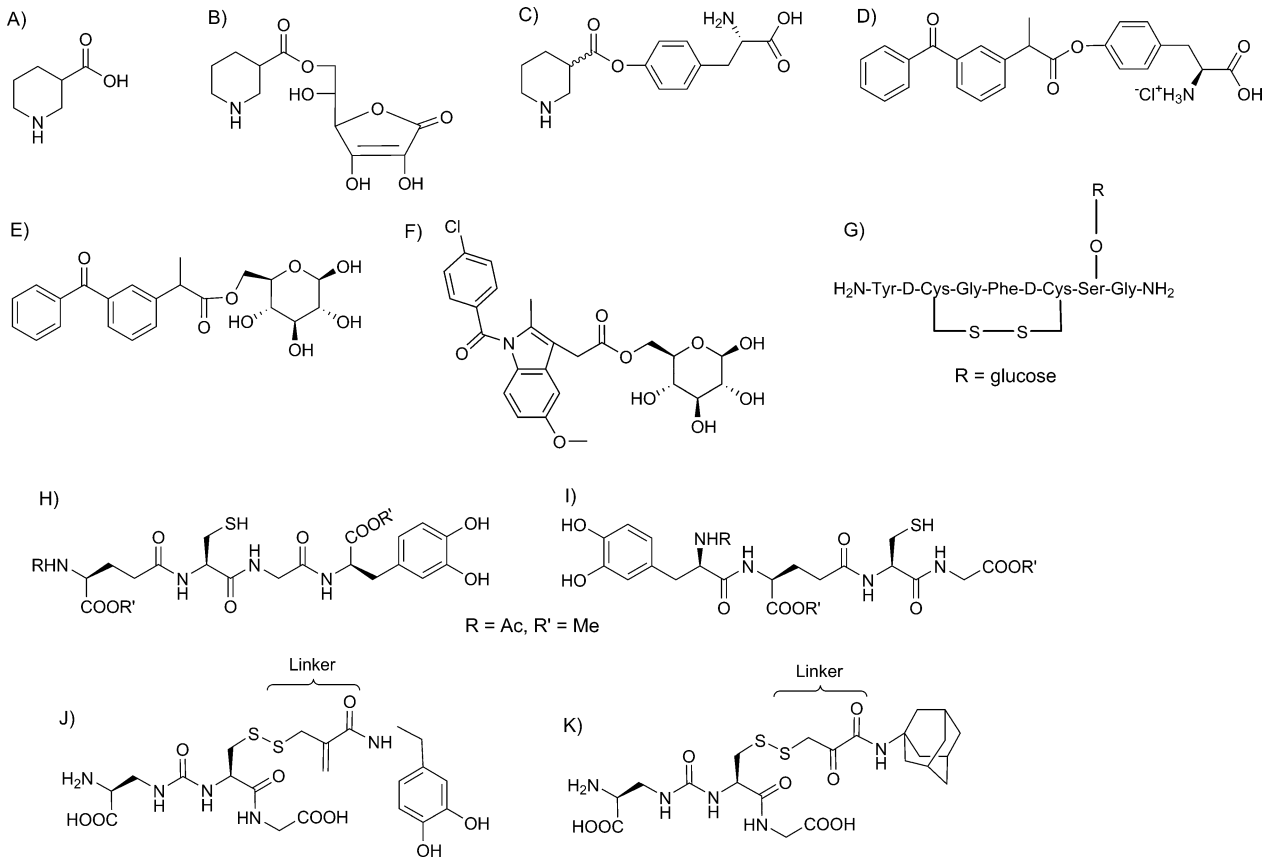

Figure 5. Chemical structures of drugs or shuttle-drug constructs for carrier-mediated transport.

The permeability of dendrimers conjugated to glucosamine (2-amino-2-deoxy-D-glucose), as measured by a cell-based BBB model, was 3.5-times greater than that of dendrimers. The internalization of glycosylated dendrimers in glioma cells was also enhanced up to eightfold compared to dendrimers. In addition, glycosylated dendrimers loaded with methotrexate, an anticancer agent, were 4.5-fold more effective in inhibiting glioma cell growth. These results indicate that glycosylation may have a dual role for drugs: by improving their BBB permeation and uptake by glioma cells. However, in vivo studies are required to confirm these findings.[14] It was also shown that glycosylated analogues of dermorphine[54] (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), deltorphin[54,55] (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2), and [Met^5]enkephaline[56] (Tyr-Gly-Gly-Phe-Met) produced greater analgesia in mice than the parent nonglycosylated peptides. The authors concluded that the increased analgesic response by glycosylated peptides is attributable to a higher blood to brain transport, which in turn may be attributed, at least partly, to GLUT-1. Since glucose transporters are expressed in all tissues, glycosylated compounds can thus be taken up by other organs.

Glutathione (GSH) is a tripeptide with an unusual peptide linkage between the amine group of the cysteine residue and the carboxy group of the glutamic acid side chain. GSH, which enters the brain by carrier-mediated transport, is known to protect proteins against oxidation. L-Dopa was attached to GSH through an amide bond (Figure 5H), and oral administration of the resulting compounds to rats resulted in sustained delivery of L-dopa and dopamine to the brain, thereby elevating the concentration of these two compounds in this organ with respect to an equimolar dose of dopamine.[57]

More and Vince[58] used a metabolically stable urea analogue of GSH as a carrier for the delivery of dopamine and adamantamine across MDCK (Madin-Darby canine kidney) cell monolayers. The carrier GSH analogue was coupled to dopamine and adamantamine through a linker, and the resulting molecules (Figure 5I and J) crossed the cell monolayer. The lower transport of GSH across MDCK cell monolayers in the presence of the conjugates showed that their transport mechanism was GSH-transporter-mediated and not passive diffusion.

Coupling a drug to an endogenous carrier substrate may affect the affinity of the endogenous substrate for its transporter; therefore, uptake of the drug in the brain can be affected by an unconjugated endogenous substrate that has a greater affinity.

4. Molecular Trojan Horses

Endogenous ligands for specific BBB receptors, also known as Trojan horses, have the capacity to shuttle drugs into the brain.[16] Vasoactive intestinal polypeptide (VIP) participates in the regulation of cerebral blood flow; however, in vivo studies showed no neuropharmacological effect as a result of low transport of peptide to the brain, which is attributable to the presence of the BBB. A VIP analogue (Ac-HSDAVFTDNYTRLRKQ-Nle-AVRRYLNSALN-NH_2, VIPa) was coupled to OX26 by means of the avidin–biotin system to increase uptake of the peptide in the brain (Figure 6). Biotin is a small natural vitamin (H or B_7) that binds with high affinity to avidin proteins. OX26 is a murine monoclonal antibody (MAb) that crosses the BBB by means of the transferrin receptor (TfR; Figure 7). Following systematic administration of the biotinylated VIPa conjugated to avidin-OX26 in rats, the conjugate was detected in the brain and resulted in a 65% increase in cerebral blood flow, whereas biotinylated VIPa alone did not cross the BBB and had no effect on this flow.[59]

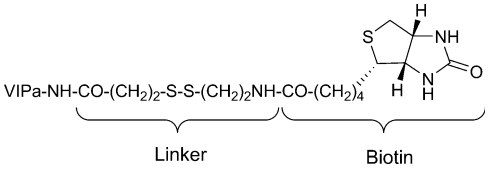

Figure 6. Chemical structure of biotinylated VIPa.

