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Overcoming MDR at the blood–brain barrier

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Abstract. The brain capillaries represent at major barrier preventing entry into the brain for xenobiotics and many drugs, which otherwise may be therapeutically active in the central nervous system. The ABC transporter, p-glycoprotein, which is predominantly located in the lumenal surface of capillary endothelial cells, is a key player in this barrier function. Thus, particular efforts have been made to overcome the barrier or to circumvent this efflux pump. At present various options are under consideration: (a) direct modulation of the transporter by specific inhibitors, (b) interference with the regulatory cascade of protein transcription, translation and function, and (c) encapsulation of substrates into delivery systems, which are able to by-pass p-glycoprotein. Among the inhibitors being under development to block p-glycoprotein, the non-immuno-suppressive cyclosporin A-analogue PSC-833 has gained most attention. In-vivo data in nude mice with an intracerebrally transplanted human glioblastoma demonstrated, that administration of the otherwise non-effective cytostatic Paclitaxel results in a dramatic decrease of tumor size after co-administration of PSC-833. Understanding the cascades underlying the regulation of p-glycoprotein expression and function, such as endothelin-receptor regulated activity or the influence of nuclear transcription factors such as pregnane X receptor, on p-glycoprotein expression may offer novel therapeutic options to control drug transport across the blood–brain barrier. Several strategies designed to by-pass p-glycoprotein without direct inhibition have been described. The applied systems use antibody-coupled immunoliposomes, or albumin-associated liposomes and nanoparticles, respectively, to move the encapsulated drug through the lumenal plasma membrane of capillary endothelial cells avoiding direct contact with p-glycoprotein. Within the past decade it became increasingly clear that the brain microvascular endothelium is a dynamic barrier, with p-glycoprotein being a central modulator of barrier function. Although rapid progress has been made in identifying multiple mechanisms that modulate p-glycoprotein action, still much more remains to be discovered, including practical aspects of inhibitor development, modification of regulatory pathways or development of specific delivery systems to lower the barrier function in brain capillaries.

Keywords: Blood–brain barrier; p-glycoprotein; PSC-833; Paclitaxel; Brain tumor; Endothelin receptor; Nuclear transcription factor; Pregnane X receptor; Immunoliposome; Nanoparticle

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1. Introduction

A major problem in the management of CNS diseases is the limited entry of drugs into the brain. The basis for that restricted access is the blood–brain barrier, which is formed by the brain capillary endothelium. This endothelium exhibits a variety of structural features, which makes it different from peripheral capillaries. Whereas those are fenestrated with gaps up to 50 nm width, cerebral endothelial cells are closely connected by tight junctions and zonulae occludentes, resulting in extremely high *trans*-endothelial resistances of approximately 1500 to 2000 Ω cm^2. The capillaries are surrounded by a continuous basal membrane enclosing pericytes, which have been postulated to be involved in defense mechanisms. The outer surface of the basement membrane is covered by astrocytic or glial foot processes [1,2] and there is evidence that secretion of soluble growth factors by astrocytes may play a role in endothelial cell differentiation.

Functionally, the blood–brain barrier has two major elements, which protect the central nervous system from potentially hazardous xenobiotics, but simultaneously represent an obstacle to therapeutic drugs targeting sites of action inside the CNS. The first is a passive diffusional element, which reflects the physical properties of the tight junctions and the cells exhibiting a low rate of endocytosis. The second element reflects the activity of solute carrier proteins (transporters) embedded in the plasma membranes of the endothelial cells (Fig. 1).

Multispecific xenobiotic transporters play an outstanding role in the protective function of the blood–brain barrier. At present, mRNA for 15 drug transporters including the organic anion transporting polypeptide (OATP), organic anion transporter (OAT), multidrug-resistance protein (MDR), multidrug-resistance-associated protein (MRP), organic cation transporter (OCT), concentrative nucleoside transporter (CNT) and equilibrative nucleoside transporter (ENT) subfamilies have been identified in brain capillaries or brain capillary endothelial cell lines. Eight transporters have been immunolocalized within brain capillary endothelial cells, four of them being ATP-driven drug export pumps on the luminal (blood-side) plasma membrane: p-glycoprotein (the MDR1 gene product), breast cancer resistance protein (BCRP), MRP2 and MRP4. Together these

