Drug Delivery Across the Blood-Brain Barrier

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Abstract: The blood brain barrier, formed by brain capillary endothelial cells, represents the major obstacle for drug entry into the central nervous system. Efforts are ongoing to overcome this barrier without causing permanent damage to brain tissue. The present review attempts to provide key information on cerebral microvessel anatomy, features contributing to barrier function and current approaches in overcoming the blood-brain barrier using cellular and molecular methodologies to transfer drugs to the brain as well as intelligent drug delivery systems.

Keywords: Blood-brain barrier, p-glycoprotein, Drug delivery systems, Nanoparticles, Immuno-liposomes.

INTRODUCTION

A major difficulty in treatment of many central nervous system (CNS)-related diseases is delivery of therapeutic agents to the brain. Diseases, such as Morbus Parkinson, neurodegenerative disorders, epilepsy, HIV-related encephalopathy, infections or brain tumors, represent an enormous burden for public health, with immense socioeconomic consequences. Often, the limiting factor to treatment is restricted access of drugs to the CNS, with distribution into the brain limited by the blood-brain barrier, formed by the brain capillary endothelium.

Indeed, more than 98% of small molecule drugs and almost all larger drug molecules, including recombinant proteins, monoclonal antibodies and gene therapeutics, do not cross the blood-brain barrier [1]. To enable entry into the CNS, these drugs need to be reformulated and intelligent drug delivery systems exploited.

In the last decade a variety of approaches have been developed, including manipulation of export proteins at the blood-brain barrier, targeting with antibody-linked liposomes, nanoparticles, redox systems, or protein vectors, all facilitating enhanced drug uptake into the brain. The benefits of such innovative delivery systems include a decreased dose of otherwise poorly permeable drugs, continuous therapeutic drug levels, prolonged viability of pharmaceuticals with short half-life, less invasive routes of administration, fewer side effects and, as a consequence, higher patient compliance. In addition, improving brain microvessel cellular and molecular knowledge may lead to further innovation in drug delivery design for targeting drugs to the central nervous system.

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Anatomy of the Blood-Brain Barrier

The term "Blut-Hirn-Schranke" or blood-brain barrier was first coined in 1900 by Lewandowsky, who studied penetration of potassium ferrocyanide into the brain. Further experimental evidence of this physical barrier between brain and blood was demonstrated by Paul Ehrlich [2]. Injecting water-soluble dyes (e.g. the aniline dye coerulean-S) into rats, he noted a conspicuous absence of blue stain in the brain and spinal cord. Experiments by Bouffard [3] and Goldmann [4] confirmed these findings. They showed that injecting trypan blue into the spine of animals only stained brain and cerebrospinal fluid (CSF), but not the whole animal.

The endothelium surrounding the network of brain capillaries forms a selective barrier separating blood from brain. Compared to peripheral capillaries, which are fenestrated with openings up to 50 nm, cerebral endothelial cells are closely connected by tight junctions (*zonulae occludentes*) resulting in extremely high trans-endothelial resistances of up to 1500 to 2000 Ωcm^2 [5]. The capillaries are encircled by a continuous basal membrane, enclosing an intermittent cell layer, the pericytes, postulated to be involved in brain defense mechanisms. The outer surface of the basement membrane is covered by astrocytic or glial foot processes [6,7]. Most likely, astrocytes secrete soluble growth factors and thus play a role in endothelial cell differentiation.

Cerebral Microvessel Functional Properties

With regard to barrier function, brain microvessels exhibit two major characteristics: the first is a diffusion driven or passive component, reflecting physical properties of both, tight junctions between brain capillary endothelial cells, representing a seal to intercellular diffusion, and the cells themselves, exhibiting low endocytotic activity.

More recently, a second, more selective and metabolism-driven element was identified. It is the result of solute

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transport proteins embedded in the plasma membrane of endothelial cells. Together, these two barrier elements protect the CNS from potentially toxic xenobiotics, whilst concurrently denying therapeutic drugs entry to the brain and access to CNS-sites of action.

As most drugs of choice penetrate the blood-brain barrier poorly, treating certain brain diseases has remained difficult. Certainly, understanding how the different barrier components function and how they can be modulated is an important first step in meeting the challenge of efficient and selective CNS drug delivery.