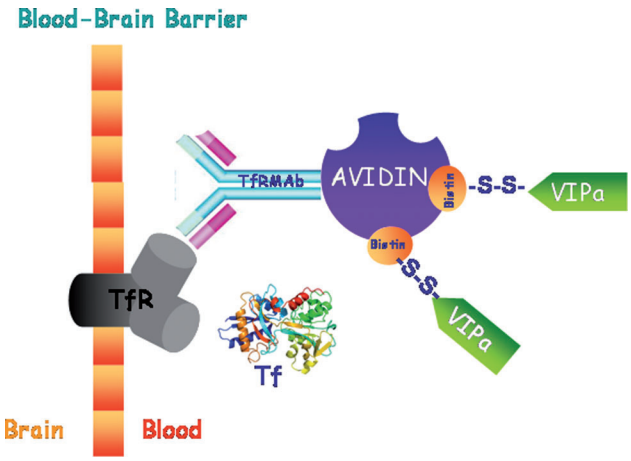

Figure 7. Delivery of biotinylated VIPa through the BBB by using a transport vector composed of a conjugate between avidin and the OX26 antitransferrin receptor monoclonal antibody (TfRMAb). Tf, circulating transferrin; TfR, BBB transferrin receptor.

Brain-derived neurotrophic factor (BDNF) is a potential neuropharmaceutical for the treatment of a variety of neurodegenerative disorders. However, BDNF—similar to other neurotrophins and protein-based therapeutics—does not undergo significant transport through the endothelial wall of brain capillaries. The uptake of biotin-PEG^{2000}-BDNF conjugated to OX26/streptavidin (SA) in the brain was seven- and twofold higher than that of biotin-PEG^{2000}-BDNF and morphine (a neuroactive molecule), respectively. It was hypothesized that this increased uptake in the brain was due to improvement of the BBB permeation through the transferrin receptor. In addition, the conjugation did not alter the biological activity of BDNF, as shown by in vitro assays.[60] OX26/SA has also been used to deliver VIPa to rat brain. Intravenous injection of the VIPa coupled to the OX26/SA vector in rats resulted in at least a tenfold increase in uptake

by the brain and an enhanced brain blood flow by 60%, while unconjugated VIPa did not cause any change in this flow.[61]

A variety of preclinical studies have demonstrated that the endogenous polypeptide human basic fibroblast growth factor (bFGF) has potent neuroprotective effects. Given that bFGF shows poor brain penetration, a high dose is required to produce neuroprotection in this organ. However, at high doses, bFGF causes peripheral side effects. The conjugation of monobiotinylated bFGF (bio-bFGF) to OX26/SA did not affect the binding affinity of bFGF for its receptor in cultures of BHK-21 cells. The bFGF-OX26 conjugate showed not only higher uptake in the brain but also decreased bFGF distribution in the peripheral tissues compared with bio-bFGF after i.v. injection in rats.[62]

Poly(ethyleneglycol)-poly(ε-caprolactone) (PEG-PCL) polymersomes (POs) conjugated to OX26 (designated OX26-PO) showed an uptake in the brain 2.6-fold that of the POs following i.v. administration in rats. NC-1900, a peptide fragmented from vasopressin, caused memory improvement when injected intracerebroventricularly in rats suffering from scopolamine-induced memory impairment. However, the ability of NC-1900 to cross the BBB is poor, thereby limiting its application. To study the ability of OX26-PO to deliver drugs to the brain, it was loaded with NC-1900. While NC-1900 and NC-1900-POs did not improve learning and memory deficits in rats with scopolamine-induced memory impairment, i.v. administration of OX26-PO-NC-1900 improved memory function in these animals.[63]

Radioactively labeled epidermal growth factor (EGF) peptide is a potential imaging agent for the detection of brain tumors. However, this peptide cannot be used alone for this purpose because of its inability to cross the BBB. The biotinylated peptide was conjugated to OX26/SA through bis(aminohexanoyl) [$(CH_2)_5NHCO(CH_2)_5NHCO$] as a linker to enhance the delivery of EGF to the brain. This conjugation resulted in no binding to the EGF receptor, as determined by in vitro assays. Replacement of the linker by poly(ethyleneglycol) (PEG) with a molecular mass of 3400 Da (PEG^{3400}) restored the EGF binding affinity.[64] In vivo studies revealed that the detection of brain tumors in rats is possible only when EGF is conjugated to OX26/SA through a PEG^{3400} spacer.[65]

Radiolabeled $A\beta^{1-40}$ (the first 40 amino acids of the β-peptide of Alzheimer's disease) can be used for imaging the Aβ amyloid fibrils in brains affected by Alzheimer's disease. However, ^{125}I-$A\beta^{1-40}$ does not cross the BBB in vivo.[66] Moreover, ^{125}I-$A\beta^{1-40}$ is rapidly removed from the blood by metabolism. Uptake of monobiotinylated ^{125}I-$A\beta^{1-40}$ conjugated to the OX26/SA in the brain after i.v. administration in rats was twofold higher than that of morphine, while ^{125}I-$A\beta^{1-40}$ showed negligible uptake in the brain. In addition, the conjugation enhanced the metabolic stability of the ^{125}I-$A\beta^{1-40}$ and did not affect its binding affinity to the amyloid plaques of Alzheimer's disease.[67] By using the same drug-delivery system, the uptake in the brain of a biotinylated polyamide nucleic acid (bio-PNA), a potential anti-HIV compound, increased 28-fold compared with unconjugated bio-PNA following i.v. injection in rats.[68]

Chitosan nanoparticles (NPs) did not gain access to the brain after i.v. injection in mice; however, chitosan NPs coupled to OX26 were detected in this organ, thereby indicating that OX26 enabled these particles to cross the BBB.[69] OX26 conjugated to lipid nanocapsules (LNCs) was still recognized by transferrin receptor in vitro; however, the conjugate (LNC-OX26) showed only a twofold higher accumulation in the brain than unconjugated LNC following i.v. injection in mice. The uptake of the conjugate in the brain did not increase significantly, possibly because of its rapid elimination from the blood by the liver.[70] OX26 also transported loperamide-loaded human serum albumin nanoparticles (HAS NPs) across the BBB after i.v. injection in mice and induced significant antinociceptive activity.[71]

