Future perspectives for brain pharmacotherapies: implications of drug transport processes at the blood–brain barrier

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Abstract: Years ago, ischemic stroke was regarded as a model disease for the development of neuroprotective therapies by the pharmacological industry. Results were disappointing. There are still no treatments available allowing the rescue of brain tissue once a stroke has occurred. Study failure is not only a problem in the stroke field. In other neurodegenerative conditions and in non-degenerative brain disorders, progress in drug development was also rather scarce until recently. An important factor in drug failure is the blood–brain barrier, which expresses active transporters that eliminate drugs from the brain. These transporters exhibit strong variations between different animals, which make it difficult to predict brain concentrations of drugs over species barriers. This paper claims that more detailed knowledge about: (1) the biology of blood–brain barrier transporters; (2) their regulation in brain disease, (3) the affinity of transporters to candidate drugs; and (4) the accumulation of drugs in brain tissue is needed for the overall success of clinical trials to be improved. An alternative strategy could be the use of disease-modifying treatments that do not have to enter the brain to exert their function. As such, restorative and anti-inflammatory strategies acting at the blood–brain interface might gain therapeutic potential in the future.

Keywords: ABC transporter, biodistribution, pharmacology

Introduction

For many years, ischemic stroke was regarded as a model disease of degenerative brain illnesses. Based on its high prevalence and socioeconomic relevance, and the well-defined character of the stroke insult characterized by a sudden onset of the neurodegenerative process, strong efforts were made by pharmaceutical companies to establish neuroprotective drugs that prevent the progression of injury once a stroke has occurred. Until now, not a single compound has been shown to be successful in human patients [Savitz and Fisher, 2007; Shuaib *et al.* 2007; O'Collins *et al.* 2006]. The only efficacious therapy in the acute stroke phase is thrombolysis; for example, using tissue-plasminogen activator [Hacke *et al.* 2004; NINDS Stroke Study Group, 1995].

In early stroke studies a number of pitfalls explain why drugs failed to show efficacy. As such, candidate drugs were selected that exhibited serious side effects, that were given at wrong dosages or

that were administered outside windows of opportunities [Feuerstein *et al.* 2008; Segura *et al.* 2008; Savitz 2007; O'Collins *et al.* 2006; Stroke Therapy Academic Industry Roundtable, 1999]. NMDA receptor antagonists are a good example of these issues, inducing severe memory disturbances at clinically relevant dosages [Villmann and Becker, 2007; Albers *et al.* 2001; Davis *et al.* 2000] and being efficacious mainly in the first few minutes after stroke in animal studies [Hossmann, 2006, 1994; Mies *et al.* 1994, 1993], which is clearly before patients enter the hospital.

In addition, patient cohorts were rather small in early stroke trials, too small to reveal significance in heterogeneous patient cohorts [Stroke Therapy Academic Industry Roundtable, 2005, 2001; Wahlgren and Ahmed, 2004]. Neuroimaging possibilities were still limited in early stroke studies, and refined magnetic resonance imaging (MRI) techniques [MR Stroke Collaborative Group, 2006] did not exist. Efficacy assessments were based on rough clinical

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readouts that were perhaps inadequate to reveal modest drug actions [Donnan, 2008; Wahlgren and Ahmed, 2004].

Despite improvements in study designs, recent stroke trials were still not successful [Hermann and Bassetti, 2007a, 2007b; Savitz and Fisher, 2007]. The continued failure of stroke studies has swept away enthusiasm in the pharmaceutical industry, which has stopped its research programs in the meantime.

Lack of disease-modifying treatments in clinical neurology

That a journal focusing on neurological diseases outside the cerebrovascular field promotes a discussion on reasons of stroke study failures is most probably related to the fact that the issue of neuroprotection is also unresolved in other degenerative brain diseases. Similar to stroke, there are no survival-promoting drugs available for conditions like Parkinson's disease [Kiebertz and Ravina, 2007], amyotrophic lateral sclerosis [Festoff *et al.* 2003] or multiple system atrophy [Wenning *et al.* 2004] that are unequivocally effective.