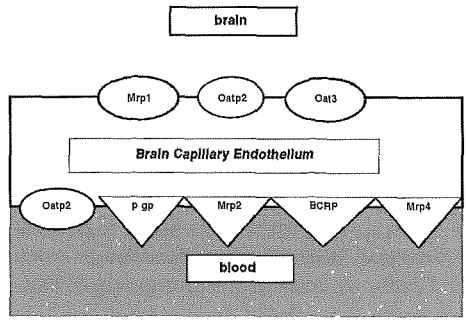

Fig. 1. Distribution of xenobiotic transporters at the blood–brain barrier. Only transporters with known localization are shown. They account for about half of the xenobiotic transporters expressed. Abbreviations: organic anion transporter (OAT), multidrug-resistance-associated protein (MRP), p-glycoprotein (p-GP), organic anion transporting polypeptide (OATP), breast cancer resistance-associated protein (BCRP).

Table 1
Examples of drug classes and individual drugs transported by p-glycoprotein

Ca^{2+} channel blockers
Verapamil and analogues
Diltiazem
Mibefradil
Nifedipine, Nitrendipine, Felodipine
Antineoplastic agents
Taxanes
Vinca-alkaloids
Etoposide and analogues
Anthracyclines
Imatinib
Lonafarnib
HIV protease inhibitors
Ritonavir
Saquinavir
Amprenavir
Nelfinavir
Lopinavir
Lipid lowering agents
Atorvastatin
Lovastatin
Simvastatin lactone
Antibiotics
Erythromycin
Rifampicin
β-Adrenoceptor antagonists
Carvedilol
Celiprolol
Talinolol
Bunitrolol
Carazolol
Immunosuppressive drugs
Cyclosporine A and derivatives
Tacrolimus (FK506) and derivatives
Sirolimus (Rapamycin) and derivatives
Opioids
Morphine-6-glucuronide
Methadone
Loperamide
Fentanyl
Asimadoline

(continued on next page)

Table 1 (*continued*)

Diverse
Prazosin (anti-hyptertensive)
Amytripiline (anti-depressant)
Digoxin (cardiac drug)
Midazolam (Benzodiazepine)
Ranitidine (H2-receptor antagonist)
Methotrexate (antirheumatic)
Ivermectin (anthelmintic)
Octreotide (somatostatin analogue)

transporters can handle a very wide range of anionic (MRP2 and MRP4), cationic (P-glycoprotein and BCRP) and uncharged (all four) xenobiotics. All four types of carrier proteins belong to the large class of ABC (ATP binding cassette) transport proteins. They are present at high levels in a variety of normal tissues with various physiological functions, such as the adrenal cortex, the brush border membrane of renal proximal tubules, the apical membrane of enterocytes in the gut, testis, endometrium of pregnant uterus and the blood–brain barrier.

At the blood–brain barrier, p-glycoprotein has gained the highest attention. Its location, multispecificity and potency have combined to make it the most critical barrier to entry of a large variety of therapeutic drugs into the CNS. Some examples are listed in Table 1. The clinical relevance of p-glycoprotein has impressively been shown for HIV-protease inhibitors: Several in vitro, in situ and in vivo studies have demonstrated that Indinavir, Ritonavir, Saquinavir, and others are substrates of p-glycoprotein [3,4]. Other clinically relevant drugs, which have been shown to be actively transported by p-glycoprotein, include anticancer drugs, such as vinca alkaloids, doxorubicin or taxanes. Similarly to the HIV protease inhibitors, the consequence of p-gp at the blood–brain barrier is a diminished drug permeation and hence a lower therapeutic efficacy in the chemotherapy of brain tumors [5–7].

The outstanding role of p-gp makes it an obvious target for therapeutic approaches to overcome the blood–brain barrier. Several strategies have been tested, some of them with remarkable success. Changes in p-glycoprotein activity could result from: (a) direct modification of the excretory function by inhibitors, (b) alterations in the regulatory cascades of the protein resulting in altered transporter synthesis and function, or (c) by-passing the export pump by encapsulation of substrates in delivery systems not being recognized by the carrier protein.