Regulation of Blood-Brain Barrier Function

Brain levels of CNS therapeutics can be increased by direct manipulation of the blood-brain barrier. As mentioned above, the functional barrier has two elements: relatively impermeable tight junctions that restrict the flow of solutes between cells and drug efflux transporters that selectively prevent cellular xenobiotic accumulation from the blood. Clearly, devising strategies to bypass these elements of the barrier, while limiting CNS entry of toxic chemicals and preserving an optimal extracellular environment, is a substantial challenge. Meeting that challenge will require an understanding of the basic mechanisms contributing to the blood-brain barrier. However, compared to other barriers and excretory tissues that largely determine drug distribution in the body, our understanding of mechanisms driving xenobiotics into and out of the CNS is at an early stage.

Opening Tight Junctions at the Blood-Brain Barrier

In 1949, Broman and Olsson first described the "possible injurious effects" on cerebral blood vessels of injection of media for cerebral angiography [8]. This phenomenon was again observed by Stanley Rapoport in 1970, who for the first time postulated that drug penetration across the blood-brain barrier could be transiently increased by intra-arterial infusion of concentrated solutions [9]. Since then, a number of substances have been studied for their ability to modify the barrier. Today, intracarotid infusion of a hypertonic mannitol solution is the most commonly used method in preclinical and clinical studies. Animal studies show that osmotic disruption of the blood-brain barrier increases drug delivery to the brain by 10- to 100-fold [10]. At first glance, the mechanism responsible for barrier opening seems simple: intracarotid infusion of hypertonic mannitol increases osmolality within brain capillaries, drawing water out of the endothelial cells. Subsequent shrinkage then opens intercellular tight junctions. The effect lasts 20-30 minutes, during which drugs that normally do not cross the blood-brain barrier enter the brain [11]. However, detailed cellular mechanisms responsible for blood-brain barrier opening may well be more complex, since osmotic stress is known to affect second messenger systems as well as the cytoskeleton [10].

Recent studies show that tight junction permeability can also be increased non-osmotically. Erdlenbruch *et al.* used alkylglycerols such as 1-*O*-pentylglycerol to open the blood-brain barrier in rats with implanted brain tumors and demonstrated an increase in delivery of methotrexate to the brain [12]. They also demonstrated accumulation of FITC-dextran 40,000 within the lumens of isolated rat brain capillaries during incubation with alkylglycerols. Using isolated rat brain capillaries, we recently demonstrated that 100 mM mannitol, 100 mM sucrose and 30 mM 1-*O*-pentylglycerol increased permeability of Texas Red (~600 Daltons) to the same extent, approximately 4-fold [13]. Use of alkylglycerols looks promising, considering the potential for exact regulation of barrier opening and lack of long-term toxicity [12]. The exact mechanism of barrier opening by alkylglycerols remains to be determined. It has been suggested that alkylglycerols induce fluidization of cellular membranes and thus indirectly change tight junctional integrity.

It should be noted that there are serious limitations to the use of blood-brain barrier disruption aiming at increased drug delivery to the CNS. First, the technique is invasive and its clinical use requires considerable expertise [11]. Second, tight junctional disruption is non-selective and thus enhances entry of many blood-borne substances, such as albumin, which may well lead to unwanted side effects [14]. Opening the barrier leaves the CNS unprotected. Finally, increased blood-brain barrier permeability may be limited spatially, thus complicating drug delivery to the whole organ or specific loci [15].

Targeting Drug Efflux Transporters at the Blood-Brain Barrier

If specific transporters at the blood-brain barrier are important determinants of drug delivery to the CNS, it should be possible to target these transporters and improve drug entry. Three strategies are currently available to manipulate transport protein activity: 1) direct inhibition of transporter function using specific inhibitors, 2) short-term (minutes) regulation of transporter function through intracellular signaling, and 3) longer-term (hours to days) regulation of transporter expression through specific manipulation of transcription or translation. All of these strategies have been explored experiments with animal models. In all studies the target transporter selected was p-glycoprotein, a drug efflux pump highly expressed in brain capillary endothelial cells and recognized as an important element of the blood-brain barrier, selectively restricting access of therapeutic drugs to the CNS [16].

Direct Inhibition of P-glycoprotein. P-glycoprotein plays an important role in multidrug resistance in cancer chemotherapy and there are many direct p-glycoprotein inhibitors. The first generation of p-glycoprotein inhibitors included compounds such as verapamil, quinidine, quinine, cyclosporine A, amiodarone and nifedipine [17-20]. However, these compounds often caused toxic side-effects *in vivo* due to their low potency, weak effectiveness and poor selectivity and newer p-glycoprotein inhibitors were developed solely for the purpose of reversing drug resistance [21]. Second generation inhibitors include valspodar (PSC833) and biricodar (VX-710) and third generation inhibitors include elacridar (GF120918), tariquidar (XR-9576), zosuquidar (LY-335979), and laniquidar (R-101933) [20, 22-24]. Animal studies indicate that of the few relatively specific p-glycoprotein inhibitors available, PSC833 (valspodar) is the most effective in increasing brain drug levels [23,25].