Taking a closer look, today's difficulties in pharmacological development may even go beyond neuroprotection. Drug development in clinical neurology was highly successful in the 1960s and 1970s, when symptomatic treatments for Parkinson's disease (i.e. L-Dopa, dopaminergic agonists; e.g., see Cotzias *et al.* 1969) and epilepsy (i.e. anti-convulsants; e.g. see Livingston *et al.* 1967) entered everyday practice. Ever since, rather little progress was made in the development of new drugs, particularly of disease-modifying drugs that not only attenuate symptoms, but also counteract pathological processes.

The development of anti-inflammatory strategies in the treatment of multiple sclerosis (e.g. natalizumab) is a significant exception to that rule. Natalizumab is a humanized monoclonal antibody directed against α 4-integrin, which prevents the invasion of lymphocytes into the brain [Polman *et al.* 2006; Rudick *et al.* 2006]. In patients with relapsing-remitting multiple sclerosis, natalizumab reduces relapse rate by more than 65% [Polman *et al.* 2006]. The primary target of natalizumab is located on immune cells, thus natalizumab does not necessarily need to enter the brain to exert its function.

What are the reasons for the failure of drug therapies in other neurological diseases? Are there any perspectives to escape the problem of drug failure? Have we perhaps overestimated our ability to influence disease mechanisms? Or do we follow the wrong strategies? The present review aims to provide answers to these questions.

Complexity of biological systems

Biological systems, including the brain, have been optimized during phylogeny. As a consequence, signaling processes respond in a well-adapted way to pathological insults (see Table 1). Most signal factors are involved in not just one but a variety of biological processes. As such, pharmaceutical interventions specifically influencing one signaling pathway will invariably disturb other pathways as well. This may lead to undesired drug actions (i.e. side-effects) and exacerbate brain damage (Table 1). Drug side-effects have frequently been reported in clinical trials in the past [e.g., see Albers *et al.* 2001; Davis *et al.* 2000].

If biological processes are so well optimized, one might take a nihilistic position questioning whether it is possible to attenuate disease processes in a clinically relevant way. Studies in animal models refute that view. Several animal studies have shown that neuroprotective compounds may reduce histological injury and improve neurological deficits after stroke [Feuerstein *et al.* 2008; O'Collins *et al.* 2006; Wahlgren and Ahmed, 2004]. Why don't these compounds, which are protective in animals, work in patients? Are our animal models able to predict drug actions in clinics?

Reliability and validity of animal models

Whether animal studies are suitable for predicting drug actions is evaluated by the criteria of reliability and validity. At first glance, animal models in the stroke field fulfill both criteria to large extent. However, on closer examination, some criticisms have to be raised (Table 1).

In the field of neuroprotection, intraluminal middle cerebral artery (MCA) occlusions are widely employed for evaluating drug actions. In this model MCA blood flow is interrupted either permanently or transiently with a monofilament that is advanced through the skull base via the internal carotid artery [Hermann *et al.* 2001;

Table 1. Problems in translational neurology and suggestions for future trials.

Criticism	Remark	Suggestion
Complexity of biological systems	Biological processes optimized, respond to pathophysiological insults in a well-adapted way	Consider side actions of therapeutic interventions more carefully. Make use of system-oriented multi-parametric instead of single readouts in experimental studies. Include clinically relevant functional endpoints to evaluate relevance of effects seen.
Reliability and validity of animal models	Models artificial, not properly mimicking disease processes. Models optimized for reproducibility of injury (patients, on the other hand, exhibit highly variable pathologies) Experimental studies mostly using young, inbred animals	Use clinically relevant models in preparation for clinical trials. Use animals of different strains, include old animals or animals with preexisting pathologies (e.g. atherosclerosis), include outbred animals in studies. Although this approach requires larger animal groups, the clinical impact of observations should greatly be improved with this strategy.
Heterogenous patient cohorts	Patients with variable localization and severity of injury, patients exhibiting variable age, genetic status and risk factor profile	Perform sufficiently powered studies to compensate for data variability. Use well-defined patient cohorts in early Phase II trials. Thereby, the size of studies may considerably be reduced. Select therapeutic strategies that are likely relevant also to other disease manifestations, so that translation of findings from such well-defined cohorts to larger cohorts may successfully be achieved later on.
Species differences of drug targets	Specificities of drug targets (e.g. mutations) causing inefficacy of drugs in certain species	Test animals across species barriers. Include surrogate markers to evaluate drug actions. When inbred strains are used, examine more than one strain if possible to account for genetic specificities in a chosen animal. Include more than one species into preclinical testing.
Species differences of drug transporters	Specificities of drug transporters affecting brain accumulation of pharmacological compounds	Perform careful biodistribution studies in preparation for clinical trials. Evaluate drug concentrations in the target (i.e. brain) tissue. Stringently perform concentration measurements in all relevant species and wherever possible also in humans.

