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Review Article

Novel delivery systems for drug targeting to the brain

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CONTENTS

Abstract

The development of new drugs is expensive and time-consuming. Most of the potential compounds lack therapeutic efficacy due to their inability to reach the target site. An alternative to developing new drugs is to focus on delivering drugs of potential therapeutic value to the target site. The brain is one of the organs where the targeting of drugs is difficult. Transport of drugs from circulating blood into the central nervous system (CNS) is restricted by the blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier, which are formed by tight junctions connecting the cerebral endothelial and epithelial cells of the choroid plexus, respectively. Several strategies have been developed to circumvent the BBB, of which chemical delivery systems and novel delivery systems are the most important. The objective of this article is to provide updated information on the utilization of known transport systems across the BBB for the development of novel delivery systems that deliver drugs to the brain at a desired rate under various pathological conditions, including cerebral ischemia, brain tumors and Alzheimer's disease. The novel delivery systems include microspheres, niosomes, liposomes, nanoparticles, solid lipid nanoparticles, lipid microspheres, polymeric micelles, vector-mediated delivery, noninvasive gene therapy and nasal administration.

Introduction

The development of new drug molecules is expensive and time-consuming. The average cost and time for the development of a new chemical entity are much higher (approximately $500 million and 10-12 years, respectively) than those required to develop a novel drug delivery system (NDDS; $20-50 million and 3-4 years, respectively). Hence, there is a need for evolving an existing drug molecule from a conventional form to a novel delivery system that can significantly improve its performance in terms of patient compliance, safety and efficacy by targeting to the specific site.

The failure to reach the brain in sufficient levels is often responsible for the therapeutic failure of drugs. The transport of compounds from the circulating blood into the central nervous system (CNS) is restricted by the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier, which are formed by tight junctions connecting the cerebral endothelial and epithelial cells of the choroid plexus, respectively (Fig. 1). The BBB represents an insurmountable obstacle for many drugs, including antibiotics, antineoplastic agents and a variety of

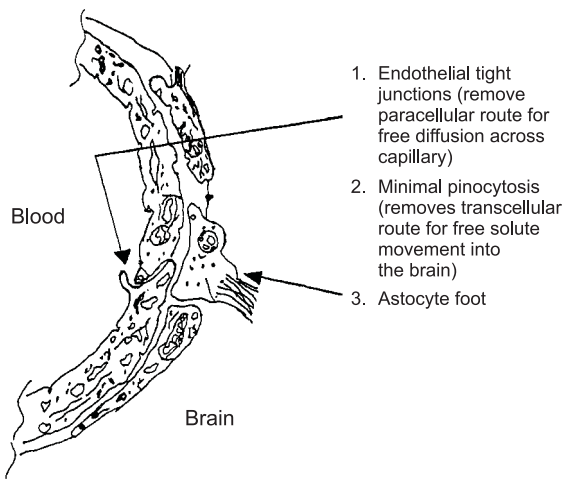

Fig. 1. General scheme of anatomic specializations of the brain capillary endothelium.

CNS-active drugs, especially neuropeptides. The inability of certain drugs, such as peptides and proteins, to cross the BBB raises problems in treating disorders of the brain, one of the leading causes of illness and death. For example, Alzheimer's disease is currently the third largest medical problem in the United States, afflicting some 4 million people at an annual cost of over $50 billion.

A wide range of strategies to increase the clinical utility of therapeutics for neurological disorders have been developed, as reviewed elsewhere (1), including osmotic disruption of the BBB (2), infusion pumps delivering drugs into the CSF (3-5), intravenous injection of surfactant-coated nanoparticles (6,7), liposomes (8), solid lipid nanoparticles (9, 10), coupling of drugs to a carrier undergoing receptor-mediated transcytosis through the BBB (11), implantation of biodegradable polymers, tissues or cells (12), and gene therapy (13-16).

Transport mechanisms across the blood-brain barrier (Fig. 2)

Passive diffusion

1. Paracellular aqueous pathway

This transport system involves the passage of compounds between cells by opening the tight junctions that link the endothelial cell membranes. Biochemical opening of these tight junctions may provide a pathway for the transport of compounds that are of neurobiological interest.

Paracellular passage of compounds across the BBB can be increased through the intracarotid infusion of hyperosmotic saccharide solutions (17). Osmotic opening of the BBB for the delivery of therapeutics to the brain is thought to cause a physical shrinkage of endothelial cells, which in turn pulls the tight junctions apart, forming pores in the barrier through which the drug can pass. However, osmotic opening of the BBB is associated with neurotoxicity in rats and seizures in human (18).

Intracarotid infusion of RMP-7, an analogue of bradykinin, to rats with brain tumors increased the uptake of various compounds (100-70,000 Da) to tumor as well as nontumor areas (19). In mice, RMP-7 facilitated paracellular transport by altering the integrity of tight junctions (20). Similarly, intracarotid arterial injection of the leukotriene LTC_4 has been found to open the brain tumor BBB, but not the normal BBB (21). These methods can improve brain delivery of water-soluble drugs such as carboplatin (22).

A potential drawback of osmotic and biochemical opening of the BBB is a lack of specificity, increasing the access of all compounds that are circulating in the blood to the brain. Another limitation is that passage through the

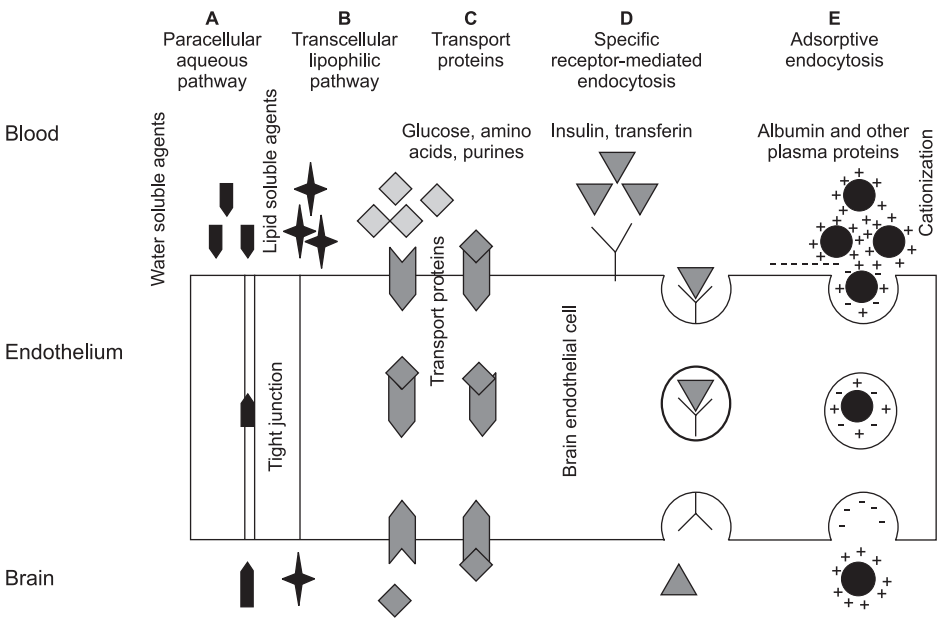

Fig. 2. The blood-brain barrier showing the routes across the brain endothelium. (A) Normally, the tight junctions permit the diffusion on only very small amounts of water-soluble compounds (paracellular aqueous pathway), while (B) the large surface area of the lipid membranes of the endothelium offers an effective diffuse route for lipid-soluble agents (transcellular lipophilic pathway). (C) The endothelium contains transport proteins for glucose, amino acids, purine bases, nucleosides, choline and other substances. (D) Certain proteins such as insulin and transferrin are taken up by specific receptor-mediated endocytosis. (E) While most native plasma proteins, such as albumin are poorly transported, cationization can increase their uptake by absorptive endocytosis and transcytosis.

openings may be size-selective and therefore limit passage to low-molecular-weight compounds.

2. Transcellular lipophilic pathway

This pathway involves the transport of compounds through the capillary endothelial cells of the brain. Peptides and proteins must be modified to increase their permeation across the BBB. This can be achieved by increasing the lipophilicity of the protein, and thus enhance its ability to penetrate the endothelial cell membrane and cross the BBB. Bodor *et al.* (23) reported increased delivery to the brain of an enkephalin analogue that had been modified to increase both its liphophilicity and resistance to proteolysis. However, the efficiency of this approach is unclear, as less than 0.01% of the modified peptide appeared to have penetrated the BBB following systemic administration.