Anti-Aβ antibodies prevent Aβ aggregation and disaggregate the Aβ amyloid fibrils of Alzheimer's disease. Thus, these antibodies can be considered a new approach for the treatment of this brain disorder.[72,73] It was shown that a recombinant mouse/rat chimeric MAb against the mouse transferrin receptor, namely cTfRMAb, crossed the BBB in mice.[74] Fusion of a single chain fragment variable (ScFv) anti-Aβ MAb to cTfRMAb enabled it to penetrate the BBB in mice following i.p. injection, while retaining its binding affinity to Aβ amyloid peptide.[75] Moreover, cTfRMAb delivered glial-derived neurotrophic factor (GDNF), a neurotrophin that, like BDNF, does not cross the BBB, to the brain after i.v. administration in mice. However, the fusion of GDNF to cTfRMAb did not affect the affinity of GDNF to its receptor.[76] The MAbs mentioned earlier (OX26 and cTfRMAb) cannot be used in humans because they do not interact with human BBB transferrin receptor.[77] This inability to recognize human BBB TfR may explain why OX26 and cTfRMAb have not been used in clinical trials for the delivery of drugs to the human brain. However, it was observed that a murine MAb is recognized by the human insulin receptor (HIR) and it crosses the BBB via this receptor. Therefore, the capacity of this HIRMAb as a shuttle was evaluated for the delivery of some drugs to the brain.

Boado et al.[77] showed that fusion of BDNF to the HIRMAb produced a fusion protein (BDNF-HIRMAb). The concentration of this protein in the brain following i.v. administration to Rhesus monkeys was >10-fold higher than the endogenous concentration of BDNF in this organ. Moreover, the fusion protein retained the binding affinity of BDNF for its receptor and also the neuroprotection activity of BDNF. Mucopolysaccharidosis Type I or Hurler's Syndrome is a lysosomal storage disorder that affects the brain. Treatment of this condition involves enzyme replacement therapy by using a recombinant enzyme. However, the enzyme required, α-L-iduronidase (IDUA), does not cross the BBB (Figure 8). The fusion of IDUA to the HIRMAb allowed the transfer of the enzyme across the BBB following i.v. injection in Rhesus monkeys, while HIRMAb-IDUA presented acceptable enzyme activity compared to IDUA.[78] ^{125}I-$A\beta^{1-40}$ was conjugated to the HIRMAb by using of SA-biotin technology without altering the binding affinity of ^{125}I-$A\beta^{1-40}$ to amyloid plaques of Alzheimer's disease in vitro. Intravenous injection of the conjugated ^{125}I-$A\beta^{1-40}$ in monkeys resulted in a 10-fold greater uptake than that of unconjugated peptide in the brain.[66]

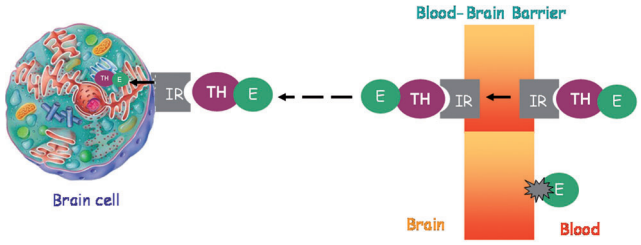

Figure 8. Delivery of a lysosomal enzyme (E) to the brain by using a chimeric MAb to the human insulin receptor (IR) as a molecular Trojan horse (TH). Alone, the lysosomal enzyme IDUA does not cross the BBB. However, following the fusion of the IDUA to the TH, the enzyme crosses the BBB and brain cell membrane, by trafficking on the IR, which is expressed in both membranes.

The ScFv anti-Aβ MAb was fused to HIRMAb and the resulting molecule was referred to as a fusion antibody. The anti-Aβ MAb did not cross the BBB in the Rhesus monkeys; however, the fusion antibody was distributed in the brain following i.v. administration. Moreover, the antibody disaggregated amyloid plaques in transgenic mouse brain.[79] The human tumor necrosis factor receptor (TNFR) may provide a potential treatment against inflammation in tissues. However, it cannot be used to treat brain disorders because it is unable to cross the BBB. TNFR fused to HIRMAb—called the HIRMAb-TNFR fusion protein—retained its binding affinity to HIR and TNFR activity, as measured in in vitro assays.[80] Moreover, the uptake of this fusion protein by the brain of adult Rhesus monkeys was 13-fold greater than TNFR following i.v. administration.[81]

In addition to being expressed in the brain, HIR is found in other organs. Thus, the drug coupled to HIRMAb may be taken up by these tissues and produce side effects.[5]

The poor BBB permeation of quantum dots (QDs) limits their application as imaging agents for the diagnosis and treatment of brain disorders. To deliver QDs to the brain, they were encapsulated in the core of PEG-poly(lactic acid) (PEG-PLA) NPs decorated with wheat germ agglutinin (WGA). Following intranasal administration in mice, the conjugates were distributed in body tissues; however, a greater distribution was found in the brain.[82] The conjugation of transferrin (Tf) to quantum rods also enabled them to transport across an in vitro BBB model via the transferrin receptor.[83] Lactoferrin (Lf) is a protein of the transferrin family that acts as a transporter through the BBB via the Lf receptor. The conjugation of Lf to PEG-PLA NPs enhanced the accumulation of NPs in the brain after i.v. injection in mice. Furthermore, the Lf-NPs did not show any toxicity against mouse brain endothelial cells in an in vitro assay.[84]

5. Colloidal Carriers

Colloidal drug carriers such as liposomes and nanoparticles have been applied for the delivery of drugs to the brain. Colloidal particles are easily eliminated from the blood by the liver and the spleen. Modification of colloidal particles by PEG can increase their stability in the blood. In addition, to improve the uptake of these particles in the brain, they can be attached to specific transporters such as monoclonal antibodies and transferrin.[85,86]

5.1. Nanoparticles

The use of NPs for the delivery of drugs to the brain is an interesting approach because these particles can carry a wide range of drugs to the CNS and release them in a sustained manner. The small size of NPs (200 nm) allows them to be taken up by cells. Materials such as poly(alkylcyanoacrylates) (PACAs), polyacetates, polysaccharides, and copolymers can be used for the preparation of NPs. Drugs can be entrapped, adsorbed, or attached to NPs. Poly(*n*-butylcyanoacrylate) (PBCA) NPs coated with polysorbate 80 (surfactant) have been widely used to deliver drugs to the CNS which otherwise cannot cross the BBB.[85,87]

To deliver the nonpenetrating enkephalin analogue dalargin[88] to the brain, Kreuter et al.[89] bound the drug to PBCA NPs coated with polysorbate 80. After i.v. injection into mice, NPs coated with polysorbate 80 were found in the brain. An analgesic response was also observed, as measured by using the tail-flick test. However, a control consisting of the three components (drug, NPs, and surfactant) mixed directly before i.v. injection showed no analgesic effect.