Hata *et al.* 2000a, 2000b; Endres *et al.* 1998; Longa *et al.* 1989]. Intraluminal MCA occlusion closely reflects the pathology of ischemia and reperfusion. However, ischemia is not induced by a blood clot (i.e. thrombus), but by mechanical occlusion. Thus, this model does not mimic thrombus interactions with the vasculature.

Besides intraluminal stroke models, models of cerebral thromboembolism have been established in recent years, in which standardized amounts of clot material are injected into the cerebral vasculature which are then resolved by means of thrombolytic drugs [Zhang *et al.* 2005; Kilic *et al.* 2000, 1998; Busch *et al.* 1998]. These

models much more closely reflect clinical embolic stroke. Yet the procedures of stroke induction are much more time-consuming and require strong surgical skills, which is why these models are less often used. For more detailed discussion of model systems, the reader is referred to Bacigaluppi and Hermann [2008].

Most animal models are optimized in a way that induces very standardized and reproducible lesions (Table 1). This enables researchers to detect drug effects with small animal numbers. To fulfill these requirements, inbred animals are widely used (Table 1). Inbred animals have the advantage that their molecular biology is well

characterized, which allows evaluation of mechanisms of drug action in a reliable way.

Unfortunately, the homogeneity of animal models insufficiently reflects the heterogeneity of disease processes under clinical conditions, where the patients' age, genetic status and risk factor profile, as well as the severity of damage exhibit huge variability (Table 1) [Wahlgren and Ahmed, 2004]. In the stroke field, most animal studies are performed in young animals with otherwise intact vascular systems. These studies hardly reflect stroke pathophysiology in aged animals [Petcu *et al.* 2008; Badan *et al.* 2003] and in elderly patients (Table 1) [Wahlgren and Ahmed, 2004].

The large variability regarding patient age, genetic status and risk factor profile raises the question whether it is desirable that clinical trials should always assess from the beginning the entire spectrum of disease manifestations. Early Phase II trials with more select patient groups could help to reduce the size of clinical trials, while at the same time increasing the effect strength of a drug of choice (Table 1).

Differences in drug efficacy between animal species

The usefulness of animal studies has been questioned in view of observations that some drugs exhibit efficacy in some but not other animal species or strains [Sauter and Rudin, 1995]. As such, the lack of efficacy in some species was linked with specificities in the biology of target molecules that affect drug responsiveness (Table 1). This led to the recommendation that stroke therapies should always be evaluated in more than one species before entering clinical trials [Stroke Therapy Academic Industry Roundtable, 1999]. This important recommendation was followed in several of the more recent stroke studies.

Although it is obvious that molecular signaling processes may vary between species, it seems questionable whether altered drug responsiveness alone accounts for the failure of many of the earlier trials. In fact, cell death pathways are highly conserved during evolution [Kornbluth and White, 2005; Yan and Shi, 2005], major alterations in death-related genes invariably questioning the survival of the organism either via uncontrolled apoptosis or proliferation of cells.

Ancient forms of caspases for example are already present in early invertebrates, such as *Drosophila* and *Caenorhabditis elegans* [Kornbluth and White, 2005; Yan and Shi, 2005].

That pharmacological compounds properly interact with their target structures can simply be tested in human cell cultures. New candidate drugs are broadly screened in cell culture before entering clinical studies [Hermann and Bassetti, 2007a]. Thus, genetic mutations of target structures hardly explain the lack of drug efficacy in human patients.

Transport processes influencing drug efficacy

While drug interactions with target structures achieved strong interest in the context of drug development, the question as whether a drug is at all able to reach its target tissue was sometimes neglected. As a matter of fact, all drugs that are systemically delivered need to pass the blood-brain barrier (BBB), a physiological barrier aiming to prevent the entry of blood-borne substances into the brain [Abbott *et al.* 2006; Begley, 2004]. The BBB invariably affects the accumulation and efficacy of drugs. It represents a confounding factor, which may account for negative findings [Hermann and Bassetti, 2007a].