Active diffusion

1. Carrier-mediated transport pathway

Extensive research work has been carried out on carrier-mediated transport and various systems have been identified.

a. Hexose transport system

At the luminal membrane of the brain capillary endothelial cells, the Na^+-independent glucose transporter GLUT1 takes up D-glucose and mannose, but not L-glucose, from plasma (24). GLUT1 is also involved in the transport of the oxidized form of vitamin C, dehydroascorbic acid, across the BBB, which accounts for increasing antioxidant potential in the CNS (25). GLUT1 is expressed abundantly (about $6x10^6$ molecules per brain capillary endothelial cell [BCEC]) and has a very large value for maximum transport rate, or V_{max}. Keeping this in mind, other workers have synthesized glucose-chlorambucil derivatives (26) and methylsulfonyl derivatives of glucose (27) to develop antitumor agents that readily cross the BBB. Other studies demonstrated that β-D-glucosyliso-phosphoramide mustard (D-19575), an alkylating agent, and cycasin (methylazoxymethanol-β-D-glycoside) are transported via the sodium-dependent glucose cotransporter system. Cycasin is proposed to be a significant etiological factor for the prototypical neurodegenerative disorders Western Pacific amyotrophic lateral sclerosis and Parkinson's dementia complex. Its uptake via the sodium-dependent glucose cotransport system was significantly inhibited by α-methyl-D-glycoside, as well as by phlorizin (an inhibitor of the sodium-dependent glucose cotransport system), replacement of extracellular NaCl with LiCl and by dinitrophenol (an inhibitor of energy metabolism). These findings indicate that cycasin transport across the BBB is via the Na^+/energy-dependent glucose cotransport system (28, 29).

b. Amino acid transport systems

The large neutral amino acid transport system is symmetrically distributed in both luminal and abluminal membranes of BCEC. The transport of large neutral amino acids is Na^+-independent. Phenylalanine and leucine are transported by this system. This transport system has the highest permeation rate in terms of V_{max}/K_m (30, 31), where K_m represents the Michaelis constant. An Na^+-dependent system, transporting small neutral amino acids such as alanine, is present only at the abluminal side. Another Na^+-cotransporter system is present only at the abluminal membrane and capable of transporting analogues of *N*-(methylamino)isobutyric acid (MeAIB) and large neutral amino acids (32). Recently, glucose-coupled L-tyrosine (GcpY), which has a free carboxylic function, and 2-(L-tyrosylamide)-2-deoxy-D-glucose (Y-2DG), which has a free amino function, were synthesized and using *in situ* brain perfusion techniques, the inhibitory effect of GcpY on [^{3}H]-L-tyrosine uptake by the large neutral amino acid transport system was found to be greater than that of *N*-methyl-L-phenylalanine or *N*-acetyl-L-phenylalanine, whereas Y-2DG did not affect it. These results indicate that a free amino group is not required for substrate recognition of the large neutral amino acid transport system, provided that the modified amino group is able to take a positive charge (33).

Several amino acid mimetic-drugs, such as L-dopa, α-methyldopa, α-methyltrypsin, baclofen, gabapentin and phenylalanine mustard, are considered to be taken up by the neutral amino acid transport system (30, 31). The design of amino acid analogues may therefore be useful in developing CNS-active drugs. The delivery of amino acid-mimetic drugs by utilizing the large neutral amino acid transport system with its relatively low K_m value may be subject to competitive inhibition.

Glutamate and aspartate are transported via the acidic amino acid transport system. The glutamate transport system is Na^+-independent and distributed in the luminal membrane of BCEC. A strategy for carrier-mediated brain delivery of drugs by conjugation with L-glutamate was successful in the case of the BBB-impermeable D-melphalan (34).

Arginine and lysine are transported through the basic amino acid transport system. When compared to the hexose and monocarboxylic acid transport systems, the maximum transport rate (V_{max}) of substances through this transport system is lower (11).

β-Alanine and taurine are transported via the β-amino acid transport system. Orally administered and endogenous taurine is taken up by the β-alanine transport system at both the luminal and abluminal membrane of BCEC in an Na^+- and Cl^--dependent manner (35).

c. Monocarboxylic acid transport system

The monocarboxylic acid transport (MCT) system transports lactate, pyruvate, short-chain monocarboxylic acids (MCAs) such as acetate and ketone bodies such as β-hydroxypyruvate and acetoacetate, which are essential for brain metabolism (30).

Acidic drugs bearing a monocarboxylic acid moiety cross the BBB via the monocarboxylate transporter(s). Salicylate, benzoate and probenecid were suggested to be transported via the monocarboxylate transport system from the brain interstitial fluid to plasma across the BBB (36). HMG-CoA reductase inhibitors (antihyperlipidemic drugs), such as simvastatin, pravastatin and lovastatin, all of which contain a carboxylic acid moiety, permeate through the BBB via the monocarboxylate transporter. Simvastatin is known to cause sleep disturbances whereas pravastatin apparently does not, and the absence of CNS side effects of pravastatin may be ascribed to very low affinity for the monocarboxylate transporter (37). Therefore, a strategy to avoid undesirable side effects may be to reduce the affinity of drugs for the responsible transporter at the BBB.

Lactic acid is transported across cell membranes by at least two well-characterized monocarboxylic acid transporters: MCT1 and MCT2. The MCT1 transporter is localized primarily on the capillary endothelial cells, as evidenced by electron microscopy. In contrast, MCT2 is specifically localized to the glial foot processes, just external to the capillary basal lamina. The presence of two different isoforms in slightly different locations may represent a mechanism for selective translocation of MCT substrates. The MCT1 transporter may have higher affinity for monocarboxylic acids, which may be used as fuel by the brain (such as ketone bodies). The location of the MCT2 transporter suggests that it is perhaps involved in the removal of monocarboxylic acids such as lactate from the brain parenchyma (38). However, Gerhart *et al*. (39) reported that MCT1 was found on both the luminal and abluminal membranes of BCEC. MCT1 at the BBB is involved in the bidirectional transport of lactic acid and other monocarboxylate compounds. Under normal physiological conditions in adult rats, however, the MCT1 presumably facilitates the efflux of lactate, which is produced from glucose in the brain (39). Valproic acid, a monocarboxylate, is transported through the medium-chain fatty acid transport system (40).

d. Choline transport system

There are at least two different carrier-mediated transport mechanisms specific for choline and amine drugs, as demonstrated by studies using the brain uptake index (BUI) method for the uptake of the hydrophilic amine [^{3}H]-choline. The uptake of choline was inhibited by amine compounds (eperisone, scopolamine, thiamine, isoproterenol and hemicholinium-3), whereas zwitterionic or anionic compounds were not inhibitory (41).

e. Amine transport system

Mepyramine is transported into the brain via the amine transport system. From uptake and transport studies using monolayers of primary cultured BCEC, saturable uptake of the classical H_1 antagonist [^{3}H]-mepyramine into the brain was observed (42). The uptake was inhibited by amine drugs such as chlorpheniramine, diphenhydramine and others, but not by choline, hemicholinium-3 or anionic drugs. Several H_1 antagonists (azelastine, ketotifen, cyproheptadine, emedastine and cetrizine) competitively inhibited the uptake of [^{3}H]-mepyramine, which suggests that they share common transport mechanisms with mepyramine (42). The lowest inhibitory effect was observed with cetrizine, which has a carboxylated side-chain. Introduction of an anionic moiety within the molecule may decrease affinity for the transporters, which would be desirable for drugs with CNS side effects.

f. Nucleoside transport system

The nucleoside transport system is the transport system for purine bases such as adenine and guanine, but not pyrimidine bases. The BBB has a 6-(4-nitrobenzyl)-thio-9-β-D-ribofuranosylpurine (NBMPR)-sensitive transport system that can transport deoxycytidine and uridine, but guanosine, monocarboxylates, hexose or amino acids are not substrates for this transport system (43). Tiazofurine, a nucleoside analogue, is transported from the blood into the brain via carrier-mediated transport, and partially by the nucleoside transport system (44). Thus, this transport system may provide a pathway for delivery of anticancer nucleoside derivatives to the brain for the treatment of brain tumors.

g. Erythrocyte binding transport system

Certain drugs may be bound to erythrocytes, either directly on the red cell membrane or to intracellular proteins. In the past, it has been shown that erythrocyte-bound drugs are able to dissociate and cross the BBB to gain entry to the neuropil. This phenomenon has been demonstrated in the rat BBB for the anticonvulsant drugs zonisamide, progabide and felbamate, as well as other drugs such as imipramine. Because the transit time through the brain capillary is approximately seconds, the rate of dissociation of these drugs from erythrocyte binding sites is presumed to be more rapid, or some additional serum factors promote *in vivo* dissociation of these drugs from their erythrocyte binding sites (38).