Olivier et al.[90] found that addition of polysorbate 80 to PBCA NPs loaded with dalargine led to complete desorption of dalargine from the NPs. These authors therefore considered dalargine-loaded polysorbate 80-coated PBCA NPs as a simple mixture. In addition, they observed that PBCA NPs coated with polysorbate 80 or uncoated opened the tight junctions of an in vitro BBB model. They also reported that administration of PBCA NPs coated with polysorbate 80 in mice caused occasional mortality. Furthermore, these NPs induced an analgesic effect in these animals. Therefore, it was proposed that this analgesic response was related to the opening of the tight junction as a result of the toxicity of the NPs. In contrast, Kreuter et al.[91] found that i.v. injection of neither free dalargine followed by injection of PBCA NPs coated with polysorbate 80 after 5 and 30 min nor a mixture of dalargine and PBCA NPs coated with polysorbate 80 produced analgesia. However, dalargine bound to PBCA NPs coated with polysorbate 80 showed strong antinociceptive activity following i.v. injection into mice. Furthermore, examination of bovine brain capillary endothelial cells (BBCEs) incubated with uncoated PBCA NPs and those coated with polysorbate 80 by electron microscopy showed no abnormality in the cell morphology or tight junctions. These results thus provide evidence that PBCA NPs coated with polysorbate 80 do not disturb the integrity of the BBB.

Intravenous administration of PBCA NPs coated with polysorbate 80 and loaded with the NMDA (*N*-methyl-D-aspartate) receptor antagonist MRZ 2/576 (8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]quinoline-5-oxide choline salt) in mice produced a longer anticonvulsive response compared to free MRZ 2/576. These results indicate that PBCA NPs coated with polysorbate 80 carried MRZ 2/576 into the brain and released it in a sustained manner.[92] It

was observed that the concentration of tacrine (Figure 9A) and rivastigmine (Figure 9B), both anti-Alzheimer's drugs, in rats brain increased fourfold when loaded on these NPs.[87,93]

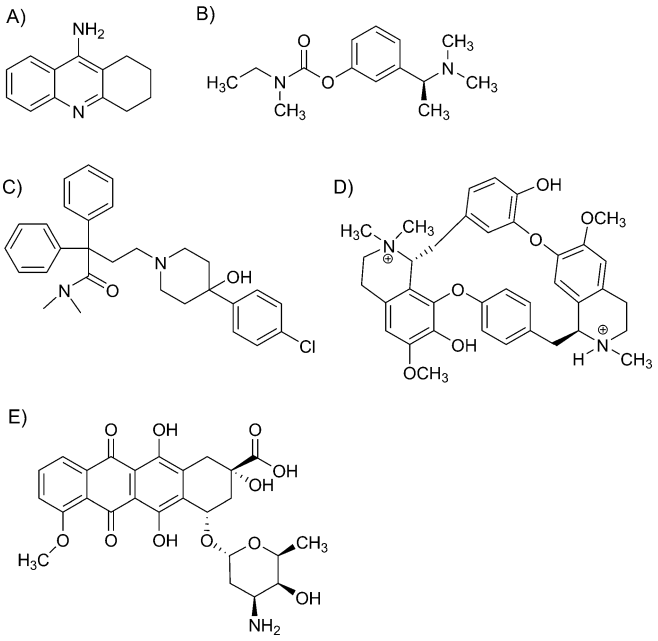

Figure 9. Chemical structure of drugs for colloidal carriers.

These NPs also delivered doxorubicin to rat brains after i.v. injection, while doxorubicin alone did not have the capacity to cross the BBB.[94] Other studies showed that these particles transported loperamide (Figure 9C) and tubocurarine (a quaternary ammonium salt, Figure 9D), which do not cross the BBB, into the brain after i.v. injection in mice and rats, respectively.[95,96]

Given that PBCA NPs, even when coated with polysorbate 80, are easily captured by the liver, spleen, and lungs, their long-term administration may be toxic to these organs. In addition, further studies are required to examine the effect of PBCA NPs on the brain because rapid degradation of the PBCA polymer can release toxic compounds.[90]

Of the various mechanisms proposed to explain the permeation of NPs coated with polysorbate 80 across the BBB, endocytosis appears to be the most relevant. These NPs adsorb apolipoproteins B and E from the blood after injection and thus mimic lipoprotein particles that could be taken up by the brain capillary endothelial cells through receptor-mediated endocytosis.[97] This mechanism is supported by studies showing that NPs made of human serum albumin (HSA) covalently bound to apolipoprotein E (ApoE) were detected in mice brains after i.v. injection, but pegylated albumin NPs were not observed in the brain.[98] Furthermore, loperamide-loaded HSA-NPs coupled to ApoE3 through an avidin/biotin linker induced an antinociceptive response in mice after i.v. injection. In contrast, loperamide-loaded HSA-NPs and free loperamide did not have a significant analgesic effect.[99] In addition, HSA-NPs covalently attached to ApoE3, A-I, and B-100 through a PEG linker delivered loperamide to the mouse brain.[100] In vitro assays demonstrated that at concentrations ranging from 0.1 to 2 $mg\,mL^{-1}$ neither HSA-NPs nor HSA-NPs-ApoE had a toxic effect on mouse brain endothelial cells.[98]

It was found that cationic bovine serum albumin (CBSA) conjugated to PEG-PLA NPs (CBSA-NPs) crossed the BBB in vivo more than native bovine serum albumin (BSA) conjugated to PEG-PLA NPs.[101] NC-1900 loaded in CBSA-NPs was fourfold more stable in plasma than NC-1900. Moreover, NC-1900-loaded CBSA-NPs produced a significant improvement in memory following i.v. administration in mice, while NC-1900 did not have this effect.[102] In addition, cationic human serum albumin (CHSA) carried leu-enkephalin across the mouse BBB following i.p. administration and produced analgesic effects, as evaluated by the tail-flick test. In contrast, noncationic HSA-Fmoc-Leu-enkephaline did not show antinociceptive effects.[103] PLA polymers with low toxicity are considered safe for human use. Results of MTT assays on brain capillary endothelial cells (BCECs) revealed that 80% cell viability was obtained using 0.1, 1, and 1.5 $mg\,mL^{-1}$ of CBSA, CBSA-NPs, and NPs respectively. In contrast, viability dropped sharply to 50% with 1 $mg\,mL^{-1}$ CBSA. These results indicate that although CBSA had a toxic effect on the cells, its toxicity decreased when coupled to NPs.[101]

Poly(D,L-lactide-*co*-glycolide) NPs (PLGA-NPs) did not penetrate the brain in mice; however, when trimethylated chitosan (TMC) was covalently coupled to these NPs, the resulting molecules (TMC/PLGA-NPs) were taken up by this organ. Moreover, injection of coenzyme Q_{10} (Co-Q_{10}) entrapped in TMC/PLGA-NPs in a transgenic mouse model induced a decrease in memory impairment and inhibited the deposition of β-amyloid fibrils in the brain. In contrast, free Q_{10} and Q_{10}-loaded PLGA did not significantly reduce memory deficits. Since TMC is a cationic polysaccharide, the authors concluded that it crosses the BBB through adsorptive-mediated endocytosis. However, TMC, PLGA-NPs, and TMC/PGLA-NPs were slightly toxic against cells, as shown by MTT assays.[104] Hombach and Bernkop-Schnürch[105] reported that MDCK cells treated with chitosan solution or chitosan particles exhibited significantly lower transepithelial electrical resistance (TEER) compared to untreated cells. The reduced TEER can be due to chitosan solution or particles causing the opening of tight junctions. Moreover, although the chitosan solution demonstrated low toxicity against MDCK cells and red blood cell lysis, chitosan particles showed much higher toxicity.