In a recent study p-glycoprotein inhibition improved therapy in an animal model of brain cancer [26]. Malignant brain tumors (higher-grade gliomas) are rarely cured by surgery or radiotherapy and chemotherapy has been of limited value, as long as the blood-brain barrier remained intact. In addition, the brain is a sanctuary for metastases in cancer patients otherwise responsive to cytostatic drugs [27]. Taxol and its derivatives are active against various tumors and have been used in treatment of malignant glioma and brain metastases [28]. However, for brain tumors taxol's therapeutic benefit has been low and variable, primarily because of limited entry to the CNS. In a combined *in vitro*/*in vivo* approach we identified p-glycoprotein as the major factor in limiting taxol's access to the CNS and using that knowledge a strategy designed to increase the drug's effectiveness against brain tumors in an animal model was validated [26]. Using cultured brain capillary endothelial cells and isolated brain capillaries we demonstrated that taxol is a p-glycoprotein substrate and that luminal accumulation of taxol in capillaries is concentrative, specific and blocked by the p-glycoprotein inhibitor, PSC833. We then demonstrated that PSC833 pretreatment of mice not only increased taxol brain levels (iv dosing), but that combined PSC833-taxol therapy produced a dramatic therapeutic effect on taxol-sensitive transplanted human U-118 MG glioblastoma, decreasing volumes by 90% (animals dosed twice over a five-week period) [26]. In contrast, taxol itself did not affect tumor volume and neither did taxol or PSC833-taxol therapy affect the volume of implanted U-87 MG tumors, derived from a non taxol-sensitive cell line. These findings and the findings of others suggest that co-administration of p-glycoprotein inhibitors with chemotherapeutics can be beneficial for the therapy of brain tumors sensitive to p-glycoprotein substrate cytostatics [23, 25].

Short-term regulation of transporter activity. Another strategy to improve drug delivery to the brain and increase CNS drug levels is to manipulate the endothelial cell's regulatory systems to turn-off transporter function transiently. This strategy's advantage is that only selected solutes will cross the barrier and CNS protection will be preserved for the most part.

In general, regulation of transporter function may occur as a result of intracellular events, for example phosphorylation-dephosphorylation of transporters within the plasma membrane, or indirectly through endocytotic insertion and retrieval of transporters from the membrane. Until recently, signals causing rapid changes in drug export pump function have been described for liver and kidney [29-32]. In contrast, at the blood-brain barrier, we know little about regulation of p-glycoprotein activity and much of what we do know concerns mechanisms that work over hours to days rather than minutes [33-35].

In renal proximal tubule, the polypeptide hormone, endothelin-1 (ET-1), activates a signaling pathway that rapidly reduces p-glycoprotein and Mrp2 function [30-32]. We have demonstrated a similar signaling pathway affecting p-glycoprotein activity at the blood-brain barrier using isolated intact rat brain capillaries [13, 30-32]. In both, proximal tubule and brain capillaries, subnanomolar to nanomolar concentrations of ET-1 act through an ET_B receptor, NO synthase and protein kinase C to reduce p-glycoprotein-mediated transport. In brain capillaries, ET-1 reduced transport to the same level as PSC833, suggesting a complete loss of p-glycoprotein function. Moreover, when ET-1 was removed, p-glycoprotein transport function fully recovered within 30 minutes. ET-1 did not increase capillary permeability, e.g. due to opening of tight junctions. For the first time these results show that brain capillaries are able to rapidly modulate p-glycoprotein transport function. Clinical relevance of these findings might be high, since many CNS disorders have an inflammatory component and ET-1 is released during inflammation [36-38]. Indeed, our recent experiments show that lipopolysaccharide (LPS), which induces an inflammatory response, and tumor necrosis factor α (TNFα), a cytokine released during inflammation, both decrease p-glycoprotein transport function over the short-term (Hartz et al, unpublished data). As with ET-1, removal of LPS and TNFα restores p-glycoprotein function. Interesingly, blocking ET-1 signaling attenuates the effects of both LPS and TNFα, suggesting a common signaling pathway. It remains to be seen whether these signaling pathways, once fully revealed, can be manipulated in a clinically useful way.