2. Overcoming MDR

2.1. Direct inhibition of p-glycoprotein

Several inhibitors of p-gp have been developed and came into clinical trails. Having found diverse rather unspecific "first generation" inhibitors like verapamil or cyclosporine A, "second generation" inhibitors have been developed with a better side effect profile: Valspodar (PSC-833), elacridar (GF 120918, GG918; a substituted isoquinolinyl acridoecarboxamide), biricodar (VX-710, Incel; an amino-keto-pipecolinate derivative)

or dexniguldipine (a dihydropyridine derivative). Inhibitors with even higher selectivity and less influence on P_{450} 3A4 drug metabolism belong to the "third generation" of p-gp inhibitors, among them ONT-093 (a diarylimidazol compound), zosuquidar (LY335 979; a difluorocyclopropyl dibenzosuberane), tariquidar (XR9576, an anthranilic acid derivative) or laniquidar (R101933) a benzacepine-3-carboxylate derivative [8–10].

Whereas most of these inhibitors have been tested in various cancer indications, primarily PSC-833, a non-immunosuppressive cyclosporin A-derivative, has been shown to be most effective in increasing brain toxin levels [11,12]. For example, this has been demonstrated in vitro and in vivo by our recent studies with the chemotherapeutic, paclitaxel (taxol) [5]. Taxol and derivatives are active against various tumors and have been used to treat malignant glioma and brain metastases [13]. However, higher-grade gliomas and other brain tumor are rarely cured by surgery or radiotherapy and as long as the blood–brain barrier remains intact, also chemotherapy shows only very limited success. In addition, the brain is a sanctuary for metastases in cancer patients otherwise responding to cytostatic drugs.

We first were able to show in isolated brain capillaries that accumulation of taxol in the lumenal space of the vessels is a concentrative and specific process, which can be blocked by PSC-833 in a dose-dependent manner (Fig. 2). Subsequently, we demonstrated that oral administration of PSC-833 to nude mice did not only increase CNS levels of taxol after i.v. dosing, but that it also produced a dramatic therapeutic effect on a paclitaxel-sensitive orthotopic transplanted human glioblastoma. When taxol was given alone, it did not affect the volume of the intracerebrally implanted U-118 MG tumors, but the combination of PSC-833 and taxol decreased tumor volume by 90% (Fig. 3), when the animals were dosed twice over a 5-week period. In contrast, neither taxol nor the combination of PSC-833 and taxol had any effect on the volume of implanted U-87 MG tumors, derived from a cell line, which was not taxol-sensitive. Neither glioblastoma cell line exhibited a multidrug-resistance phenotype. These observations suggest that co-administration of PSC-833 or other p-glycoprotein inhibitors may be of clinical benefit for chemotherapy of brain tumors sensitive to cytostatics, which are substrates of P-glycoprotein. It remains to be determined in clinical studies, whether this approach turns out to be a general strategy for increasing brain permeation of p-glycoprotein substrates.

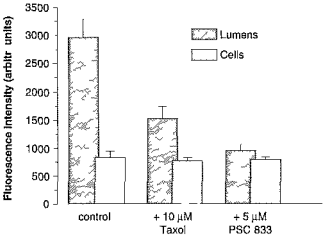

Fig. 2. Inhibition of p-glycoprotein mediated excretion of BODIPY-Paclitaxel into the lumen of isolated, functionally intact porcine brain capillaries by unlabeled paclitaxel or the p-glycoprotein blocker PSC-833 (Valspodar). Capillaries were incubated with 1 µM fluorescent paclitaxel derivative in the absence and in the presence of paclitaxel and PSC-833, respectively. The tissue was examined by confocal laser scanning microscopy [5].

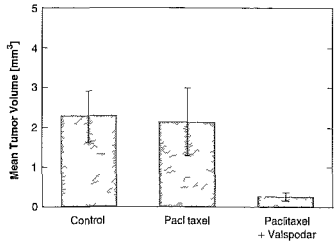

Fig. 3. Effect of valspodar/paclitaxel co-administration on the intracerebral growth of the human U 118 MG glioblastoma in nude mice Controls received no medication, the paclitaxel group received only paclitaxel, the paclitaxel–valspodar group received 50 mg/kg valspodar p.o. before i.v.paclitaxel. Animals were treated in this way on day 8 (3 mg/kg paclitaxel) and day 15 (2 mg/kg paclitaxel) post-implantation of tumors. 35 days after tumor implantation, the brains were collected and tumor volumes determined morpho-metrically. Mean values±S.E.M. (n=5).