Following i.v. injection of doxorubicin-loaded chitosan-poly(acrylic acid) (PAA) hollow nanospheres into mice, doxorubicin was detected in several organs, including the brain, while i.v. injection of free doxorubicin resulted in the biodistribution of doxorubicin in organs other than the brain. Treatment of cells with chitosan at concentrations higher than 0.1 $mg\,mL^{-1}$ led to cell viability around 60%; however, in contrast, chitosan-PAA nanospheres did not have a toxic effect.[106]

5.2. Liposomes

Liposomes are small vesicles composed of biocompatible and biodegradable phospholipid bilayers surrounding aqueous compartments.[85] Liposomes loaded with daunorubicin, an anticancer drug, were conjugated to transferrin and also to *p*-aminophenyl-α-D-mannopyranoside (MAN), which crosses the BBB by means of GLUT-1. Transport studies across murine brain microvascular endothelial cells (BMVECs) revealed that these conjugates were more permeating than free daunorubicin, daunorubicin liposomes, and daunorubicin liposomes coupled to MAN or transferrin. Their i.v. administration to rats bearing C6 glioma cells confirmed in vitro results, while the length of survival of rats treated with free daunorubicin (19 days), daunorubicin liposomes (18 days), daunorubicin liposomes/MAN (18 days), and daunorubicin liposomes/transferrin (18 days) was not significantly different. However, rats injected with daunorubicin liposomes conjugated to both MAN and transferrin survived longer (22 days).[107] Similar results were obtained when epirubicin-loaded liposomes were functionalized with tamoxifen (TAM) and transferrin (Tf). Liposomes loaded with epirubicin, a potential anticancer agent derived from doxorubicin, were functionalized with tamoxifen and transferrin. TAM was introduced into liposomal bilayers because it was considered to have the ability to inhibit the efflux of epirubicin from the brain and brain tumor cells. Liposomes modified with TAM and Tf were more effective carriers for epirubicin across rat-brain microvascular endothelial cells (BMEC) than unmodified liposomes and liposomes modified with TAM or Tf. Results from in vivo studies were consistent with those from in vitro experiments. Rats with brain glioma that were administered epirubicin-loaded liposomes modified with TAM and Tf survived longer (23 days) than those given epirubicin-loaded liposomes modified with TAM (18 days) or Tf (19 days), epirubicin-loaded unmodified liposomes (17 days), and free epirubicin (15 days). Moreover, epirubicin-loaded liposomes modified with TAM and Tf had the strongest inhibitory effect on tumor volume. These findings suggest that liposome modification with TAM and Tf not only improved the concentration of epirubicin in the brain but also targeted the drug to the tumor cells.[108]

The low permeability of nerve growth factor (NGF) across the BBB limits its clinical application. Encapsulation of NGF in liposomes improved its capacity to cross an in vitro model of the BBB and the BBB in rats in vivo. However, higher in vitro and in vivo permeability was obtained when liposomes were chemically conjugated to RMP-7 or physically mixed with this compound. RMP-7, a ligand for the B_2 receptor on the BBB, is an analogue of an endogenous peptide named bradykinin. It has been reported that RMP-7 improves uptake of drugs in the brain through opening of the tight junctions. It was thus considered an invasive approach and phase III clinical trials with this drug were abandoned. However, in this study, the authors found that while liposomes carrying NGF physically mixed with RMP-7 caused 33% mortality in rats after 14 days, rats administered NGF liposomes conjugated to RMP-7 showed no mortality.[109]

Encapsulation of topotecan, a potential antitumor compound, by liposomes slightly improved its permeation across BMVECs. However, topotecan liposomes with TAM incorporated into their lipid bilayer membrane and conjugated to WGA showed a greater ability to cross BMVECs than did topotecan liposomes. Moreover, i.v. administration of these liposomes into rats with brain tumors showed that those modified with TAM and WGA enhanced rat survival by six days compared to rats administered topotecan liposomes alone. The authors concluded that improved delivery to the brain is related to inhibition of efflux in the BBB with TAM and enhanced BBB transport with WGA through adsorptive-mediated endocytosis.[110]

Delivery of low levels of free daunomycin, a drug used in chemotherapy (Figure 9E), and liposomes to the brain is related to their rapid clearance from blood. Pegylation of the liposome reduced its plasma clearance significantly; however, this effect was at the expense of abolishing its uptake by the brain. In contrast, coupling of OX26 to PEG liposomes caused a fivefold increase in plasma clearance. However, PEG liposomes conjugated to OX26 was delivered at higher levels to the brain than free daunomycin, liposomes, and PEG-conjugated liposomes.[111]

Procationic liposomes (PCLs) are more stable than cationic liposomes (CLs) in blood because they are neutral or negatively charged at physiological pH values; however, PCLs can be converted into CLs by enzymes of BCECs and thus cross the BBB by adsorptive-mediated endocytosis. The permeability coefficient of PCLs loaded with coumarin-6, a fluorescent dye, across BCECs was 6.8-times greater than that of conventional liposomes loaded with coumarin-6, whereas PCLs did not increase the concentration of coumarin-6 in the brain compared to conventional liposomes after i.v. administration into mice. To obtain higher levels of coumarin-6 uptake in the brain, PCLs were modified with Lf. However, Lf-modified PCLs enhanced only the uptake of coumarin-6 2.3-fold, which cannot be considered an effective delivery of the drug to the brain. The authors hypothesized that the increased uptake of Lf-modified PCLs in the brain was related to its unidirectional transfer with Lf receptors on the BBB; in addition, PCLs that were brought into contact with BCECs by means of Lf were converted into positively charged liposomes and taken up by adsorptive-mediated endocytosis. In addition, toxicity studies with MTT assays showed more than 80% viability of BCECs treated with PLCs or Lf-PLCs, with the total lipid concentration ranging from 40 to 320 μM. This viability was considerably higher than with CLs.[112]