Transcriptional regulation of transporter expression and function. A third possibility to open the blood-brain barrier is to modulate transporter expression at the level of transcription or translation. These are mechanisms that would work over the long-term, i.e. hours to days rather than minutes. Transcriptional regulation of MDR1 gene expression is not completely understood [39,40]. Even less is known about the regulation of other drug efflux pumps that are also expressed at the blood-brain barrier, such as, Mrp1, Mrp2, Mrp4 or BCRP [40].

Recently, several members of the superfamily of ligand-activated transcription factors, so-called orphan nuclear receptors, have been identified as key determinants in liver drug efflux transporter and drug metabolizing enzyme transcriptional regulation [41,43]. These nuclear receptors are thought to coordinately regulate a network of drug transporters and drug metabolizing enzymes as a defense against xenobiotics [44,45]. One of these receptors is the pregnane X receptor (PXR; *NR1I2*), first discovered by Kliewer *et al.* [46]. PXR defines a novel steroid signaling pathway, as it is activated by naturally occurring steroids, e.g. pregnenolone and progesterone, and synthetic glucocorticoids (dexamethasone) and antiglucocorticoids (RU486). Importantly, PXR is also activated by a wide range of xenobiotics, including dietary compounds, toxicants and a large number of commonly prescribed drugs [47,48]. Therefore, PXR is considered to be a 'master regulator' of xenobiotic removal [49]. Efflux transporters regulated by PXR include organic anion transporting polypeptide isoform 2 (*SLCO1A4*), bile salt export pump (*ABCB11*) and multidrug resistance-associated protein isoforms 2 and 3, Mrp2 and Mrp3 (*ABCC2, ABCC3*). Recently, Geick *et al.* discovered a complex regulatory cluster of several binding sites for PXR in the 5'-upstream promoter region of the human MDR1 gene [50]. Importantly, PXR is the only ligand-activated nuclear receptor known to control transcription of *MDR1*, and thus expression of p-

glycoprotein. Mrp2 on the other hand has been reported to be regulated by not only PXR, but two more nuclear receptors, the farnesoid X receptor, FXR, and the constitutive androstane receptor, CAR [51].

For the first time we detected PXR mRNA and protein in isolated brain capillaries; immunostaining confirmed PXR protein expression in capillary endothelial cells [35]. When isolated rat brain capillaries were exposed to the PXR ligands, pregnenolone 16α-carbonitrile (PCN) or dexamethasone, p-glycoprotein protein levels increased (quantitative immunostaining, Western blots). Transport of a fluorescent p-glycoprotein substrate into the lumens of isolated capillaries was increased in parallel. Moreover, dosing rats with PCN and dexamethasone increased p-glycoprotein expression in plasma membranes from liver, kidney and brain capillaries. P-glycoprotein-specific transport in capillaries was also upregulated. Initial experiments also show upregulation of Mrp2 in liver and brain capillaries [35] as well as of phase II metabolizing enzymes (Bauer *et al*. unpublished data). This suggests coordinate regulation of drug metabolism and efflux transport at the blood-brain barrier. However, to what extent metabolizing enzymes are involved in barrier function remains to be determined.

These findings indicate that exposure to PXR ligands should result in barrier tightening for drugs that are p-glycoprotein and Mrp2 substrates. This is potentially a serious problem, since PXR is activated by a wide range of prescription and over the counter drugs (e.g. St. John's worth). PXR is also activated by endogenous metabolites produced during organ malfunction, for example liver failure. All these factors activating PXR have the potential to effectively change the way that multiple organs distribute drugs throughout the body.

Clearly, a better understanding of drug efflux transporter regulation at the blood-brain barrier holds the promise for more efficient treatment, e.g. PXR antagonists, dietary modifications, designed to down regulate transporter expression. However, at present, there is no practical way to intervene at the level of PXR-gene interactions. First, there are no PXR-antagonists available yet and second, it is not clear to what extent p-glycoprotein expression can be downregulated by removal of PXR ligands from diet or from blocking PXR function.