2. Specific receptor-mediated endocytosis (RME)

The concept of receptor-mediated endocytosis (RME) was formulated in 1974 by Goldstein and Brown to

explain the regulation of cellular cholesterol metabolism by the observed surface binding, internalization and subsequent intracellular degradation of plasma low-density lipoprotein (LDL). The importance of their findings was honored with the Nobel Prize in 1985. In the following years, RME was recognized as a mechanism by which animal cells also internalize many other macromolecules. Receptor-mediated transport processes can be further distinguished by the fate of the ligand and the receptor after cellular internalization. In principle, the internalized material is stored, degraded or recycled back to the cell surface (45). Specific receptors on brain capillaries have been identified for insulin, insulin-like growth factors (IGF-I, IGF-II), angiotensin II, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), interleukin-1 (IL-1) and transferrin (11). Various other receptors that are overexpressed on brain tumor cells are folic acid, epidermal growth factor (EGFR), platelet-derived growth factor (PDGFR) and peripheral benzodiazepine receptors (46-48). Transcytosis across the BBB *in vivo* has to date only been shown for insulin and transferrin. Iron is transported to the brain via receptor-mediated endocytosis. In addition to iron, the transferrin receptor also binds divalent cations, such as copper, zinc, gallium, manganese and aluminium (49).

The unusually high expression of transferrin and insulin receptors on the surface of a normal BBB provides a potential advantage for the delivery of drugs into the brain. Most endogenous peptides are transported into the brain via RME. Apart from receptor-mediated transport into the brain, peptides also utilize adsorption-mediated transcytosis (AMT; see below). Interestingly, peptides such as insulin, IGF, cationic albumin and transferrin cross the BBB utilizing RME. Specific receptors facilitate the movement of these compounds across the luminal capillary membrane, through the cytoplasm and across the abluminal membrane to the neuron. This process involves binding of the receptor and peptide at one side of the BBB (*i.e.,* the luminal membrane), translocation of the receptor-peptide complex through the cytoplasm, and dissociation of the peptide from the receptor on the external surface of the abluminal membrane (38). Thus, receptor-mediated transport systems that normally transport endogenous peptides and proteins into the brain could also be exploited for drug delivery to the brain.

3. Adsorption-mediated transcytosis (AMT)

Adsorption-mediated transcytosis (AMT) through the BBB is functionally similar to RME, except that the initial triggering of the endocytotic event at the luminal side of the BBB is accomplished through an electrostatic interaction between a positively charged moiety of the drug substance and a negatively charged moiety of the blood-brain membrane. Although specific targeting by AMT to the CNS cannot be expected because of nonspecific operation in other tissues, the lower affinity and higher capacity of AMT compared with RME should be favorable for the delivery of peptides to the brain.

Ebiratide, a synthetic peptide analogous to adrenocorticotropic hormone (ACTH) used to treat Alzheimer's disease, is a positively charged drug and is able to cross the BBB in an intact form utilizing AMT (50). Similarly, E-2078, an analogue of dynorphin, is transported to the brain via AMT following systemic administration (51, 52). Other large molecules that penetrate the BBB via AMT include various polycationic proteins such as β-endorphin-cationized albumin complex (53), histone (54) and avidin (55).

Efflux transport systems

The efflux transport systems are expressed on the capillary endothelial cells of the BBB and the blood-cerebrospinal fluid barrier to actively efflux toxic metabolites and xenobiotics out of the brain while maintaining the functional integrity of the brain. The P-glycoprotein and organic anion transporters are recognized as important determinants of drug distribution to, and elimination from, various anatomical parts of the brain.

P-Glycoprotein (P-gp)

P-Glycoproteins are *N*-glycosylated membrane proteins of about 1,280 amino acids. The polypeptide chain consists of two similar halves, each containing six putative transmembrane segments and an intracellular ATP-binding site. Hydrolysis of ATP provides for active drug export, which can occur against large concentration gradients (56-60). The ATP-dependent drug transport protein P-gp is predominantly found in the apical membranes of a number of epithelial cell types in the body, including the blood luminal membrane of the BCEC that make up the BBB. P-Glycoprotein present in the endothelial cells of the BBB is functionally active in transporting drugs from the brain (or basolateral) side to the blood (apical or luminal) side of these cells (Fig. 3).

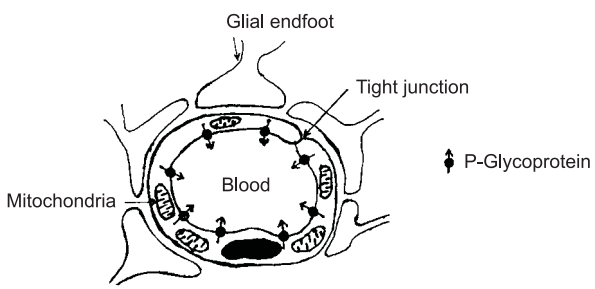

Fig. 3. Neural capillary: section of brain blood capillary balls; arrows indicate the localization of P-glycoprotein and the direction of drug transport in the luminal membrane of the brain capillary endothelial cells.

Based on the relationship between the octanol/water partition coefficient (PC) divided by the square root of the molecular weight ($PC/MW^{1/2}$) and the BBB permeability coefficient (PS), one can classify the substrates into three different groups: 1) substrates exhibiting a good correlation between PS and lipophilicity; 2) compounds exhibiting a significantly greater PS value than indicated by their lipophilicity; and 3) substrates exhibiting a significantly smaller PS value than indicated by their lipophilicity.

The transport mechanisms for the first two groups are passive diffusion and facilitated transport, respectively (61). The absolute cut-off molecular weight for substrates in the third group for significant transport across the BBB, regardless of lipophilicity, is 400 Da. This molecular weight threshold hypothesis was proposed to explain the mechanism operating in the case of the third group (62). Another possible mechanism is that P-gp in the BBB may play an important role in restricting the permeation of the last group of compounds by pumping them out of the brain into the circulating blood. This is supported by several lines of evidence. Firstly, the compounds of the third group, including vinca alkaloids, doxorubicin, epipodophyllotoxin and ciclosporin, are well-known substrates for P-gp (63, 34). Secondly by immunoelectron microscopy of brain sections using the monoclonal antibody MRK16 in human brain (64, 65) and the monoclonal antibody C219 in bovine brain (66), P-gp was detected exclusively at the luminal membrane of the BCEC. An excellent and thorough study by Beaulieu *et al*. (67) employing a novel technique provided the most convincing evidence so far that in capillary endothelial cells of rat brain, P-gp is predominantly, if not exclusively, localized to the luminal membrane of the BCEC. P-Glycoprotein expression can also occur in astrocytes, especially in certain pathological states (67, 68).

The real impact of the P-gp transport system on the BBB only became evident with the generation of knockout mice lacking the mdr1a P-glycoprotein (*mdr1a*$^{-/-}$ mice). These mice, which behave normally under normal laboratory conditions, turned out to be almost 100-fold more sensitive to the neurotoxic pesticide ivermectin. This was discovered by chance, as the mice were sprayed with ivermectin to treat a mite infestation but nearly all *mdr1a*$^{-/-}$ mice died. Subsequent analysis demonstrated that these animals died due to accumulation of 100-fold more [^{3}H]-ivermectin in their brains than wild-type mice. Ivermectin was therefore discovered to be a good substrate for mouse mdr1a and human MDR1 P-glycoprotein (69). The impact of several drugs known to be P-gp substrates was therefore studied using mdr1a knockout mice. The anticancer drug vinblastine accumulated 20-fold more in the brain of mdr1a knockout mice, whereas plasma levels were only increased 2-fold (70). Similar results were obtained with the [^{3}H]-labeled drugs digoxin and ciclosporin, while more moderate increases in brain concentrations were found for [^{3}H]-morphine and [^{3}H]-dexamethasone (69). Digoxin accumulated in the brain of mdr1a knockout mice for a period of 3 days after a single intravenous bolus injection, resulting in 200-fold higher brain levels as compared to wild-type mice (71). Therefore, major CNS toxicity for digoxin in humans without functional P-gp in their BBB can be expected. P-Glycoprotein in the BBB would limit the entry of potentially toxic compounds from the blood into the brain by pumping them actively back into the blood (72, 73).

The molecular weight of lipophilic drugs is a good predictor of the degree of brain penetration (62). However, the use of lipophilicity as an index of BBB transport is indicated only when the molecular weight is less than approximately 400-600 Da (30, 62). Several hydrophobic molecules between 400 and 700 Da were found to enter the brain far less efficiently than expected, whereas highly hydrophobic molecules with a molecular weight above 700 Da did not appear to an appreciable extent in the brain. It now appears that P-gp in the BBB is a major factor determining the low apparent brain penetration of hydrophobic compounds larger than 400-700 Da.

Quinidine transport into the brain was increased by PSC-833 (10 mg/kg), an inhibitor of P-gp in normal mice, whereas its transport was unaffected in *mdr1a*$^{-/-}$ mice. This clearly indicates that P-gp efflux is responsible for low levels of quinidine in the brain of normal mice (74).