It is believed that the BBB is permeable to phagocytic cells of the immune system, such as monocytes and neutrophils. Therefore, these phagocytic cells are candidates as carriers for the delivery of drugs to the brain. Since liposomes are recognized and phagocytized by these cells in the circulating blood, the drug of choice can thus be encapsulated in liposomes. However, the drug should be stable in the lysosome of these cells, to be released from them once inside the brain. Serotonin (5-hydroxytryptamine) is a neurotransmitter that does not penetrate the brain. When serotonin was encapsulated in liposomes, its concentration in the brain was

twofold higher than that of free drug in solution following i.v. injection in rats. Incubation of liposomes with isolated human monocytes and neutrophils resulted in the accumulation of liposomes within these cells; in addition, liposomes were detected in circulating monocytes and neutrophils in blood after i.v. injection in rabbits. However, since i.v. injection of alendronate liposomes in rabbits increased the number of neutrophils and depleted circulating monocytes, which was associated with no uptake in the brain, the authors proposed that the enhancement of uptake of serotonin in the brain is related to liposome transport into the brain mainly by monocytes. Since the uptake of serotonin in the brain increased only twofold, the clinical application of this approach is limited. However, increased transport of immune cells through the BBB in several brain diseases, such as multiple sclerosis and Alzheimer's disease, has been reported. Therefore, monocytes and neutrophils may be more effective in brain-associated inflammatory disorders.[113] Ferulic acid (4-hydroxy-3-methoxycinnamic, FA) is an important active compound of traditional Chinese medicine that has been used for the treatment of neurovascular and cardiovascular diseases. However, application of FA for brain disorders is restricted due to its poor uptake in the brain. Intravenous injection of FA-loaded liposomes in rats with brain inflammation resulted in a twofold higher concentration of FA in the brain compared to the injection of FA solution. It also led to a significantly higher concentration of FA in the liver and spleen. When liposome-loaded FA was coated with RGD peptide (Arg-Gly-Asp), the FA concentration in the brain increased up to three- and sixfold of that of uncoated liposome-loaded FA and FA solution, respectively. In contrast, while the FA concentration in the liver and spleen was reduced compared to uncoated liposome-loaded FA, it was still higher than that of FA solution. In addition, in vitro studies showed a higher uptake of RGD-coated liposomes than uncoated liposomes by monocytes and neutrophils. These results indicate that RGD-coated liposomes and uncoated liposomes loaded with FA are delivered to the brain by these phagocytic cells. The higher uptake in the brain of FA loaded into RGD-coated liposomes can be attributed to the RGD peptide being a substrate for receptors present on monocytes and neutrophils, thus facilitating the endocytosis of RGD-coated liposomes by these cells.[114]

Since a single liposome can carry $>10\,000$ drug molecules, the use of these vesicles coupled to specific transporters increases the drug-carrying capacity of these transporters by up to four orders of magnitude. However, since liposomal formulations can lose the entrapped drug, they should be administered shortly after being produced. In addition, although several liposomal formulations have been approved by the US Food and Drug Administration (FDA), none of them target the brain.[86,111,115]

6. Peptide-Vector-Mediated Strategy

The other approach for the delivery of neuropharmaceuticals is the use of small naturally derived peptides that cross cellular membranes efficiently, for example, pegelin and penetratin peptides (18 and 16 amino acids, respectively). SynB1 and pegelin (RGGRLSYSRRRFSTSTGR; molecular mass 2099 Da) are derived from natural peptides called protegrins. They have an amphipathic structure in which the positively charged and hydrophobic residues are separated in the sequence. Replacement of the four cysteine residues with serine residues leads to linear peptides (pegelin). Penetratin peptides (such as D-penetratin (rqikiwfqnrrmkwkk, the amino acids are in the D form; molecular mass 2245 Da) are derived from the transcription factor antennapedia. The potential of this approach as an effective delivery system for transporting drugs across the BBB has been demonstrated in animal models.[29]

Rousselle et al.[29] used SynB1 and D-penetratin for drug delivery into the brain. As a model system they coupled the anticancer agent doxorubicin to these peptides through a chemical linker (Figure 10A and B) and studied its ability to cross the BBB by using the in situ rat brain perfusion technique. The uptake of doxorubicin in the brain is normally very low because of efficient efflux by the P-glycoprotein (P-gp) pump within the BBB. However, vectorization of doxorubicin led to a 20-fold increase in the amount transported into brain parenchyma. These vectors are non-invasive, as no opening of the tight junctions was observed. This enhancement in the uptake in the brain was also observed after i.v. administration to mice. In addition, free doxorubicin was accumulated at higher concentrations in tissues such as heart, lungs, and kidney than in the brain, which could cause several side effects. However, the uptake of vectorized doxorubicin was lower in these tissues compared to the brain and consequently may reduce side effects associated with the administration of this drug.

The capacity of SynB1 to deliver the antibiotic benzylpenicillin (B-Pc) to the brain was evaluated by in situ rat brain perfusion. The efficiency of β-lactam antibiotics for the treatment of CNS infections is limited because of their poor penetration across the BBB. Uptake of B-Pc in the brain was improved greatly following coupling to the SynB1 vector through a glycolamidic ester linker (Figure 10C). This finding thus showed the efficiency of SynB1 as a potential transporter for the delivery of drugs to the brain.[116]

It was found by using in situ rat brain perfusion that dalargin was taken up very poorly by the brain; however, uptake by this organ improved considerably when this drug was conjugated to SynB1 or SynB3 (RRLSYSRRRF, molecular mass 1395 Da) through a disulfide linker (Figure 10D). The analgesic effect was evaluated by the hot-plate test following administration of free or vectorized dalargin. While the former produced little analgesic activity, the conjugation of dalargin to SynB1 and SynB3 led to a considerable enhancement of the analgesic activity immediately after i.v. injection.[117]

Intracerebroventricular injection of M6G, an active metabolite of morphine, resulted in a 100-fold higher analgesic response than morphine. However, since the capacity of M6G to cross the BBB is lower than that of morphine, its systemic administration showed similar antinociceptive activity to morphine. Temsamani et al.[118] demonstrated that the conjugation of M6G to SynB3 through a