Beside these molecular and cell biological approaches to getting access to the CNS a variety of more technological systems have been developed to improve transfer of drugs into the brain:

Prodrug Approaches to Overcoming the Blood-Brain Barrier

One type of chemical targeting was investigated by the group of Bodor and is applicable to a large variety of drugs, including steroids, antibiotics and antiviral drugs, neurotransmitters, antidementia and anticancer drugs as well as several peptides [52-54]. The so-called retrometabolic drug design uses a sequential metabolism approach. First a precursor or compound moves freely into the brain before it is metabolically converted and trapped. The drug carrier systems are based on the redox conversion of a lipophilic dihydropyridine into a positively charged, lipid insoluble pyridinium salt. The dihydropyridinium-type carrier is sufficiently lipophilic to passively diffuse into the brain, where it undergoes enzymatic oxidation, forming the ionic pyridinium compound, which is retained in the brain [52].

Transporters and Protein Vectors

As discussed above ATP-binding cassette (ABC)-transporters are a major limiting factor to drug entry into the brain [55]. The MDR1 gene product p-glycoprotein is most highly expressed and responsible for efflux of a wide range of compounds. Other important transporters are the multidrug-resistant associated (MRP) and breast cancer resistance (BCRP) proteins.

One option to overcome the blood-brain barrier is by-passing the protective ABC-transporters, without direct inhibition of export pumps. For example, the drug of interest can be coupled to a vector, recognized by a surface receptor and subsequently internalized. One of the best studied vector candidates is transferrin. In mammalian cells transferrin is involved in iron uptake *via* the transferrin receptor. The transferrin receptor is highly expressed at the luminal membrane of the blood-brain barrier and thus transferrin-coupled vectors may be able to transport impermeable drugs across the blood-brain barrier or at least mediate uptake into the capillary endothelium [56-58].

A drawback in using transferrin itself, however, is competitive inhibition by endogenous transferrin present in the blood. This can be overcome using an antibody against the transferrin receptor. For example, conjugation of methotrexate or vasoactive intestinal peptide to a transferrin receptor directed antibody, and i.v. administration in rats led to an increased accumulation of drug and greater pharmacological effect [59,60].

An alternative to transferrin is the iron binding protein p97 (or melanotransferrin), also able to cross the blood-brain barrier [61]. A recent study suggests that p97 has significant potential to be an effective vehicle for delivery of therapeutic drugs to the brain. *In vivo* experiments in mice showed that transport of adriamycin-p97 conjugates was 6 to 8 fold higher than transport of albumin or lactoferrin [62].

Another brain drug delivery technology, designated 2B-Trans™, makes use of the membrane bound precursor of heparin-binding epidermal growth factor, also known as the diphtheria toxin receptor. Expression is amplified in disease conditions and therefore the receptor is suitable for site-specific disease targeting. Since there are no endogenous ligands, competition is unlikely. A non-toxic mutant of diphteria toxin (CRM197; [63]), already marketed for human use in vaccination programs, with a proven safety profile, serves as a receptor specific carrier protein. Guinea pig *in vivo* data, using horseradish peroxidase as model protein drug, demonstrated efficient uptake of diphteria toxin receptor targeted delivery into the brain [64].

SynB vectors represent a further type of CNS-targeted delivery system. These are small peptide vectors, able to enhance brain uptake of various anticancer drugs, analgesics, antibiotics and others [65-69]. Use of these vectors with 10-

27 amino acid residues could significantly enhance brain uptake of molecules, without opening blood-brain barrier tight junctions. The mechanism of uptake for these vectors is not yet fully understood, but there is evidence that entry into the brain is mediated by a receptor-independent process [70,71].

Recently, small single domain antibodies (~14 kDa) were shown to transmigrate across cerebral endothelial cells *in vitro* and the blood-brain barrier *in vivo* [72], with the ability to shuttle molecules, up to 10 times their size, into or across the target tissue. The efficacy of this antibody delivery system was improved by introducing avidity by multimerization [73]. Cationized albumin or monoclonal antibodies against the insulin receptor were also tested as carriers, trying to deliver drugs across the blood-brain barrier. This chimeric peptide strategy delivers drugs *via* receptor mediated endocytosis [74-76].

Liposomal Drug Delivery Systems Targeting the Central Nervous System and Brain Tumors

The major disadvantage of all the protein based strategies is that few drug molecules are directly coupled to the signal molecules. In contrast, liposomal or nanoparticulate conjugation of vector molecules potentially allows the transfer of up to 30 000 drug molecules per liposome [77].

Intravenously administered liposomes often bind unspecifically to blood components, the opsonins, and are subsequently trapped by the reticuloendothelial system (RES) of liver, spleen, lung or bone marrow. A significant improvement is hydrophilisation of the liposomal surface, attaching polyethyleneglycol (PEG) chains (stealth® liposomes). Through coupling of signal molecules to ends of PEG residues, such as antibodies against surface receptors, targeting is improved and the RES avoided.