P-Glycoprotein in the BBB has a decisive effect on the clinical application of drugs such as domperidone and loperamide. Both domperidone and loperamide are efficiently transported by mdr1a and MDR1 P-glycoprotein (75). Domperidone, a dopamine antagonist, does not pass the BBB and hence cannot be used as a neuroleptic (antipsychotic) drug, but it can be used as an antiemetic drug due to its selective peripheral activity. When domperidone was administered to *mdr1a*$^{-/-}$ mice, they displayed extreme passivity and a total lack of spontaneous movement, indicative of CNS activity for domperidone, which was not observed in wild-type mice given similar doses (75). These results suggest that P-gp is a major determining factor for the clinical use of domperidone (Table I).

Although loperamide is structurally an opiate, it lacks central effects and displays only peripheral opiate-like effects on the gastrointestinal tract leading to constipation. When loperamide was administered to *mdr1a*$^{-/-}$ mice, they displayed a full-blown picture typical of opiate

Table I: Drugs affected by P-glycoprotein in the BBB (63, 76).

Drug	Molecular weight	Therapeutic category
Ciclosporin	1203	Immunosuppressant
Dexamethasone	392	Glucocorticoid
Domperidone	426	Antiemetic
Doxorubicin	544	Antineoplastic
Digoxin	781	Cardiotonic
Erythromycin	734	Antibiotic
Indinavir	614	HIV protease inhibitor
Loperamide	477	Antidiarrheal
Nelfinavir	568	HIV protease inhibitor
Paclitaxel	854	Antineoplastic
Quinidine	324	Antimalarial
Saquinavir	671	HIV protease inhibitor
Vinblastine	811	Antineoplastic

effects in the CNS in mice: pronounced excitement, compulsive circling movement interrupted by bouts of immobility, a crouched appearance and Straub tail. Upon administration of [^{3}H]-loperamide, the *mdr1a*$^{-/-}$ mice accumulated 13-fold higher levels of radioactivity in the brain, whereas the plasma levels were only 2-fold higher than in wild-type mice (75). These results suggest that in the absence of P-gp in the BBB, loperamide would act as a centrally active opiate.

Considering the potential clinical importance of P-gp-mediated drug transport in the multidrug resistance (MDR) of cancer cells, the oral bioavailability, excretion and brain penetration of drugs, there is currently a flurry of activity to develop highly effective and specific P-gp blockers. PSC-833, a nonimmunosuppressive cyclosporin analogue, is an effective and well-characterized representative of these blockers which is currently being tested in phase III trials for the chemotherapy of acute myeloid leukemia. Even more efficient and specific P-gp blockers may be in the pipeline (77-79).

By regulating the activity of P-gp, it is possible to improve the entry of certain drugs into the brain that currently display insufficient brain penetration for effective therapy. In order to examine the BBB efflux transport mechanism under *in vivo* conditions, the intracerebral microinjection technique was developed and the brain efflux index (BEI) established (80). The BEI value is defined as the relative percentage of drug effluxed from the ipsilateral cerebrum to the circulating blood across the BBB compared with the amount of drug injected into the cerebrum, *i.e.*:

$$\text{BEI (\%)} = \frac{\text{Amount of drug effluxed at the BBB}}{\text{Amount of drug injected into the brain}} \times 100$$

The advantages of the BEI method are its ability to allow determination of the apparent *in vivo* drug efflux rate constant across the BBB, monitoring the concentration dependency of the test drug and the performance of inhibition studies. The limitation of the BEI method is that only one data point can be obtained for a single intracerebral microinjection (80). The BEI method can be used to characterize previously identified BBB efflux transport and evaluate the significance of efflux transport activities under *in vivo* conditions.

Organic anion efflux transport system

Since P-gp preferentially accepts hydrophobic neutral or cationic compounds, the presence of an efflux transporter for organic anions on the BBB was identified and studied using cefodizime, a third-generation cephalosporin antibiotic, as a model compound. Time profiles of the cefodizime concentration in the brain and CSF after i.v. administration, along with those of the CSF concentration of cefodizime and mannitol, a marker for passive diffusion, after intracerebroventricular (i.c.v) administration, were simultaneously analyzed using a spatially distributed model, the most precise pharmacokinetic model for describing drug disposition in the CNS (81, 82). The results indicated that the efflux permeability coefficient (PS) of cefodizime from the brain to the blood (2.4×10^{-4} ml/s/g brain) exceeds the influx from the blood to the brain (5.2×10^{-5} ml/s/g brain), implying the presence of an active transport system for organic anions across the BBB (81).

Cefodozime and 1-naphthyl-β-glucuronide are substrates for the canalicular multispecific organic anion transporter (cMOAT), a primary active transporter which mediates the excretion of organic anions into the bile across the bile canalicular membrane (74, 83). It was hypothesized that cMOAT and/or related protein(s) (such as multidrug resistance-associated protein, or MRP) may be expressed on the luminal membrane of cerebral endothelial cells. The BEI method revealed the existence of a carrier-mediated efflux organic anion transport system for compounds such as *p*-aminohippuric acid, zidovudine (AZT), 2',3'-dideoxyinosine (ddI), taurocholic acid, methotrexate, BQ-123 (an endothelin receptor antagonist) and estrone sulfate. Moreover, cerebral neurotransmitters such as γ-aminobutyric acid, L-glutamic acid and L-aspartic acid are transported from the brain to the circulating blood in intact form via a carrier-mediated efflux transport system (63). A review published recently provides an overview of several drug efflux transport families present in the CNS and their clinical importance for drug delivery to the CNS (84).

Strategies for improving drug delivery to the brain

The two main reasons for the failure of drug delivery to the brain are: 1) poor penetration of the drug across the BBB, and 2) back-transport of drugs from the brain to the blood by an efflux transport system.

For a long time, the BBB was considered to be a physical barrier, mainly represented by the cerebrovascular endothelium. Later, the BBB was regarded as a dynamic rather than a rigid barrier, which could be influenced by astrocytes and probably also by neuronal and hormonal stimuli; diseases of the CNS also affect its properties. This may offer new strategies for targeting drugs to the brain. Drug delivery to the brain can be achieved by the following methods: 1) chemical delivery systems; 2) novel delivery systems; or 3) direct administration of drugs into the brain. This review will primarily discuss advances related to developing novel delivery systems such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, vector-mediated delivery to the brain following systemic administration, and also a brief note on nasal administration.

Novel delivery systems for drug targeting to the brain

A promising strategy for improving drug delivery to the brain involves the development of suitable drug carrier

systems. Colloidal carrier systems have attracted increasing attention for targeted drug delivery. These systems include microspheres, liposomes, lipid microspheres, polymer micelles, nanoparticles, solid lipid nanoparticles, niosomes, etc.

Aspects to be considered for selecting a suitable colloidal carrier system include: 1) drug loading capacity; 2) possibility of drug targeting; 3) acute and chronic toxicity; 4) *in vivo* fate of the carrier; 5) scale-up of production; 6) physical and chemical storage stability; and 7) overall cost.

The cerebral distribution of drugs after administration depends on several factors and these should be considered and optimized. Important factors determining cerebral distribution of drugs are: 1) plasma protein binding; 2) cerebral blood flow rate; 3) influx and efflux rates at the BBB; 4) influx and efflux rates at the blood-cerebrospinal fluid barrier; 5) diffusion rate in the brain parenchyma; 6) rate of metabolism in the brain; 7) tissue binding in the brain; 8) flow rate of CSF; and 9) influx and efflux rates at the brain parenchyma.

Systemic administration

1. Magnetic particles

A magnetic cationic microsphere delivery system prepared from the polysaccharide chitosan and containing oxantrazole (OX) was examined for its ability to enhance brain delivery of OX compared to administration of OX in solution (OX-S). Magnetic chitosan microspheres containing OX (MCM-OX) significantly increased (100-fold) OX brain concentrations compared to those achieved with OX-S following intraarterial administration to male Fischer rats (OX doses of 0.1 mg/kg), with a magnetic field of 6000 G applied to the brain for 30 min. Even in the absence of the magnetic field, MCM-OX was retained in the brain, possibly through cationic-anionic interactions with the BBB (85).

Later, in an effort to determine the influence of particle size on blood-tumor barrier uptake, small uncharged magnetic particles (SMP) were used and evaluated for their ability to target intracerebral rat glioma RG-2. Small magnetic particles (10-20 nm) were injected intraarterially (4 mg/kg SMP) to rats bearing RG-2 tumors with a magnetic field of 6000 G applied to the brain for 30 min. The results indicated that in the presence of a magnetic field, SMP localized in brain tumor tissue at levels of 41% and 48% dose/g tissue after 30 min and 6 h, respectively, values significantly greater than in nontarget tissues. In comparison with larger (1 μm) diameter magnetic particles, SMP concentrated in brain tumor at significantly higher levels than previously studied magnetic neutral dextran (p = 0.0003) and cationic aminodextran (p = 0.0496) microspheres. These concepts led to the design of new, small, loaded particles as targeted drug delivery systems for brain tumors (86).