From this example it becomes clear that direct inhibition of ABC transporters may be of therapeutic benefit in situations where acute dosing is indicated. However, it is uncertain whether chronic administration of blocking agents is feasible given the protective role of p-glycoprotein and other ABC transporters in the blood–brain barrier and other organs.

2.2. Regulatory pathways modulation p-glycoprotein function and expression

Another strategy of modifying p-glycoprotein may result from understanding the regulatory pathways underlying the synthesis and function of the export pump. For CNS therapy, it would clearly be advantageous to modulate p-glycoprotein function during a short time interval, while maintaining continuous long-term protection. One approach would be a transient decrease of p-gp mediated efflux by rapid regulation of transporter function.

In renal proximal tubule, recent studies have shown that Endothelin-1 (ET-1), signaling through an ET_B receptor, nitric oxide synthase (NOS), guanylyl cyclase, protein kinase G (PKG) and PKC, rapidly reduces transport mediated by p-glycoprotein and MRP2 [14,15]. We could show that ET-1 also regulates p-glycoprotein at the blood–brain barrier [16]. In intact rat brain microvessels, subnanomolar to nanomolar concentrations of ET-1 rapidly and reversibly decreased p-glycoprotein-mediated transport, as visualized by the lumenal accumulation of the fluorescent cyclosporine analogue, NBDL-CS. With 1–100 nM ET-1, transport was reduced in a similar way as with PSC-833, suggesting almost complete loss of function.

ET-1 effects on p-glycoprotein-mediated efflux could be imitated by sarafotoxin 6c an ET_B receptor agonist. The ET_B receptor was immunolocalized to both the luminal and abluminal surfaces of the capillary endothelium. Sodum nitroprusside (SNP), which generates NO, also reduced p-glycoprotein-mediated transport, presumably mimicking the effects of NOS activation. Inhibition of protein kinase C (PKC) blocked the effects of ET-1 and of SNP, while inhibition of NOS blocked the effects of ET-1 but not of PKC activation. These data are indicative for a minimal signaling pathway being linear from the ET_B receptor to NOS to PKC (Fig. 4).

These results show for the first time a loss of p-glycoprotein excretory function in brain capillaries via a regulatory pathway and its rapid recovery when the stimulus is

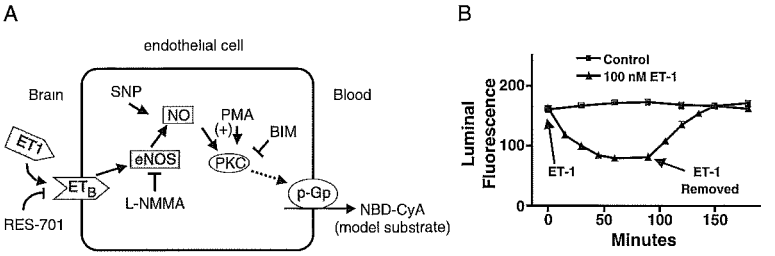

Fig. 4. ET-1 signaling of ABC transporter function in rat brain capillaries (A). Reduced p-glycoprotein-mediated luminal accumulation of a fluorescent cyclosporine A derivative (NBD-CSA) upon exposure to ET-1 and rapid recovery when the hormone was removed (B). (LNMMA=L-NG-monomethyl-arginine; SNP=sodium nitroprusside; NOS=NO synthase; PMA=phorbol ester, BIM=Bisindolylmaleimide)

removed. However, the use of ET or its agonists in patients is not feasible. It remains to be seen whether signaling can be manipulated in such a way as to make it useful in the clinic.

Beside, this fast functional regulation p-glycoprotein may also be affected by transcriptional modulation, which is a complex and not yet completely understood process. Recently [17], a regulatory cluster of several binding sites for the ligand-activated nuclear receptor, pregnane X receptor (PXR), was found in the 5′-upstream region of *hMDR1*. Reporter gene assays confirmed that this cluster of response elements is responsible for PXR-mediated *hMDR1* induction.