Immunoliposomes, coupled to a transferrin receptor antibody, were shown to internalize *in vivo* and *in vitro* and deliver p-glycoprotein substrates efficiently to the brain [77,78]. Binding to the transferrin receptor, highly expressed at the luminal surface of blood-brain barrier endothelium, these liposomes are internalized by receptor mediated endocytosis and drugs are released into the CNS. Using liposomes coupled to a monoclonal murine transferrin receptor antibody, small molecule drugs and even exogenous gene material was delivered into the brain of mice [79].

As yet, it has not been clarified whether intact liposomes pass the blood-brain barrier *in vivo* [80]. But, in cultured endothelial cells luminal p-glycoprotein was bypassed and following endosomal release of a model p-glycoprotein substrate, only small amounts were pumped back across the luminal surface [81].

A similar strategy uses liposomes coupled to cationized albumin [82], which also undergo endocytosis at the blood-brain barrier [74,83]. These liposomes bind to the luminal surface of capillaries and accumulate within cells. Uptake is inhibited by free cationized albumin, phenylarsine oxide, nocodazole and filipin, but not by dansylcadaverine, suggesting a caveolae mediated incorporation process.

Liposomal delivery seems a very promising strategy for therapy of certain brain tumors, e.g. glioblastomas. Using cationized liposomal carriers the herpes-simplex-thymidin kinase gene was targeted to tumor cells [84]. Following entry of the liposome-gene-complex into tumor cells, the gene is incorporated into cellular chromosomes. Cells then start to synthesize thymidin kinase, normally not produced by human cells. Thymidin kinase makes tumor cells vulnerable to ganciclovir, a drug currently undergoing registration for treatment of certain viral diseases. Since the liposome-gene complex predominantly enters rapidly proliferating cells one assumes that normal cells synthesize less viral thymidin kinase and thus are not significantly affected by ganciclovir treatment. Initial clinical trials, using this approach with stereotactically guided intratumoral convection-enhanced delivery, are ongoing [85,86].

Nanoparticulate Delivery Systems

Nanoparticle drug carriers and liposomes are used in similar ways. Nanoparticles are colloidal polymer particles made from biodegradable materials such as polylactides/glycolides or poly-cyanoacrylates, ranging from 10 nm to 1 μM in size. Drugs are attached to the carriers by adsorption, incorporation or covalent linkage.

A critical aspect is nanoparticle surface charge. Whereas neutral nanoparticles and low concentration anionic nanoparticles can be used as colloidal drug carriers for delivery into the brain, cationic nanoparticles seem to disrupt the blood-brain barrier and thus exert toxic effects [87].

Similar to liposomes, nanoparticles can absorb opsonins, rendering targeting to the brain more difficult. Incorporation of surface active components, such as polysorbate 80, facilitates movement across the blood-brain barrier and nanoparticles, taken up by endothelial cells, can deliver drugs of otherwise low permeability into the brain [88-90]. Drugs delivered include loperamide, doxorubicin, kytorphin or dalargin. The analgesic hexapeptide drug dalargin, incorporated into poly(butyl-cyanocarylate) particles, was able to induce a significant antinoceptive effect after administration to rats [90,91]. Apparently polysorbate-precoating and apolipoprotein B or E over-coating enhanced observed effects, indicating that apolipoproteins act as mediators of drug delivery. One hypothesis is that polysorbate 80-coated particles absorb blood-borne apolipoproteins after injection, mimicking lipoprotein particles, and are then taken up by the brain capillary endothelial cells *via* receptor mediated endocytosis. Internalited drugs would then reach the brain by diffusion or transcytosis. These types of nanoparticles may be particularly useful for chemotherapy of disseminated and aggressive brain tumors [89,92].

Perspectives

Drug targeting has recently seen a remarkable boom and the technologies described above have spurred numerous market applications. After 25 years of rather disillusioning clinical trials applying potential drug candidates directly or incorporating drugs into conventional liposomes, new vector technologies and innovative chemical drug modifications offer promising tools.

Modified liposomes and nanoparticles, loaded with vaccines, anti-inflammatory drugs, new antibiotics, cytostatics or genes, are now beginning to yield satisfactory results in *in vivo* experiments and even clinical trials. Albeit, broad use of the technologies mentioned will require some time, but continuous optimization of carrier systems will lead to new composites, offering further possibilities for drug delivery to target cells, tissues and organs.

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