2. Niosomes

Large multilamellar (100-125 nm) vesicles (niosomes) containing methotrexate (MTX) were prepared from a nonionic surfactant, cholesterol and diacetyl phosphate and methotrexate-containing niosomes were administered intravenously to mice. Despite the large amounts of drug taken up by the liver, higher levels of MTX were observed in the brain. The increased MTX levels in brain may be due to the effect of niosome components on the permeability of the BBB. Niosomes not only improved drug delivery to the brain, but also altered the metabolic profile of MTX by preventing rapid formation of 7-hydroxylmethotrexate, and reduced its excretion in urine and bile (87, 88).

3. Liposomes

Liposomes were introduced as drug delivery vehicles in the 1970s. Liposomes are concentric bilayered structures made of amphipathic phospholipids. Depending on the number of bilayers, liposomes are classified as multilamellar (MLV), small unilamellar (SUVs) or large unilamellar vesicles (LUVs). Owing to the large size of liposomes, even small unilamellar vesicles on the order of 40-80 nm do not undergo significant transport through the BBB (89-91); large multilamellar vesicles on the order of 0.3-2 μm in size accumulate in the brain following peripheral administration due to embolization within the brain microvasculature producing toxicity (89). Apart from the size of the liposomes, their composition is also known to affect transport through the BBB. From the literature, it is evident that negatively charged liposomes prepared from dipalmitoylphosphatidylcholine, with a size in the range of 50-150 nm, are known to cross the BBB to a significant extent, especially entrapping water-soluble drugs. Much work has been carried out in the last two decades recognizing liposomes, stealth liposomes and surface-modified liposomes as potential carriers for drug delivery to the brain under various pathological conditions (92-97).

Citicoline is a therapeutic agent widely used in the treatment of brain disorders. Due to the strong polar nature and charged nitrogen group, citicoline is unable to cross the BBB. An animal study revealed that brain uptake of citicoline is 0.5% with oral dosing, which is increased to ~2% following intravenous administration. In an effort to treat brain disorders, citicoline was trapped efficiently in liposomes. Citicoline-loaded liposomes improved the therapeutic efficacy, reflected in a 24% increase in the survival rate of rats with cerebral ischemia (98). Further increases in survival rate up to 66% compared to free citicoline were observed when citicoline was encapsulated in ganglioside GM1-associated liposomes (99). In a preliminary study on the *in vitro* metabolism of citicoline in human plasma, the V_{max} and K_m values varied greatly among individuals and it was assumed that

citicoline delivery to the brain may vary from individual to individual; extension of this work is in progress (100).

In another study, Jain *et al.* (101) developed dopamine hydrochloride bearing positively charged small liposomes and assessed their *in vivo* performance by periodic measurement of chlorpromazine-induced catatonia in Sprague-Dawley rats. The results were compared with free dopamine hydrochloride, dopamine and levodopa + carbidopa. These studies showed that dopamine can be effectively delivered to the brain and incorporation into liposomes could prevent its degradation in the circulation.

Conventional liposomes are normally rapidly cleared from the circulation by the reticuloendothelial system (RES). The uptake of liposomes by the RES can be avoided by developing sulfated liposomes. These sulfated liposomes were administered via the internal carotid artery in rats along with osmotic treatment with mannitol for successive delivery to the brain (91). Interestingly, the delivery of amphotericin B to the brain in cerebral inflammation was increased when osmotic opening of the BBB and stealth liposome technology were merged. RMP-7 is known to cause the osmotic opening of the BBB by selectively reacting with bradykinin B_2 receptors on the capillary blood vessels of the brain. When RMP-7 and pegylated amphotericin B liposomes were administered together intravenously, there was little increase in the concentration of amphotericin B in the brain because RMP-7 and the liposomes could not reach the BBB at the same time, but when RMP-7 was inserted on the surface of pegylated amphotericin B liposomes, concentrations in the brain were several times higher (102).

The half-life of the liposomes in the circulation can also be prolonged if ganglioside- or polyethylene glycol (PEG)-derivatized lipids are inserted within the bilayer of conventional liposomes. Liposomes, which are coated with the inert and biocompatible polymer (*e.g.*, PEG), are not readily recognized by macrophages and are often referred to as sterically stabilized, or stealth, liposomes. Recently, liposomes (85 nm) were sterically stabilized with a 2,000-Da PEG that contains a lipid at one end and a maleimide at the distal end to the liposome (103).

Liposome-based target recognition is a critical prerequisite for ligand-mediated targeting and the selected ligands should have a well-defined propensity, avidity and specificity towards receptor portals expressed selectively on the selected target cell(s). Stealth liposomes are large enough that they typically do not leak out of the bloodstream and into tissues through normal healthy blood vessels. Although stealth liposomes are retained in blood for a longer time, they cannot cross the BBB. The accelerated clearance of stealth liposomes upon repeated administration was observed by Ishida *et al.* (104) and they noted that the first dose reduced the circulation half-life of a second subsequent dose. The long circulating half-life of pegylated liposomes, possibly leading to a mild and continuous stimulation of lymphocytes or macrophages, is one of the reasons for the induction of an accelerated effect. The accelerated clearance of the pegylated liposomes is reduced by extending the dosing interval for 5 weeks between the first and second dose (105).

In the mid-1990s, liposome technology, pegylation technology and brain targeting technology were merged to allow targeted drug delivery to the brain. The various possible locations at which monoclonal antibodies are attached to the PEG liposomes were discussed by Mastrobattista *et al.* (106). It is desirable to yield high protein levels on the surface of PEG liposomes and this is achieved by coupling with a sulfide linkage to the maleimide moiety of stealth liposomes to form immunoliposomes (106). The overexpression of a transport receptor on the BBB enables the selection of a specific ligand for binding, which undergoes RME; potential receptors overexpressed on tumor cells include transferrin (TfR), folate, insulin (IR), EGFR and PDGFR. The OX26 monoclonal antibody (anti-transferrin receptor monoclonal antibody) was linked to stealth liposomes because this antibody binds to the BBB TfR and has been successfully used as a vector in the delivery of other large molecules across the BBB (103). No brain uptake of PEG-conjugated liposomes containing radioactive daunorubicin was observed. However, brain delivery of immunoliposomes containing [^{3}H]-daunorubicin was successful, and it was reported that the coupling of 30 OX26 antibodies per liposome resulted in optimal brain delivery (103). Since a single liposome may carry up to 10,000 drug molecules, the immunoliposome delivery system has the ability to dramatically increase brain drug delivery by up to 4 orders of magnitude. In contrast to the low delivery of pegylated radiolabeled daunorubicin to the brain, long-circulating pegylated glucocorticosteroid liposome uptake into brain was 4.5-fold greater than free glucocorticosteroids and may be due to the formation of a fixed aqueous layer thickness (FALT) around the pegylated liposomes which alters the pharmacokinetics of drug (107, 108).

In another study, methotrexate-containing G22 MAb-coupled liposomes (immunoliposomes) showed selective cytotoxicity towards glioma cells which was 100-fold greater when compared with free methotrexate. This system could be utilized for targeting chemotherapeutic agents to glioma cells (109).

The recent discovery that TfRs in the cerebral endothelium are increased, with a peak on the first day, after 90-min transient middle cerebral occlusion, returning to control levels by 6 days, has opened up new strategies for delivering drugs under such conditions. Transferrin-coupled pegylated liposomes could be utilized as an efficient drug delivery tool to the brain after stroke (110). Pegylated liposomes were coupled covalently to thiolated bovine serum albumin (BSA) and cationic BSA, and studied to evaluate their targeting efficiency to brain and also to elucidate the mechanism of transport in BCEC. It was found that, in contrast to BSA-coupled liposomes, cationic BSA liposomes were rapidly taken up by BCEC by caveolae-mediated transport. Therefore, cationic BSA-coupled liposomes could be utilized as a carrier for drug delivery to the brain (111).

Polysaccharide-anchored liposomes were developed by coating the liposomal surface with natural or hydrophobized polysaccharides (*e.g.,* mannan, pullunan, amylopectin, *etc.*, or their palmitoyl or cholesteroyl derivatives). Improving the physical and biochemical stability of liposomes, as well as the ability to target liposomes to specific organs and cells, were the major attributes of the polysaccharide-anchored liposomes. Polysaccharide-anchored liposomes could be employed as carrier constructs onto which site-specific sensing molecule(s), such as an MAb against tumor-surface antigens, could be physically or chemically attached (112).