Pharmacological compounds rarely cross the BBB unless they are highly lipophilic. Most lipophilic drugs, on the other hand, do not find their access to the brain parenchyma without encountering a shuttle mechanism, formed by transmembranous ATP-binding cassette (ABC) transporters, which actively eliminate them from the brain tissue in an ATP-dependent way [Hermann *et al.* 2006; Löscher and Potschka 2005; Higgins and Linton, 2004].

ABC transporters bind a large number of chemically unrelated pharmacological molecules [Hermann and Bassetti, 2007a and Hermann, 2007; Löscher and Potschka, 2005; Gottesman *et al.* 2002], suggesting that their biological role is keeping the brain clean from hazardous environmental influences. ABC transporters exhibit considerable overlap in the spectrum of substrates they eliminate [Hermann and Bassetti, 2007a].

ABC transporters represent a family of 48 proteins, at least 8 of which are expressed along the BBB [Hermann and Bassetti, 2007a; Hermann *et al.* 2006; Löscher and Potschka, 2005].

Figure 1. Structure and function of ABC transporters. (a) ABC transporters consist of two transmembrane domains, adjacent to which nucleotide-binding domains (NBDs) are located. There are two major models explaining the function of ABC transporters. Based on (b) the *ATP switch model*, the two NBDs of an ABC transporter dimerize upon ATP binding, forming a so-called sandwich configuration. Hydrolysis of ATP provides energy for the NBD dissociation, resulting in a switch of the transmembrane domains that mediates drug elimination. The ATP switch model well reflects that drug elimination is an ATP-dependent process. It insufficiently explains why ABC transporters extrude such a broad spectrum of chemically unrelated lipophilic compounds from cells. This aspect is better represented by (c) the *hydrophobic vacuum cleavage model*, which postulates that the configuration change in the ABC transporter induces a negative pressure in the inner leaflet of the lipid bilayer that pulls lipophilic material from the cytosol into the bilayer and from the bilayer into the extracellular space. As such, the function of ABC transporters resembles a vacuum cleaner. The hydrophobic vacuum cleavage model offers a good explanation for the broad substrate affinity of ABC transporters and for the fact that ABC transporter affinity is so closely linked with a substrate's lipophilicity. GS=glycosylation site.

ABC transporters contain two transmembrane domains, adjacent to which nucleotide-binding domains (NBD) are located (Figure 1a). The function of ABC transporters is still to some extent enigmatic. There are two major models that are presently discussed.

Based on the ATP switch model, the two NBD of an ABC transporter dimerize upon ATP binding, forming a so-called sandwich configuration (Figure 1b). Hydrolysis of ATP to ADP provides

energy for the dissociation of the NBD, with a consecutive switch in the transmembrane domains that mediates drug elimination (Figure 1b) [Higgins and Linton, 2004].

The ATP switch model well explains why ABC transporters eliminate drugs ATP-dependently. However, it does not account for the broad spectrum of lipophilic substrates ABC transporters eliminate. Broad affinities to lipophilic compounds cannot simply be reconciled with

interactions with hydrophilic proteins, particularly when interactions take place in hydrophilic cytosolic fluids.

These criticisms led to the development of an alternative model, the hydrophobic vacuum cleavage model. As such, the configuration change in the ABC transporter induces a negative pressure in the inner leaflet of the lipid bilayer that pulls lipophilic material into the bilayer and then forwards it out of the cell, thus acting as a vacuum cleaner [Sharom, 2006; Bolhuis *et al.* 1996] (Figure 1c). The hydrophobic vacuum cleavage model offers a good explanation why ABC transporters remove drugs from the brain in such a non-selective way. It also explains why ABC transporter affinity is so closely linked with a substrate's lipophilicity.

Altered ABC transporter function in the ischemic brain

In vitro studies demonstrated some years ago that ABC transporters may be upregulated under pathophysiological conditions. As such, hypoxia [Wartenberg *et al.* 2003], oxidative stress [Felix and Barrand, 2002] and glutamate exposure [Zhu and Liu, 2004] increased the expression of the ABC transporter ABCB1 (previously called multidrug resistance transporter-1). The upregulation of ABCB1 by hypoxia was shown to occur in a hypoxia-inducible factor-1 (HIF-1)-dependent manner in capillaries [Comerford *et al.* 2002], suggesting that the ABCB1 elevation is part of a coordinated adaptive gene response taking place in the injured tissue.