The pregnane X receptor, PXR (*NR1I2*) [18,19], is a member of a superfamily of ligand-activated transcription factors, the so-called orphan nuclear receptors. Belonging to a steroid signaling pathway, it is activated by natural steroids such as pregnenolone and progesterone, and synthetic glucocorticoids and anti-glucocorticoids. Importantly, PXR can also be activated by a variety of xenobiotics including dietary compounds and a large number of commonly prescribed drugs. As a consequence, PXR regulates a number of target genes, that are involved in xenobiotic metabolism and efflux. Export proteins regulated by PXR include organic anion transporting polypeptide isoform 2 (*SLCO1A4*), bile salt export pump (*ABCB11*), multidrug-resistance-associated proteins isoforms 2 and 3, Mrp2 and Mrp3 (*ABCC2, ABCC3*) and p-glycoprotein (*ABCB1, MDR1*). Therefore, PXR has been described to act as a 'master regulator' of xenobiotic removal [20]. It is important to note that PXR is the only ligand-activated nuclear receptor known to control transcription of *MDR1*, and thus expression of p-glycoprotein.

Using RT-PCR, we detected PXR mRNA in isolated rat brain capillaries. Receptor expression in endothelial cells was confirmed by immunostaining [21]. When we incubated isolated rat brain microvessels with two PXR ligands, pregnenolone 16α-carbonitrile (PCN) and dexamethasone, mRNA (quantitative RT-PCR) and protein (Western blots and quantitative immunostaining) levels for p-glycoprotein increased. In addition, p-glycoprotein-mediated transport of fluorescent substrates into capillary lumens could be observed. Moreover, dosing rats with PCN and dexamethasone increased p-glycoprotein expression in plasma membranes from liver and brain capillaries and upregulated specific transport in the capillaries (Fig. 5).

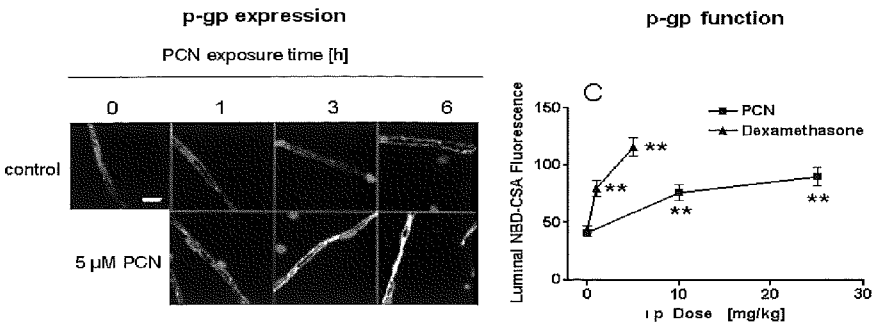

Fig. 5. Time course of increased p-glycoprotein expression in rat brain capillaries exposed to 5 µM PCN in vitro; confocal images of immunostained capillaries (A). Stimulation of p-gp mediated (PSC-833 sensitive) luminal accumulation of p-gp substrate NBD-Cyclosporin A. Each point represents a mean value from 20 capillaries [21].

These experiments provide the first evidence for PXR expression in brain and for regulation of xenobiotic transporters at the blood–brain barrier by PXR. They reveal one possibility by which the activity of p-glycoprotein, a primary gatekeeper of the blood–brain barrier, is modulated and argue for selective tightening of the blood–brain barrier in patients exposed to the wide range of xenobiotics that are PXR ligands.

Increased pump expression should result in enhanced neuroprotection. However, since many p-glycoprotein substrates are used to treat CNS disorders, increased pump expression also implies reduced access of such drugs to their site of action inside the CNS. Many p-glycoprotein substrates are also PXR ligands. Thus, it is possible that PXR activation up-regulates p-glycoprotein expression and thus drug resistance when certain substrates are given chronically. In addition, PXR action is known to be affected by multiple co-repressors and co-activators within the cell nucleus. However, at present, there is no practical way to intervene at the level of PXR-gene interactions. Up to now it is not clear to whether p-glycoprotein expression can be down-regulated by removal of PXR ligands from the diet. It has to be expected that the effects of diet modification are greatest on transporter expression in tissues of the GI tract, but there may be a benefit to the blood–brain barrier of patients scheduled to undergo pharmacotherapy for CNS disorders.