Several groups have developed liposomal constructs for brain targeting in human glioma. They employed sulfatides and MAbs as site-directing devices to endow tractability to the liposomes (113, 114). The survival of 9L glioma-implanted rats treated with carbohydrate pullulan-based liposomes loaded with cisplatin was significantly higher as compared to the average survival recorded for untreated groups (115).

Gene therapy of the brain is hindered by the presence of the BBB, which prevents the brain uptake of blood-borne gene formulations. Gene medicines can be delivered successfully to the brain via the endogenous BBB transport system. This therapy requires repeated administration, and would therefore be advantageous for noninvasive routes. Exogenous genes have been expressed in the brain after invasive routes of administration, such as craniotomy or intracarotid arterial infusion of noxious agents causing BBB disruption.

Targeting of gene medicines using endogenous BBB transport systems noninvasively to the brain requires the development of a suitable formulation of the gene therapeutic that is stable in the bloodstream. Cationic liposome/DNA complexes have been used for *in vivo* gene expression, but these formulations aggregate extensively in saline solution. In another approach, the plasmid DNA is incorporated into pegylated immunoliposomes that can cross the BBB utilizing endogenous transport systems. Pegylation of liposomes prevents rapid uptake by the RES and PEG optimizes the plasma bioavailability of the liposomes. The presence of PEG strands also provides a site for conjugation of peptidomimetic MAbs.

Widespread gene expression in the brain can be achieved using a formulation that does not employ viruses or cationic liposomes, but instead uses endogenous receptor-mediated transport pathways at the BBB. Significant expression of an exogenous gene in the brain was seen after noninvasive intravenous administration of a 6-7-kb expression plasmid encoding either luciferase or β-galactosidase packaged inside neutral pegylated immunoliposomes (pegylated liposomes conjugated with OX26 MAb). This noninvasive technology for gene targeting to the brain utilizes the endogenous BBB transferrin receptor. Luciferase gene expression in the brain peaks at 48 h after a single intravenous administration of 10 μg of plasmid DNA per adult rat, a dose that is 30-100-fold lower than that used for gene expression in rodents with cationic liposomes. However, the activity returned to baseline within 72 h after intravenous injection of pegylated immunoliposomes, which was attributed to the presence of a heterologous intron in the 3'-unsaturated region (UTR) of the luciferase expression vector, resulting in decreased gene expression *in vivo* (116). To circumvent the decreased gene expression *in vivo*, pegylated liposomes carrying a β-galactosidase gene packaged in an expression plasmid with short UTR lacking a heterologous intron were developed and showed persistent gene expression in the brain for at least 6 days following intravenous injection (15). The present approach to gene therapy could be used in humans by changing the targeting moiety of the formulation to enable targeting the human IR, which is widely distributed on brain cells.

Boron neutron capture therapy (BNCT) is an emerging approach to the treatment of brain tumors. Carlsson *et al.* (47) reviewed the potential applications, pharmacokinetics and pharmacodynamics of ligand liposomes containing large boron atoms for the treatment of gliomas and melanomas utilizing either folate or EGF receptors overexpressed on tumor cells (47). However, this system is administered intracerebrally or intratumorally, which is not of interest for discussion in this review.

4. Nanoparticles

Nanoparticles were first developed around 1970 and employed as carrier systems for vaccines and anticancer drugs (117). Nanoparticles are polymeric particles made of natural or artificial polymers (size range about 10-1000 nm [1 μm]) to which drugs are bound by sorption, incorporation and chemical binding (118). Depending on the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically uniformly dispersed.

One of the main problems in targeted drug delivery is the rapid opsonization and uptake of the injected carrier systems by the RES (by macrophages in liver and spleen). These carrier systems are removed rapidly from the bloodstream and are 90% cleared within 5 min after systemic administration (119). Nanoparticles also create a formulation problem and require stabilizers to prevent rapid release of drug from the nanoparticles (120, 121).

Surface modification of biodegradable and long-circulating polymeric nanoparticles enables permeation across the BBB. This can be achieved by two methods: 1) surface coating with hydrophilic polymers /surfactants; and 2) the development of biodegradable copolymers with hydrophilic agents. Some of the widely used surface-coating materials are polyethylene glycol (PEG), polyethylene oxide (PEO), poloxamer, poloxamine, polysorbate 80 (Tween-80) and lauryl ethers (122). Poly(butylcyanoacrylate) nanoparticles represent the only nanoparticles that have so far been successfully used for the *in vivo*

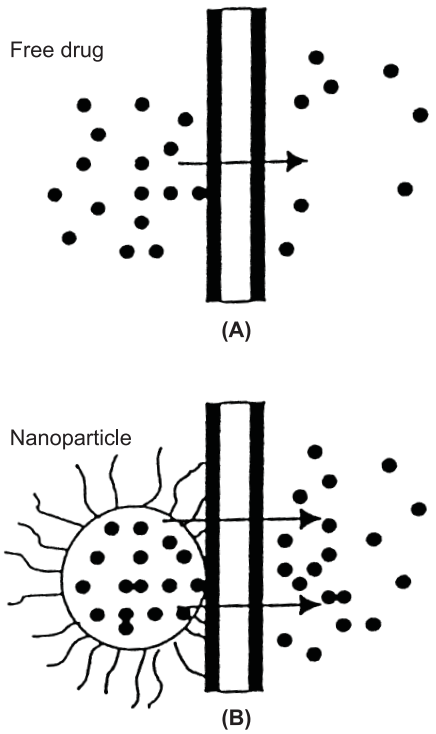

Fig. 4. Schematic representation of drug uptake through biological membranes for: (A) free drug and (B) polysorbate 80-coated nanoparticle-bound drug.

delivery of drugs to the brain. This polymer has the advantage that it is very rapidly biodegradable (123, 124). The mechanisms by which nanoparticles are delivered to the brain have been reviewed (7).

The concept of using polysorbate 80 for enhancing the delivery of a drug to the brain was discovered accidentally while attempts were being made to improve the absorption of methotrexate through the gastrointestinal tract (125). Also, Sakane *et al.* (126) showed that a 9% solution of polysorbate 80 provided enhanced passage of insulin and the dipeptide β-kyotorphin through the BBB in the brain by osmotic opening of tight junctions. Later, its importance in drug delivery to the brain was investigated and patented by Kreuter *et al.* (127).

Nanoparticle-mediated drug transport to the brain requires overcoating of the particles with polysorbates. Dalargin-loaded nanoparticles (10 mg/kg) have been coated with polysorbate 20, 40, 60 and 80, and injected intravenously to mice. The percentage of maximum analgesic effect produced was calculated and the results obtained indicate that significant levels of dalargin are achieved in the brain by coating poly(butylcyanoacrylate)-dalargin nanoparticles with polysorbates. The highest drug delivery was observed with polysorbate 80 (128) (Fig. 4). Similarly, the brain concentrations of systemically administered doxorubicin can be enhanced over 60-fold by binding to biodegradable poly(buytylcyanoacrylate) nanoparticles overcoated with the nonionic surfactant polysorbate 80 (129).

Overcoating of drug-loaded poly(butylcyanoacrylate) nanoparticles with polysorbate 80 led to the adsorption of apolipoprotein E (apo E) from blood plasma onto the nanoparticle surface. The particles then seem to mimic LDL particles and could interact with the LDL receptor, leading to their uptake by endothelial cells. Subsequently, the drug may be released in these cells and diffuse inside the brain, or the particles may be transcytosed. Polysorbate 80 appears to act as an anchor for apo E. The ability to cross the BBB of drug-loaded nanoparticles, however, is not only due to adsorption of apo E, but is probably the 'team work' of apolipoproteins such as A-I and A-IV, which prevent the nanoparticles from hepatic uptake prior to their contact with the apo E receptors at the BBB (130).

Drugs that have successfully been transported into the brain using nanoparticle carrier systems include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the NMDA receptor antagonist MRZ-2/516 and doxorubicin. The most likely mechanism seems to be endocytosis by the endothelial cells lining the brain blood capillaries (7).

Hexadecylcyanoacrylate nanospheres and polycyanoacrylate nanoparticles were pegylated to develop long-circulating nanoparticles as new drug carriers for brain delivery (131, 132). The reduced liver uptake of nanoparticles was dependent on the molecular mass and surface density of PEG.

5. Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are composed of lipids, phospholipids and cosurfactants and are prepared by high-pressure homogenization. The advantages of SLN include: 1) the possibility of controlled drug release and drug targeting; 2) increased drug stability; 3) high drug payload; 4) the feasibility of incorporating lipophilic and hydrophilic drugs; 5) a lack of biotoxicity of the carrier; 6) avoidance of organic solvents; and 7) no problems with respect to large-scale production and sterilization.