ABCB1 is upregulated under pathophysiological conditions not only *in vitro* but also *in vivo*, namely in ischemic stroke [Spudich *et al.* 2006]. Among known efflux carriers, ABCB1 handles the largest fraction of commonly prescribed drugs, up to 50% of all pharmacological compounds being substrates of this transporter [Spudich *et al.* 2006]. The large spectrum of substrates points out that ABCB1 might have considerable impact on the efficacy of brain pharmacotherapies.

In line with the elevated ABCB1 expression, the accumulation of neuroprotective drugs is increased particularly strongly in ischemic brain areas after pharmacological ABCB1 blockade [Spudich *et al.* 2006]. As such, drug levels can

be elevated in mice by more than an order of magnitude when ABCB1 is deactivated [Spudich *et al.* 2006]. The elevation of drug levels goes along with an improvement in neuroprotection efficacy. Studies in drug-resistant epilepsy [Löscher and Potschka, 2005] and brain cancer [Kemper *et al.* 2003; Gottesman *et al.* 2002] resulted in similar findings, namely an upregulation of ABCB1 in affected areas associated with drug resistance, corroborating the notion that pathophysiological processes alter drug distribution.

Multiplicity of drug transporters expressed at the BBB

ABCB1 is not the only drug transporter acting at the BBB. In rodents, the transporters ABCC1, ABCC2, ABCC4, ABCC5, ABCC6 and ABCG2 are also expressed on brain capillaries [Soontornmalai *et al.* 2006]. Besides ABC transporters, so-called solute carriers (SLCs) are found [Kusuhara and Sugiyama, 2005; Hagenbuch and Meier, 2004]. SLCs are cotransporters acting as symporters or antiporters, forming a superfamily of approximately 300 molecules subdivided into 43 families [Kusuhara and Sugiyama, 2005].

In contrast to ABC transporters, which are unidirectional efflux pumps, SLCs may carry drugs through the cell membrane both in an inward and outward direction [Kusuhara and Sugiyama, 2005]. Unlike ABC transporters, the driving force for drug transport is not ATP hydrolysis. Instead, concentration gradients of endogenous solutes (e.g. carnitine, urate, Na⁺, H⁺) mediate drug transport [Kusuhara and Sugiyama, 2005]. In view of the large diversity of SLCs, their role in drug accumulation *in vivo* is poorly understood. Based on their broad substrate affinities, the SLC21 and SLC22 subfamilies might play a major role in drug biodistribution.

The huge number of transporters expressed along the BBB is highly fascinating. It brings into the issue of drug biodistribution a complexity that still requires careful scrutiny. As such, for many transporters it is still unknown (1) what their physiological functions at the BBB are; (2) how they interact with currently prescribed drugs; and (3) how transporters are regulated in brain pathology. In the case of ABCB1 and ABCC2, it has recently been shown that they are regulated by the pregnane X receptor

(PXR) in brain capillaries *in vitro* and *in vivo* [Bauer *et al.* 2008]. Whether PXR is responsible for the upregulation of ABCB1 in stroke, drug resistant epilepsy and brain cancer remains to be shown. The fact that many transporters exhibit a strong overlap in the substrates they eliminate makes drug distribution processes difficult to predict.

Variation of drug transporters between species

The expression and functionality of drug transporters strongly differs between animal species. ABC transporters that are detectable along the BBB in one species are not necessarily present in another one, and transporters expressed in animals are not necessarily found in humans [reviewed by Hermann and Bassetti, 2007a]. In mice, considerable differences in transporter expression have been detected between different inbred strains [for comprehensive analysis see Soontornmalai *et al.* 2006]. Substrate affinity of the transporters may also differ between species. As such, Baltes *et al.* [2007] have recently shown differences in the affinity to phenytoin and levetiracetam of transgenic mouse and human ABCB1 in cell cultures.

Species- and strain-dependent changes in ABC transporter expression and functionality have profound implications for pharmacological therapies, as the altered transporter function invariably affects drug biodistribution. As a consequence, brain accumulation data from one animal may not be translated to another one, and similarly data from animals may not be adapted to humans (Table 1) [Hermann and Bassetti, 2007a]. Incorrect estimations of brain accumulation may have led to incorrect dose selections in previous neuroprotection trials, which may offer an explanation for problems in the translation of data between animal species as well as between animals and humans.