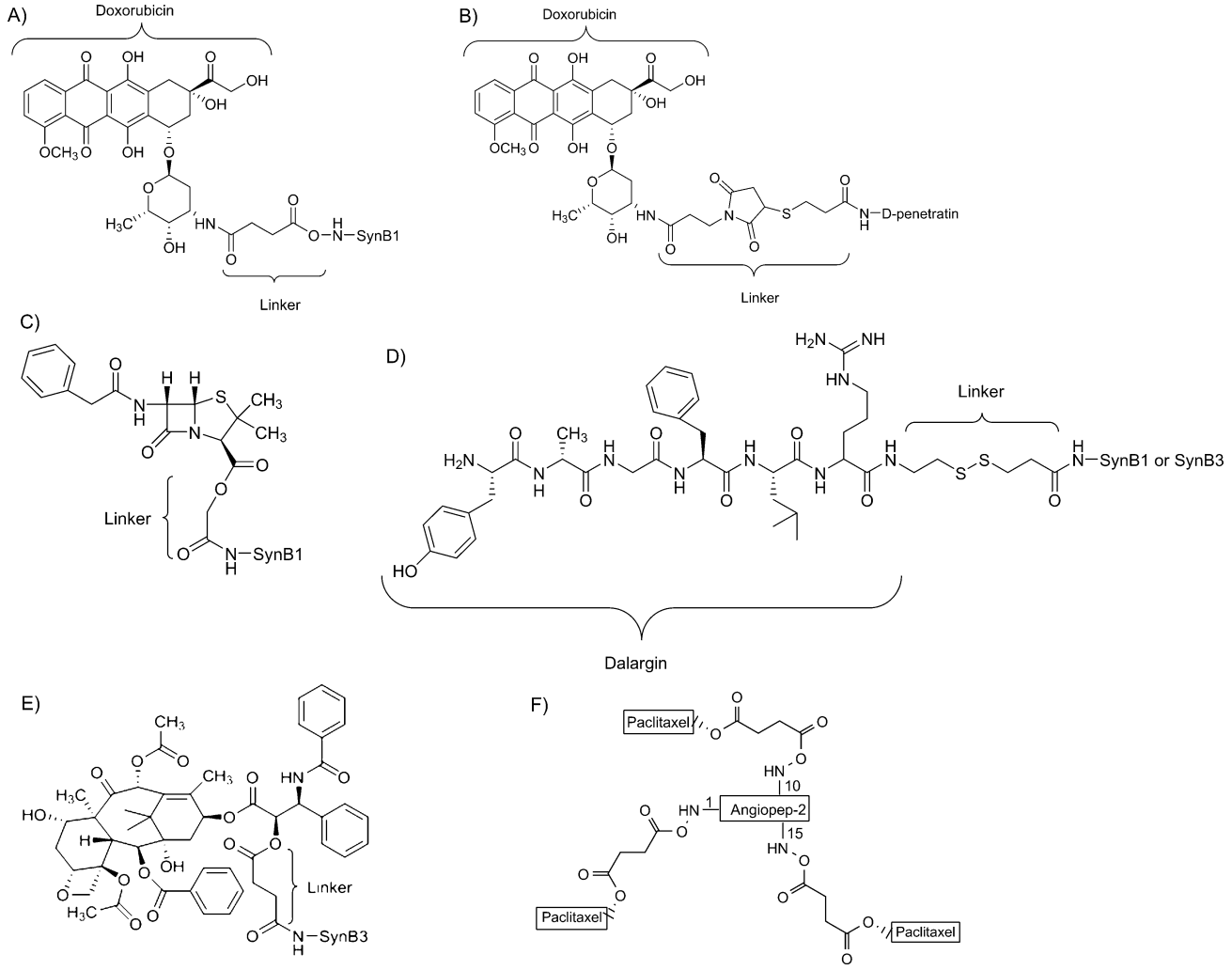

Figure 10. Chemical structure of drug-shuttle constructs for the peptide-vector-mediated strategy.

disulfide linker increased the uptake of M6G in the brain significantly, as measured by in situ rat brain perfusion. The conjugation also improved the analgesic activity of M6G, as evaluated by the tail-flick assay and the hot-plate test, thus confirming enhanced uptake of vectorized M6G in the brain. Given that conjugated M6G showed higher affinity to the μ receptors in vitro than did M6G, free M6G does not need to be released from the conjugate to produce its pharmacological activity once inside the brain.

The anticancer drug paclitaxel (PAX) has been used to treat several kinds of cancer, including malignant glioma and brain metastases. However, PAX shows a poor ability to cross the BBB, probably because it is a substrate for P-gp, and therefore its use in treatments for brain cancer is limited. To enhance the entry of PAX to the brain, Blanc et al.[30] coupled it to SynB3 by a succinate linker (Figure 10E) and studied its uptake in the brain by using the in situ mouse brain perfusion method. Delivery of conjugated PAX in the brain was enhanced 27-fold compared to free PAX, while conjugated PAX restored the in vitro PAX cytotoxicity. The conjugate bypassed the P-gp, which may explain its higher concentration in the brain. PAX is administered with an excipient (cremophor EL) to improve its aqueous solubility. The coupling of PAX with SynB3 also improved its solubility more than a 1000-fold compared to free PAX. This enhanced solubility may remove the need for the excipient and thus reduce the side effects associated with PAX. The mechanism by which vectorized B-Pc, PAX, M6G, and dalargin conjugates penetrate the brain remains unclear. However, it was shown that vectorized doxorubicin with SynB1 and SynB3 enter this organ by a mechanism involving adsorptive-mediated endocytosis.[119]

An 11-amino acid peptide from HIV trans-activating transcriptor (TAT) protein (called TAT peptide) was fused to the 116-KD β-galactosidase (β-Gal) protein. Following i.p. injection of the TAT-β-Gal fusion protein in mice, tissues such as liver, kidney, heart, lung, and brain exhibited strong β-Gal activity, while no activity was observed in the brain when β-Gal alone was injected. These results demonstrated the ability of the TAT peptide to deliver β-Gal to the brain without affecting the integrity of the BBB.[120] Ritonavir is one of the drugs approved by the FDA for use in the treatment of HIV infection. However, ritonavir is recognized by P-gp, which prevents it from entering the brain. Encapsulation of ritonavir

in either poly(L-lactide) NPs or TAT-conjugated NPs bypassed the efflux action of P-gp and enhanced its permeability in MDCK-MDR1 and MDCK-wt (wt = wild-type) cell lines. Furthermore, after two weeks of treatment with ritonavir, mice brain showed an 800-fold higher concentration of this drug with TAT-conjugated NPs than free ritonavir and about a 7-fold higher concentration than the unconjugated NPs. TAT-conjugated NPs not only overcame the BBB but also enhanced the uptake and sustained the retention of the drug in the CNS. All these features are critical for the therapeutic efficacy of anti-retroviral drug therapy.[36]

Polyester poly(D,L-lactide-*co*-glycolide) (PLGA) NPs are unable to cross the BBB; however, PLGA conjugated with a glycosylated heptapeptide H_2N-Gly-L-Phe-D-Thr-Gly-L-Phe-L-Leu-L-Ser(O-β-D-Glucose)-$CONH_2$ (g7) crosses the BBB in rats.[121] In one study, g7-NPs were loaded with loperamide to assess their ability to deliver drugs to the CNS. The nociceptive studies by hot-plate tests demonstrated that the i.v. administration of loperamide-loaded g7-NPs produced an analgesic action in rats as a result of CNS opioid activity mediated by loperamide. These authors proposed that this drug, once embedded into NPs, was transferred across the BBB and released from these particles. In contrast, control groups, including loperamide solution or g7-NPs, were ineffective. The authors hypothesized an adsorptive-mediated endocytosis mechanism to explain the crossing of g7-NPs through the BBB. Furthermore, biodistribution studies of rhodamine-123-loaded g7-NPs confirmed the localization of g7-NPs in the CNS.[122,123]