Solid lipid nanoparticles are suitable drug carrier systems for potential intravenous use due to their very low cytotoxicity relative to polymeric particles (133, 134). They possess a solid matrix for controlled release of drug, avoiding burst release as seen with fat emulsions. Hence, SLN as drug carriers possess the advantages of polymeric nanoparticles while being devoid of the disadvantages of fat emulsions.

The cellular uptake of camptothecin was significantly increased when it was incorporated in SLN and administered intravenously and orally. Solid lipid nanoparticles containing camptothecin exhibited sustained release and stability towards hydrolysis. Hence, high levels of active camptothecin lactone were maintained at the target site. Thus, SLN may be considered a good carrier for the delivery of camptothecin to the brain (9, 10). Intravenous

administration of paclitaxel-loaded SLN led to higher and more prolonged plasma levels of paclitaxel. Interestingly, both nonstealth and stealth SLN (stearic acid-PEG 2000) showed a similar low uptake by liver and spleen macrophages, as well as significantly increased uptake in the brain (135, 136). Similarly, doxorubicin stealth and nonstealth SLN provided higher concentrations of doxorubicin in the brain of two species tested following intravenous administration and the concentrations of doxorubicin increased as the concentration of the stealth agent increased, reducing the cardiotoxicity compared to doxorubicin solution (137, 138).

To overcome the limited access of 5-fluoro-2'-deoxyuridine to the brain, 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine was synthesized and incorporated into SLN. The brain area under the concentration-time curve of 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine SLN and 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine was 10.97- and 5.32-fold higher than that of 5-fluoro-2'-deoxyuridine, respectively (139).

Diminazene diacetate, a hydrophilic antitrypanosomal drug, was loaded (33% w/w) into lipid nanoparticles by creating nanoparticles from lipid-drug conjugates (LDC) without altering its activity, overcoming one limitation of SLN, *i.e.*, the limited loading capacity for hydrophilic drugs. These diminazene LDC nanoparticles were prepared using polysorbate 80 as stabilizing agent for enhancing brain delivery to treat second-stage human African trypanosomiasis (140). Extensive research is in progress to develop SLN as delivery systems for drug targeting to the brain (141-144).

6. Lipid microspheres

Lipid microspheres (LMs) are spherical globules of size ranging from 200 to 600 nm in diameter and capable of incorporating only hydrophobic drugs. The LMs are prepared by emulsifying biocompatible oil- or triglyceride-containing lipid-soluble drugs using homogenizer and/or ultrasonicator. Lecithin or phospholipids are added as emulsifying agents. The basic LM preparation has been marketed as a nutritional supplement (Intralipid, Celepid) and is given in doses of 300-500 ml. In general, the biodistribution of LMs is similar to liposomes.

Lipid microspheres have several advantages over liposomes, including low manufacturing cost, easy mass production, high drug-loading capacity and extended shelf life of 18-24 months at room temperature. Clinprost-containing LMs are reported to permeate across the BBB by endocytosis. To avoid the trapping of LMs by the RES, small LMs and negatively charged LMs are used as an advanced drug carrier system for a variety of lipophilic drugs. Another strategy for avoiding rapid clearance uses phospholipids modified with PEG or the addition of surfactants.

In addition, anchoring of specific ligands on the surface of LMs may provide a potential pathway for targeting specific sites (145, 146). In one study, TTC-909, a newly developed isocarbacyclin methyl ester, was incorporated in LMs and accessed for neuroprotective effect. TTC-909, given intravenously 10 min after transient forebrain ischemia, dose-dependently protected against ischemia-related delayed neuronal death. This finding suggests that TTC-909 incorporated in LMs exerts a neuroprotective effect in ischemic delayed neuronal death in the hippocampus (147).

7. Polymeric micelles

Amphiphilic block copolymers such as the pluronics (polyoxyethylene polyoxypropylene block copolymers) self-assemble into polymeric micelles. Pluronic micelles solubilizing haloperidol enhanced its neuroleptic activity, presumably owing to the increased circulation time in plasma resulting in increased brain uptake. Pluronic micelles of haloperidol conjugated to an antibody to a specific antigen on brain glial cells enhanced the brain uptake of haloperidol, reducing mortality compared to pluronic encapsulated haloperidol (148, 149). The brain uptake of digoxin was also enhanced by encapsulation in pluronic micelles (150). Hydrophobic drugs may be solubilized within the core of the micelle or, alternatively, conjugated to the micelle-forming polymer. Polymeric micelles bearing targeting ligands may also be used as drug-targeting agents (151). In addition, pluronics are known to modulate the P-gp efflux transport system of the BBB and could serve in the future as delivery systems for targeting P-gp substrates to the brain (152).

8. Vector-mediated BBB transport

Partridge *et al.* (153) developed BBB transport vectors (chimeric peptide neuropharmaceuticals) for the active delivery of nontransportable peptides (153). The chimeric peptide model involves coupling of the nontransportable peptide (*e.g.,* β-endorphin) to a transportable vector such as cationized albumin or monoclonal antibodies to transferrin or insulin (53, 154). Disulfide bonds are favorable linkers in the synthesis of chimeric peptides and allow the peptide moiety to be cleaved from the vector by brain disulfide reductases following transcytosis across the BBB.

There are multiple strategies for disulfide coupling of vector to peptide. There are two steps in this process: thiolation of peptide primary amino groups and formation of activated disulfide on the vector. Avidin, an egg white cationic protein, is a potential brain drug transport vector (55). Like other cationic proteins, avidin undergoes absorption-mediated binding and endocytosis in bovine brain capillaries via a process that was inhibited by another polycationic protein, protamine. The rapid clearance of the avidin/biotin complex nearly precludes the use of avidin, *per se*, as a BBB transport vector. However, the construction of avidin fusion proteins represents a viable alternative, and allows for drug delivery to the brain of

biotinylated therapeutics, and provides the added advantage of the inherent high efficiency in coupling of biotinylated drugs to avidin fusion proteins (11). An alternative approach is the use of novel bifunctional PEG derivatives to conjugate drugs to transport vectors that allow an increase in the length of the spacer arm between the drug and the transport vector, without loss of biological activity (155).

Unusually high expression of TfR and human IR on the surface of endothelial cells of the BBB provides a potential pathway for the delivery of drugs to the brain using monoclonal antibodies. Factors to be considered while designing systems to target cell surface receptors are: 1) homogeneity of receptor expression; 2) affinity and nature of the target antigen; 3) density of the target antigen; 4) the possibility of up-down regulation following exposure to the targeting ligand; 5) the epitope on the target antigen; 6) the rate of endocytosis; 7) the route of internalization of the ligand-receptor complex; and 8) the cellular fate of the receptor-ligand complex.

A monoclonal antibody to the human IR was shown to be a highly active brain delivery vector in primates and had a BBB PS product 9-fold greater than the BBB PS product in primates of an anti-TfR MAb (155). The feasibility of using anti-TfR antibodies for the delivery of drugs to the brain was tested using the rat anti-TfR antibody OX26. Immunohistochemical studies were used to confirm that this murine MAb preferentially binds to BCEC following intravenous administration (156). OX26 binds to an extracellular domain on the TfR distinct from the transferrin binding site and does not interfere with transferrin binding (156) (Fig. 5). The ability of an anti-TfR antibody to target BCEC *in vivo* and undergo receptor-mediated endocytosis was not altered following linkage to diverse 'passenger' molecules such as methotrexate (MTX) or nerve growth factor (NGF) (157, 158). Intravenously administered OX26–NGF has been shown to stimulate the survival of fetal brain cholinergic neurons grafted onto the interior chamber of the eye (159). In another study, transferrin-conjugated NGF showed 5-fold higher accumulation in brain than a biotin-NGF conjugate, which was attributed to the increased permeability surface area product, which was 8-fold higher than that of biotin-NGF (160).

Bickel *et al.* (161) demonstrated that a biotinylated vasoactive intestinal peptide (VIP) analogue coupled to a conjugate of avidin and OX26 increased cerebral blood flow after intracarotid administration in rats, while the administration of VIP was ineffective. One disadvantage with this technique is that avidin and avidin-OX26 conjugates are rapidly removed from the peripheral circulation, necessitating intracarotid delivery.

More recently, Wu and Pardridge (162) conjugated VIP to neutral avidin (Streptavidin, SA) and confirmed that this VIP-SA-OX26 conjugate increased blood flow rates after intravenous administration in the target organ (brain), and concomitantly attenuated side effects in peripheral organs such as the salivary glands (162). Similarly, radiolabeled Aβ(I-40) ([^{125}I]-Aβ[I-40]) was monobiotyinylated (bio) and conjugated to a BBB drug delivery and brain-targeting system. This complex was comprised of either the 83-14 MAb to the human IR or the R17-217 rat monoclonal antibody to the TfR, which is tagged with SA. This system proved to be effective in delivering peptide radiopharmaceuticals to the brain which could be used for imaging amyloid-mediated or other brain disorders. However, these drug-targeting systems were metabolically unstable *in vivo* owing to active biotinidase activity. In the future, brain drug targeting in mice utilizing avidin-biotin technology will need to incorporate biotin analogues that are resistant to biotinidase (163, 164).