Implications of drug transporters for pharmacological studies

As a consequence of the variability of BBB transporter expression and function, careful brain accumulation experiments are required in preparation for clinical trials in order that proper dose selections are made. In the past, drug doses were sometimes selected based on rough measures; for example, by extrapolating

concentrations determined in one species to another species based on information about an animal's body weight and the molecule's biological half-life. In view of the above presented biodistribution concepts, such strategies are no longer inadequate [Hermann and Bassetti, 2007a].

Brain concentrations of drugs should be measured in the brain tissue. Until recently, it was difficult to determine the concentrations of drugs in the human brain, owing to the lack of the possibility of performing non-invasive pharmacological measurements. With positron emission tomography (PET), it has recently become possible to measure brain accumulation non-invasively in primates [Lee *et al.* 2006; Sharma *et al.* 2005; Murakami *et al.* 2004]. PET might open new perspectives for pharmacological therapies in humans in the near future. With more information on drug accumulation, drug doses may be selected in a better way. This may preclude study failures.

Other consequences for neuropharmacology

In view of the profound influence of ABC transporters on the accumulation and efficacy of brain-targeting drugs, the question arises as to how their influence on drug distribution can be minimized. First of all, newly developed drugs should be screened carefully for ABC transporter affinity, before clinical trials are faced. The pharmaceutical industry has already been aware of this issue in the past decade, evaluating drug affinity in *in vitro* high-throughput assays [Garrigues *et al.* 2002].

Second, neurologists carrying out clinical pharmacological trials should be aware of the issue, that ABC transporter single nucleotide polymorphisms (SNP) may influence the pharmacokinetics of commonly prescribed drugs [Marzolini *et al.* 2004]. So far, 29 SNPs have been identified in the human *ABCB1* gene, out of which the C3435T SNP has attracted clinical interest, patients carrying the 3435 TT allele exhibiting reduced ABCB1 expression levels compared with other alleles [Hoffmeyer *et al.* 2000]. There are a number of reports that *ABCB1* 3435 TT indeed may exhibit enhanced responses to drug treatments [Siddiqui *et al.* 2003; Fellay *et al.* 2002]. Other studies could not confirm this finding [Nasi *et al.* 2003].

Table 2. ABC transporter inhibitors currently under evaluation.

Drug	Interaction with cytochrome P450 3A4	Transporter selectivity
Cyclosporin A (Novartis, Basle, Switzerland)	Yes	ABCB1, ABCC1, ABCC2, ABCG2
Valspodar (PSC833; Novartis, Basle, Switzerland)	Yes	ABCB1, ABCC2
Biricodar (VX710; Vertex Pharmaceuticals, Cambridge, MA, USA)	Yes	ABCB1, ABCC1
Zosuquidar (LY335979; Eli Lilly, Surrey, UK)	No	ABCB1
Tariquidar (XR9576; Xenova, Berkshire, UK)	No	ABCB1, (ABCG2)
Laniquidar (R101933; Janssen, Beerse, Belgium)	No	ABCB1
ONT093 (Ontogen Cooperation, Solana Beach, CA, USA)	No	ABCB1
Elacridar (GF120918; GlaxoSmithKline, Research Triangle Park, NC, USA)	No	ABCB1, ABCG2

Third, ABC transporter inhibitors might be used to enhance neuroprotective drug accumulation. Strong efforts were made by the pharmaceutical industry to develop selective ABCB1 blockers aiming at enhancing drug efficacy [Gerrard *et al.* 2004; Agrawal *et al.* 2003; Thomas and Coley, 2003; Rubin *et al.* 2002]. As a first compound, the inhibitor zosuquidar has in the meantime been approved by the FDA for the add-on treatment of leukemia. Other compounds acting on ABCB1 or other BBB transporters are presently under evaluation.

An overview of the most important inhibitors currently tested is given in Table 2. The first compounds that entered clinical trials (so-called first- and second-generation inhibitors) still exhibited interactions with the hepatic cytochrome P450 3A4 [Kuppens *et al.* 2005]. Cytochrome P450 3A4 affinity is an undesirable feature of many ABC transporter blockers (Table 2), via which the hepatic metabolism of other medications is altered. Cytochrome P450 3A4 binding was successfully eliminated in more recent third-generation ABC transporter blockers (Table 2) [see also Thomas and Coley, 2003].