2.3. Drug delivery systems by-passing p-glycoprotein

Another option to overcome MDR at the blood–brain barrier is by-passing p-glycoprotein without direct inhibition of the export pump. For that purpose, several systems have been described. One system uses antibody-coupled immunoliposomes to transport p-glycoprotein substrates. The strategy is to move the encapsulated drug through the lumenal plasma membrane of micovessel endothelial cells thereby avoiding direct interaction with the export pump (Fig. 6). Immunoliposomes, which are coupled to an antitransferrin receptor antibody, have been demonstrated in vivo and in vitro to be internalized by receptor-mediated endocytosis and to deliver p-gp substrates efficiently to the brain [22–24]. Similar delivery could be achieved with liposomes coupled to cationized albumin [25]. Another system utilizes drug-containing nano-particles, which pass through the lumenal membrane by endocytosis. Nanoparticles

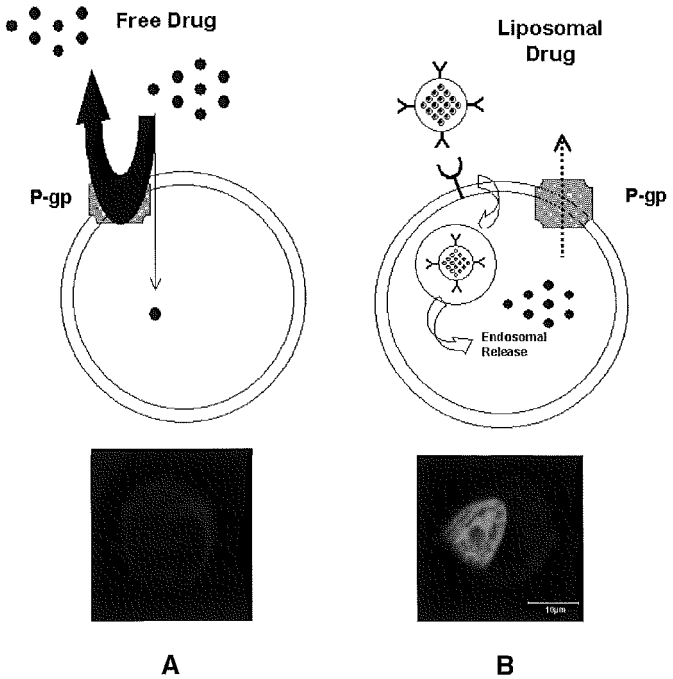

Fig. 6. Approach of by-passing p-glycoprotein with immunoliposomes. (A) Cellular accumulation of drug is severely limited by efflux mediated by p-glycoprotein. (B) Drug accumulates after membrane permeation of the drug-loaded liposome and subsequent endosomal release [modified from 23].

have been used to deliver several drugs into the brain and have been especially useful in chemotherapy of disseminated and aggressive brain tumors [26,27].

3. Perspectives

Our understanding of the complexity of the blood–brain barrier has greatly increased during the past decade. It has become increasingly clear that the cerebral microvessel endothelium is a dynamic barrier, with the properties of passive and active/ selectiveelements, which are capable of changing dramatically as a result of hormonal signaling, disease and therapy. The discovery of active carrier proteins and cytotic mechanisms andelements, which are capable of changing dramatically as a result of hormonal signaling, disease and therapy. The discovery of active carrier proteins and cytotic mechanisms and their contribution to drug permeation across the barrier is a great step forward to the successful development of efficient CNS drugs and to the understanding of unwanted CNSside effects of non-CNS drugs. Because of the key role that p-glycoprotein plays in determining xenobiotic entry into the brain, understanding how the activity of this effluxtransporter is modulated may provide practical strategies for selectively manipulating barrier permeability for a large number of drugs. From the molecular biology and pharmacology of this export pump, we may be able to identify even more specific probes to block its function or to reversibly modify its barrier

function through competition. Translational research, including optimisation of dosing protocols, finding of modifiers of nuclear receptor activity or development of even more blood–brain barrier specific delivery systems will help to define new therapeutic strategies versus CNS diseases in the future.