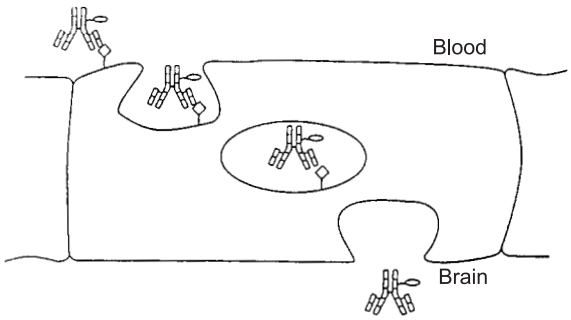

Fig. 5. Transport of anti-transferrin receptor antibody conjugates across blood-brain barrier. The antibody conjugates bind to transferrin receptors, which are present on the luminal membrane of brain capillary endothelial cells. Through the process of receptor-mediated endocytosis, the antibody conjugates are internalized into vesicular structures within the endothelial cells. The antibody conjugates are eventually transported to and released from the abluminal surface of the capillary endothelial cells, and once released into the brain, diffuse into the parenchyma.

In another study, avidin fusion proteins were employed to deliver biotinylated compounds. An antibody fusion protein consisting of an MAb specific for the TfR was genetically fused to avidin. Brain uptake of an HIV antisense drug was increased at least 15-fold when it was bound to the MAb-avidin fusion protein (anti-TfR IgG3-CH3-AV), suggesting its potential use in neurological AIDS (165). Similarly, an avidin fusion protein was employed for the delivery of a VIP analogue (VIPa) to the brain (Fig. 6). Owing to the presence of 2-3 binding sites on the avidin moiety of the avidin fusion protein, the avidin–OX26 conjugate may transport to the brain 2-3 different biotinylated therapeutics that may be biotinylated via cleavable disulfide bridges (11).

Interestingly, peripheral benzodiazepine receptors (PBR) are overexpressed on brain tumors compared to normal brain, and could serve as a target to selectively increase anticancer drug delivery through PBR ligand-drug conjugate systems. Following systemic administration, PBR-conjugated gemcitabine showed a 2-fold increase in drug levels in brain tumor tissue (48).

Equal importance should be given to development strategies for coupling drug transport vectors that result in both high-efficiency coupling and in the release of

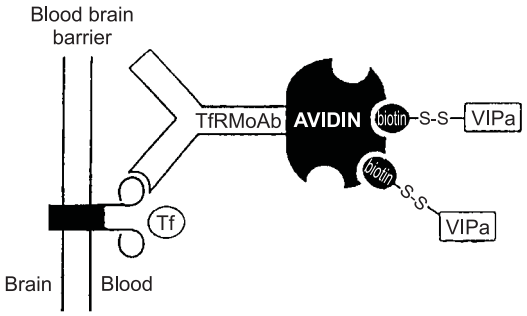

Fig. 6. Scheme of a covalent conjugate of a monoclonal antibody anti (TfR MAb) to the transferrin receptor (TfR) and avidin as a brain drug transport vector. The avidin moiety bonds the biotinylated peptide drug, in this example an analogue of vasoactive intestinal peptide, VIPa. Transferrin (Tf) does not compete with the binding side of the monoclonal antibody.

biologically active peptide following cleavage and removal of the therapeutic compound from its transport vector. Recently, the small peptide vectors —SynB vectors— penetratin and TAT have been used to deliver biologically active substances inside living cells. SynB vectors coupled to doxorubicin were shown to bypass the P-gp efflux system and drug delivery to the brain was increased 20 times by AME compared to free doxorubicin, resulting in reduced cardiotoxicity (166, 167).

Current research is using phage display technology to decipher the molecules which show selectivity for glioma cells or amyloid plaque in Alzheimer's disease and could serve as carrier ligands for efficient delivery of therapeutics or diagnostics to the target site. One such study identified three distinct families of peptides having selectivity towards malignant glioma cells, all of which contain the RGD amino acid sequence, which is known to bind to a number of integrins. Phage clones that belong to these families were internalized by RG-2 glioma cells about 63-fold more efficiently than by astrocytes, offering carrier ligands for drugs for antiglioma treatments (168).

The accumulation of Aβ peptides in amyloid plaques correlates with pathological changes that occur in the brains of patients with Alzheimer's disease. Two 20-amino-acid peptides with similar structural features that bind to the amyloid form of Aβ(1-40) but not to monomeric Aβ(1-40) were identified using phage display technology and could serve in the future as potential new carrier molecules to deliver medicines to amyloid plaques and to image plaques in the brain of Alzheimer's disease patients (169).

In addition, phage display technology has been applied to generate antiaggregating monoclonal antibodies to amyloid plaques for treating Alzheimer's disease. Restricted transport of these monoclonal antibodies across the BBB allowed the application of engineering methods to minimize their size while maintaining their biological activity (170).

Nasal administration

Targeting of drugs to the brain via the nasal route offers potential for drug development since the olfactory receptor cells are in direct contact with both the environment and the CNS. The olfactory epithelium is located on the roof of the nasal cavity adjacent to the septum and the superior turbinate. The bipolar sensory neurons, estimated to be 10-20 million in humans (171), have olfactory receptors on cilia projecting into the nasal mucus (172). The axons of these olfactory sensory cells are grouped into small bundles, which pass through the cribrioform plate of the ethmoid bone into the olfactory bulb, where they synapse with second-order neurons (173). The bundles of olfactory axons may thus provide a route of entry to the brain that circumvents the BBB.

The direct pathways for transfer of substances from the olfactory mucosa into the CNS can be broadly classified as the olfactory nerve pathway and the olfactory epithelial pathway (174). Vesicular stomatitis viruses (175), inorganic mercury (176) and agglutinin-conjugated horseradish peroxidase (177) have been shown to enter the olfactory bulb by the first pathway after entering the olfactory receptor cells. Substances absorbed by paracellular transport adjacent to the receptor cells in the nasal cavity could enter the lamina propria and then progress to the perineural space and the CNS (177, 178).

In an attempt to deliver the drug to the brain through nasal administration, radiolabeled dopamine was administered intranasally and intravenously to Sprague-Dawley rats. The bioavailability after nasal administration of [^{3}H]-dopamine was found to be 68 ± 30%. The uptake of [^{3}H]-dopamine in the brain was significantly higher after nasal administration compared to intravenous administration. The higher amount of unchanged dopamine in the brain after nasal administration of [^{3}H]-dopamine to rats indicates that a direct pathway exists for this drug from the nasal cavity along the olfactory neurons into the brain (179). Tirucherai *et al.* (180) discussed the importance of developing prodrugs having selectivity for the nutrient transport systems of the nasal mucosa for targeting the CNS (180). The nasal route to the brain would appear to offer an attractive alternative to the systemic route for the administration of drugs which are unable to pass the BBB (Table II).

Conclusions

Advances in novel drug delivery systems for drug delivery to the brain have progressed rapidly in recent years, and therapeutically efficient formulations have been developed for drugs that were unable to cross the BBB. Systemic administration of colloidal carriers enables the drug to concentrate in the brain endothelium and be endocytosed without causing any damage to the BBB. Surface modification of these colloidal carriers escapes entrapment by the RES and they remain in the blood for longer periods of time (for example, pegylated liposomes

Table II: List of reported drugs delivered to the brain via the olfactory pathways following nasal administration in rats.

Drug	Reference
Apomorphine	181, 182
Arecoline	183
Cocaine	184
Carboxylic acids	185
Cefalexin	186
Dopamine	179
Local anesthetics	187
L-Dopa butyl ester	188
Monosialoganglioside	189
NGF	178
Estradiol	190
Progesterone	191
(*S*)-UH-301	192
Dextromethorphan	193
Physostigmine	183
Propiomazine	194
Picolinic acid	195

and nanoparticles). Nanoparticles coated with polysorbate 80 lead to the adsorption of apo E from plasma and then appear to mimic LDL, leading to uptake by endothelial cells of the blood vessels in the brain. In addition, anchoring of specific ligands to the surface of colloidal carriers improves drug delivery to the brain. Phage display technology could offer molecules which possess specific binding affinity for the target site and serve as ligand carriers. In the future, novel drug delivery systems with controlled size, surface charge, surface characteristics and specific ligands will be developed to deliver drugs to the brain in therapeutic quantities and will become an alternative to the present surgical and conventional methods.

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