Overcoming drug elimination by deactivation of ABC transporters

So far, results with selective ABCB1 inhibitors in clinics were rather disappointing. In patients with leukemia, ABCB1 inhibitors influenced the accumulation of chemotherapeutics in tumor cells only to a minor extent [Gerrard *et al.* 2004; Tidefelt *et al.* 2000], which is in contrast to earlier rodent studies. The poor results with ABCB1

inhibitors may have to do with most drugs being substrates not only of a single, but sets of ABC transporters. As such, other transporters might take over the elimination of drugs once a single transporter is deactivated.

As a consequence of overlapping substrate affinities, inhibitors of BBB transporters may be needed in humans that deactivate more than one transporter. Whether such more broadly acting inhibitors can still be used without interfering with endogenous homeostatic and metabolic processes (such as cytochrome P450 3A4), still remains to be shown. In addition to xenobiotics and pharmacological compounds, most ABC transporters also possess endogenous substrates. ABCC1, for example, eliminates bilirubin from cells [Gennuso *et al.* 2004] and in this context has been supposed to exert protective functions. It is still unclear how cell injury processes are altered once such transporters are deactivated. An attractive strategy could be the use of dual inhibitors, which block sets of two ABC transporters. As such, inhibitors simultaneously acting on ABCB1 and ABCC1 (e.g. biricodar) as well as ABCB1 and ABCG2 (e.g. elacridar) are currently being evaluated [see also Hermann and Bassetti, 2007a].

Drug encapsulation strategies

It has been suggested that the accumulation of drugs with low or intermediate lipophilicity in the brain may be increased by drug encapsulation strategies. As such, liposomes have been used in experimental settings recently, allowing the delivery of drugs to the brain in an efficacious

way [Linker *et al.* 2008]. The main aim of liposomes is to facilitate passage of endothelial membranes, providing a means to escape immediate drug elimination at the luminal membrane surface. Yet, once released into the brain tissue, drugs will inevitably face active transport mechanisms. As such, encapsulation strategies may not ultimately resolve the problem of drug elimination.

Disease-modifying treatments acting outside the brain parenchyma

Does a medication modifying disease processes at all need to enter the brain in order to protect against ischemic or degenerative neuronal injury? Recent observations in multiple sclerosis, particularly with natalizumab [Polman *et al.* 2006; Rudick *et al.* 2006] suggest that pathophysiological processes in the brain might be influenced also by strategies acting in the peripheral blood. Anti-inflammatory and anti-oxidative strategies have also been used successfully in stroke models recently [e.g. Dénes *et al.* 2008; Kilic *et al.* 2008a, 2008b, 2005; Dimitrijevic *et al.* 2007; Sehara *et al.* 2007]. Recent observations point out that anti-inflammation might promote survival even at time points at which conventional neuroprotectants do not act. This might extend the therapeutic window of drugs.

In addition to pharmacological compounds, cell-based therapies (i.e. stem cells) [Bacigaluppi *et al.* 2008; Martino and Pluchino, 2006] as well as strategies promoting the remodeling of the injured vasculature [Kilic *et al.* 2006a, 2006b; Wang *et al.* 2005; Zhang and Chopp, 2002; Zhang *et al.* 2000] have recently been used to stimulate recovery in the injured brain. Whether stem cell effects depend on the ability of cells to enter the brain parenchyma or whether the cells can also promote recovery from outside the brain (either via immune or vasculature-mediated mechanisms [Bacigaluppi *et al.* 2008]) remains to be shown.

Conclusions

Developing therapies that modulate mechanisms of brain diseases in a clinically relevant way is perhaps the most challenging task in modern biology. This has to do with the fact that any kind of treatment interacts with a complex biological system, which responds itself to pathological insults in a well-adapted way.

In particular, the brain is surrounded by the BBB, which possesses active strategies in order to prevent drug access, expressing a broad variety of ATP-dependent (ABC transporters) and ATP-independent (SLC) carriers that extrude drugs back into the vascular space. In view of these transporters, a drug's target actions as well as its biodistribution deserve attention when new compounds are evaluated.

Molecular biology has shown us pitfalls in biomedical research, which offer explanations as to why previous studies failed. As stroke has been particularly intensively studied, the problems in the translation of research from bench to bedside were more eminent than in other fields. Our insights have led us to more stringent study designs, which will hopefully foster more successful studies in the future.

Conflict of interest statement

None declared.

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