Acknowledgements

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References

[1] G.W. Goldstein, A.L. Betz, The blood–brain barrier, Sci. Am. 255 (1986) 74–83.
[2] M.W. Bradbury, The blood–brain barrier, Exp. Physiol. 78 (1993) 453–472.
[3] R.B. Kim, et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest. 101 (1998) 289–294.
[4] J. Drewe, et al., HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833, J. Biochem. Pharmacol. 57 (1999) 1147–1152.
[5] S. Fellner, et al., Transport of paclitaxel (Taxol) across the blood–brain barrier in vitro and in vivo, J. Clin. Invest. 110 (2002) 1309–1318.
[6] A. Regina, et al., Multidrug resistance in brain tumors: roles of the blood–brain barrier, Cancer Metastasis Rev. 20 (2001) 13–25.
[7] J.M. Gallo, et al., The effect of P-glycoprotein on paclitaxel brain and brain tumor distribution in mice, Cancer Res. 63 (2003) 5114–5117.
[8] T. Yanigasawa, et al., An effective chemosensitizer in neuroblastoma, Br. J. Cancer 80 (1999) 1190–1196.
[9] B. Tam, D. Piwcina-Worms, L. Ratner, Multidrug resistance transporters and modulation, Curr. Opin. Oncol. 12 (2000) 450–458.
[10] H. Thomas, H.M. Coley, Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein, Cancer Control 10 (2003) 159–165.
[11] E.M. Kemper, et al., Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein, Eur. J. Cancer 40 (2004) 1269–1274.
[12] E.M. Kemper, et al., Increased penetration of paclitaxel into the brain by inhibition of p-glycoprotein, Clin. Cancer Res. 9 (2003) 2849–2855.
[13] A.A. Brandes, et al., New strategy developments in brain tumor therapy, Curr. Pharm. Des. 7 (2001) 1553–1580.
[14] R. Masereeuw, et al., Endothelin B receptor-mediated regulation of ATP-driven drug secretion in renal proximal tubule, Mol. Pharmacol. 57 (2000) 59–67.
[15] S.A. Terlouw, et al., Nephrotoxicants induce endothelin release and signaling in renal proximal tubules: effect on drug efflux, Mol. Pharmacol. 59 (2001) 1433–1440.
[16] A.M. Hartz, et al., Rapid regulation of p-glycoprotein at the blood-brain barrier by endothelin-1, Mol. Pharmacol. 66 (2004) 387–394.
[17] A. Geick, M. Eichelbaum, O. Burk, Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin, J. Biol. Chem. 276 (2001) 14581–14587.
[18] S.A. Kliewer, et al., An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway, Cell 92 (1998) 73–82.

[19] J.M. Lehmann, et al., The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions, J. Clin. Invest. 102 (1998) 1016–1023.
[20] I. Dussault, B.M. Forman, The nuclear receptor PXR: a master regulator of "homeland" defense, Crit. Rev. Eukaryot. Gene Expr. 12 (2002) 53–64.
[21] B. Bauer, et al., Pregnane x receptor up-regulation of p-glycoprotein expression and transport function at the blood–brain barrier, Mol. Pharmacol. 66 (2004) 413–419.
[22] J. Huwyler, D. Wu, W.M. Pardridge, Brain drug delivery of small molecules using immunoliposomes, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 14164–14169.
[23] A. Cerletti, et al., Endocytosis and transcytosis of an immunoliposome-based brain drug delivery system, J. Drug Target. 8 (2000) 435–447.
[24] J. Huwyler, et al., By-passing of P-glycoprotein using immunoliposomes, J. Drug Target. 10 (2002) 73–79.
[25] M. Thöle, et al., Uptake of cationized albumin-coupled liposomes by cultured brain microvessel endothelial cells and intact brain capillaries, J. Drug Target. 10 (2002) 337–344.
[26] J. Kreuter, Nanoparticulate systems for brain delivery of drugs, Adv. Drug Deliv. Rev. 47 (2001) 65–81.
[27] S.C. Steiniger, et al., Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles, Int. J. Cancer 109 (2004) 759–767.

Discussion Gert Fricker

Galla

If you directly take positively charged liposomes you could incorporate positively charged lipids. Do you need BSA as well? I think you would never know how much you need because, as you have shown, if you have an excess of BSA it reduces the uptake, because probably the negative charges on the cellular membrane are blocked.