Aprotinin, a 6500 Da protein, shows a greater ability to cross the BBB greater than transferrin. By using the in situ brain perfusion method Demeule et al.[124] found that a 19-amino acid peptide, called Angiopep-2, derived from the kunitz domain of aprotinin has a greater ability to penetrate the brain than does aprotinin. The authors concluded that Angiopep-2 crosses the BBB through receptor-mediated endocytosis. Next, they tested the ability of this peptide to shuttle drugs into the brain. PAX was conjugated to the peptide (Figure 10F), named ANG1005, and its uptake in the brain was measured by the in situ brain perfusion method. Uptake of ANG1005 in the brain was fivefold greater than that of free PAX. Given that each molecule of ANG1005 bears three molecules of PAX, the uptake of PAX in the brain may increase up to 15-fold. Moreover, this conjugate bypassed P-gp at the BBB, produced reasonable cytotoxicity against the glioblastoma cell line compared to PAX, and prolonged the survival of mice with intracerebral tumors.[125] Another study by Thomas et al.,[126] in which the in situ rat brain perfusion method was used, revealed that the delivery of ANG1005 to the brain was 10-fold higher than that of free PAX. Penetration of doxorubicin and etoposide, an anticancer drug, in the brains of mice was enhanced 2- and 13-fold respectively, when conjugated to Angiopep-2. These conjugates were not substrates for efflux by P-gp, but exhibited similar cytotoxicity in vitro as unconjugated doxorubicin and etoposide.[127]

One limitation of using peptides as drug carriers is their instability in blood. In addition, the peptides discussed above may cross the BBB by adsorptive-mediated endocytosis, which can become saturated. To overcome these problems, one option could be to use more-stable *N*-methylated peptides that can enter the brain by nonsaturable passive diffusion and shuttle small drugs to this organ. Giralt and coworkers recently found that small cyclic *N*-methylated and *N*-MePhe-rich peptides can carry small drugs, such as dopamine, baicalin, levodopa, Nip, GABA, and 5-aminolevulinic acid (ALA), in in vitro BBB models such as the parallel artificial membrane permeability assay (PAMPA) and bovine brain microvessel endothelial cells (BBMECs). The drugs tested cover a wide range of structures, including linear, cyclic, and aromatic compounds. These drugs, which are used for distinct brain disorders, show poor ability to cross the BBB. While these molecules alone do not penetrate the PAMPA barrier, the peptides mentioned above do carry them across this artificial membrane and also through BBMECs.[128–130]

7. Summary and Outlook

The drugs found by CNS drug discovery programs need to overcome several hurdles to be effective to treat brain disorders. They must have low metabolism and protein binding in plasma, be able to cross the BBB to reach their site of action (BBB permeation), and distribute sufficiently in the brain as free (unbound) compounds. Therefore, a suitable brain drug-delivery approach is required to convert these drugs into efficacious and efficient therapeutic agents. In this Review we have discussed a number of strategies that have been applied to increase the BBB permeation of drugs. The shuttle-mediated strategy, among others, is an interesting and promising approach that has attracted the greatest attention and has been evaluated for several drugs. The shuttles are compounds that can penetrate the BBB and thus can carry drugs into the brain. This approach can be subdivided into several methods depending on the type of shuttle. 1) Chemical delivery system: dihydropyridine and its derivatives can be used as shuttles for selective drug delivery to the brain; 2) carrier-mediated transport: substrates for carriers present on the BBB, such as large natural amino acid transporters or glucose transporters, can deliver drugs to the brain; 3) molecular Trojan horses: endogenous compounds, for example, antibodies or proteins that are ligands for receptors expressed on the BBB, can deliver large molecules to the brain; 4) nanoparticle vectors: NPs can carry a large number of drug molecules and release them in a slow and sustained manner; 5) liposomes: the combination of liposomes with some specific BBB transporters can provide an approach to deliver drugs to the brain with high drug-carrying capacity; 6) peptide shuttle-mediated transport: many peptides enter the brain and can, therefore, be considered novel and promising shuttles.

The use of shuttles to deliver drugs to the brain may provide a suitable solution to the obstacle posed by the BBB. However, one of the concerns that should be addressed when designing new shuttle-mediated methods is the linkage between the drugs and shuttles. Once inside the brain, shuttle-bound drugs must be released to exert their biological activity; therefore, the linkage has to be stable in circulating

blood but cleavable inside the brain. In addition, drugs must retain their affinity for target receptors after cleavage. However, in some cases shuttle–drug conjugates may still be substrates for target receptors, thus the drugs do not need to be released from the conjugates.[118]

Almost all the strategies discussed in this Review are not specific for the brain but can also deliver drugs to the peripheral tissues and may thus produce toxicity and side effects. To avoid these possible problems, a suitable approach for selective drug delivery to the brain is required.

Despite the urgent requirement for CNS drugs, there are few potent drugs for brain disorders available on the market. Additional efforts should be devoted to drug delivery and drug distribution in brain compartments to supply the growing market with new CNS drugs,.

Abbreviations

AA	ascorbic acid
AIDS	acquired immune deficiency syndrome
Apo	apolipoprotein
AZT	zidovudine
BBB	blood–brain barrier
BCECs	brain capillary endothelial cells
BCSFB	blood-cerebrospinal fluid barrier
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
β-Gal	β-galactosidase
BMVECs	brain microvascular endothelial cells
B-Pc	benzylpenicillin
CBSA	cationic bovine serum albumin
CDS	chemical delivery system
CLs	cationic liposomes
CNS	central nervous system
CSF	cerebrospinal fluid
EGF	epidermal growth factor
FA	ferulic acid
FDA	Food and Drug Administration
FUS	focused ultrasound
GABA	4-aminobutanoic acid
GDNF	glial-derived neurotrophic factor
GLUT-1	glucose transporter
GSH	glutathione
HIR	human insulin receptor
HIV	human immunodeficiency virus
HAS	human serum albumin
ICF	intercellular fluid
IDUA	α-L-iduronidase
i.p	intraperitoneal
i.v	intravenous
LAT1	large natural amino acid transporter
Lf	lactoferrin
LNCs	lipid nanocapsules
MAb	monoclonal antibody
MAN	*p*-aminophenyl-α-D-mannopyranoside
MDCK	Madin–Darby canine kidney
M6G	an active metabolite of morphine
MNPs	magnetic nanoparticles
MT	magnetic targeting
NGF	nerve growth factor
Nip	nipecotic acid
NPs	nanoparticles
PAMPA	parallel artificial membrane permeability assay
PAX	paclitaxel
PBCA	poly(*n*-butylcyanoacrylate)
PCLs	procationic liposomes
P-gp	P-glycoprotein
QDs	quantum dots
TAM	tamoxifen
TAT	trans-activating transcriptor
TEER	transepithelial electrical resistance
Tf	transferrin
TfR	transferrin receptor
TMC	trimethylated chitosan
TNFR	tumor necrosis factor receptor
VIP	vasoactive intestinal polypeptide
WGA	wheat germ agglutinin

This work was supported by MCINN-FEDER (Bio2008-00799), Generalitat de Catalunya (XRB and 2009SGR-1005), and CONSOLIDER-INGENIO 2010. We also thank Tanya Yates for her help in preparing the manuscript.

Received: October 19, 2010
Revised: January 17, 2011
Published online: June 30, 2011

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