Fricker

It's probably also possible with positively-charged liposomes. The problem is that most of the positive lipids are quite cytotoxic.

Van Tellingen

You use U-118 tumour model. We don't like to use it because we frequently see spontaneous regressions of those tumour cells in mice, suggesting that there is some immune or other response going on in those tumours, which causes the regression. I was also a little bit puzzled about your use of paclitaxel in dosages of 2 mg/Kg which is actually very low. We have used doses of 20 or 30 mg/Kg and they are still not effective.

Fricker

We didn't look at tumour regression. What we saw as an effect in the animals was a loss of weight, which was tolerable. We also compared the U-87 cell line, which you have used, and we saw that the sensitivity of the U-87 to paclitaxel is much lower than the sensitivity of the U-118 cell line, and that's the reason why we used that for in vivo experiments.

Van Tellingen

The point that many people don't realise enough is that when we use xenograph models we put the tumors in quite a hostile environment, and at the moment we are able to hit those tumours considerably it is the body which will clear off all the other tumour cells. And that is, of course, something which, unfortunately, does not occur in patients.

Fricker

Another point is, what are the consequence for other parts of the body when you block the P-glycoprotein. You have this dramatic increase of paclitaxel concentration in the rest of the brain and not only in the tumour. So the question is how useful are these approaches at all because you may end up altering the distribution of PGP substrates in other parts of the body.

Lemaire

You brought up a classification of P-gP inhibitors and I was interested in the new class number 3, the new one. Could you specify the advantages of compounds in class number 3 versus those in class number 2?

Fricker

It is claimed that they that they affect to a much lower extent the cytochrome P450 metabolism than the class 2 inhibitors. I don't whether they are really more specific for p-glycoprotein.

Van Tellingen

We have also been working with zosuquidar, which is a Lilly compound, and it has some effect. For some reason the Oncogen compound and also the Johnson compound, the R101933, they do really nothing to the distribution of paclitaxel in the brain.

Fricker

I just want to say we did some studies with elacridar and it seems to be more active than the PSCE833.

Staminirovic

Not really. ET blockers are activators of NO synthasesynthetase and of protein kinase C and we measured the effect on the cyclosporine crossing, so it's most likely that it is this pathway which regulates their functional activity, but what happens directly to the transporter, I don't know.

Fricker

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Scherrmann

My question is on the regulation of PGP at the BBB within in vivo conditions. Do you think that PGP is inducible? PGP is highly expressed in the BBB. Do you think that its level can increase?

Fricker

Certainly. The experiment with the PCN was done with isolated capillaries, where an increased immunostaining was detected, and the functional data was from intact animals. The animal was treated with the PCN and then the capillaries were isolated and the function of the P-glycoprotein was measured. Cyclosporin was used, which is a very good substrate for PGP but it doesn't significantly interact with MRP proteins, so this can be certainly attributed to the PGP function.

Whittle

In your experiments with U-118 line, did you do the experiment where you gave the animal just the valspodar alone. What was the effect?

Fricker

We gave the valspodar alone, we gave just a vehicle of the valspodar, which was a kind of micro-emulsion and we didn't see any effect on the tumours. What we saw was an effect on the weight of the animals.

Whittle

On the other hand, dexamethasone is commonly prescribed in patients with malignant brain tumours; if it up-regulates the expressional function of the PGP then it could have an adverse effect on chemotherapy. Would you like to comment on that?

Fricker

This has to be evaluated, this is very new data. We didn't actually follow pharmacokinetic profiles of PGP substrates during the dexamethasone treatment, but this should certainly be done. I think it is very likely that you have redistribution by activating the PGP expression.

De Boer

Maybe in addition to that, all those corticosteroids close the blood–brain barrier.

Du Souich

Concerning PGP inactivation, the transporter may be rapidly inactivated in at least two ways: Firstly, nitric oxide can nitrosylate tyrosine residues and this could result in inactivation; and secondly, since kinases are activated, phosphorylation of the transporter could also reduce its activity. An interesting thing is that we are always tempted to compare PGP with cytochrome P450, but the increase in expression of PGP in 6 hours is very much faster than that of most of the P450 isoforms which usually take much longer, easily 24 hours.