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The priorities/needs of the pharmaceutical industry in drug delivery to the brain

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Abstract. Drug accessibility to the central nervous system (CNS) is the limiting factor in CNS drug development because many drugs do not penetrate the brain sufficiently. The highly restrictive endothelium of the brain capillary bed, i.e., the blood–brain barrier (BBB), and the protective epithelial layer of the choroid plexus, i.e., the blood–cerebrospinal fluid barrier (BCSFB), represent insurmountable obstacles for the brain penetration of many pharmacologically active compounds. Early assessment of the ability of drug candidate to penetrate the CNS is critical and crucial during the drug discovery process. A number of in vitro and in vivo approaches are available to aid in compound profiling: in silico prediction of BBB permeation, in vitro models of BBB and BCSFB, in situ and in vivo methods. Each technique has its own application with specific advantages and limitations; the strategy used by pharmaceutical research should be a combination of different models in order to obtain the best predictability to the clinical situation. Following the identification of brain penetration characteristics and the selection of the most promising compounds, the success of a CNS drug development program may be increased by enhancing drug delivery to the CNS. Strategies for CNS drug delivery may be classified as being invasive, pharmaceutical and biochemical.

Keywords: Central nervous system; Blood–brain barrier; Blood-cerebrospinal fluid barrier; Drug delivery; Transporter

1. Introduction

Drug accessibility to the central nervous system (CNS) is the limiting factor in CNS drug development because many drugs do not penetrate the brain sufficiently. The highly

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restrictive endothelium of the brain capillary bed, i.e., the blood–brain barrier (BBB) and the protective epithelial layer of the choroid plexus, i.e., the blood–cerebrospinal fluid barrier (BCSFB) represent insurmountable obstacles for the brain penetration of many pharmacologically active compounds intended to treat diseases such as depression, schizophrenia, epilepsy, multiple sclerosis, and neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. Although more limited than the surface area developed by the vessel walls forming the BBB, the choroidal surface responsible for the exchange at the BCSFB is increased by the basolateral infoldings and apical microvilli of the choroid cells; in addition the paracellular permeability of the choroidal epithelium is higher than that of the BBB endothelium [1].

The creation of the BBB and BCSFB seals the CNS from test compounds with unfavourable physico-chemical properties, i.e., low lipid solubility and high molecular weight [2]. Plasma protein binding may be another key factor for brain penetration; a tight binding and/or slow dissociation from plasma proteins are generally considered as restricting brain uptake [3]. Additionally, a significant number of lipophilic compounds do not penetrate the brain to an extent that might be anticipated from their physico-chemical properties; these compounds are actively removed from the CNS compartment by efflux transporters located at the blood–brain and blood–cerebrospinal fluid barriers [4].

Brain drug development consists in brain discovery and brain drug targeting. In order to maximize the delivery of a therapeutic into the brain and hence optimize his neurologic activity, it is critical to initially understand the factors that co-determine the concentration of a test compound in the brain following its systemic administration. Therefore part of the brain discovery activity consists in early assessment of the ability of drug candidates to penetrate the CNS; various methods including in silico prediction, in vitro models as well as in situ and in vivo methods [5–7] are utilized to assess the brain penetration of drugs and will be discussed in the first section of this article. Following the identification of brain penetration characteristics and the selection of the most promising compounds, the efficiency of CNS drug development program may be increased by enhancing drug delivery to the CNS [8,9], strategies for CNS drug delivery will be presented in the second section of this article.

2. Assessment of brain penetration

2.1. In silico methods

Passive permeability at BBB and BCSFB is related to a molecule's chemical structure and physicochemical properties [2]. Since experimental measurements of brain penetration are not amenable to high throughput screening (HTS), there has been recently a surge in computational approaches for predicting BBB partitioning from physicochemical parameters [10–12].

Computational approaches generally use the ratio of drug concentration in blood and brain at steady-state (log BB). Correlations between log BB and a combination of physicochemical descriptors like lipophilicity, polarity-polarisability and H-bonding parameters were found to be effective [13]. Thermodynamics of membrane penetration also suggested that solvatation free energy might also be a crucial determinant for entry into the CNS [14]. Polar surface area (PSA)-based quantitative structure–activity

relationship approaches are among the most popular methods to predict brain penetration [15]. Finally, the use of ACD/log D Suite software tool [11] appears to be a promising ultrahigh throughput processing of large compound databases for log BB prediction.

Although the majority of compounds that penetrate the brain do so via passive diffusion, some compounds are affected by active transport systems for both influx and efflux [2]. Presently, computational log BB prediction cannot predict the complete set of influx and efflux components of brain penetration and future progresses in this area will depend on a better understanding of the molecular recognition processes involved in such active transport systems.

2.2. In vitro models

In silico models are limited to passive diffusion estimation, therefore in vitro models of BBB and BCSFB with functional expression of transporters and metabolism capacity are desirable. As the first in vitro model, the brain capillaries can be isolated from animal as well as human autopsy using mechanical and/or enzymatic procedures [16]. As the luminal surface of the isolated brain microvessels cannot easily be accessed, BBB permeability studies investigate only the abluminal properties and function of the BBB.

Despite several drawbacks, in vitro culture cell models are now able to mimic many key features of the in vivo BBB and BCSFB. Key features determining the value of an in vitro model are monolayer tightness, expression of specific transport processes and simplicity. No one system fulfills all these criteria and therefore multiple systems are currently used.

Essentially three types of brain microvessel endothelial cell (BMEC) culture systems are used: primary cultures, co-culture systems and cell lines. Bovine BMEC cultures are widely used. In the primary culture protocol [17], BMEC are isolated by enzymatic digestion and grown on filter membrane inserts; they form confluent monolayers that retain many morphological and biochemical properties of the BBB. BMEC monolayers allow to study the transcellular transport of test compounds and permit access to both luminal and abluminal surfaces of the BMEC monolayers. Primary BMEC cultures however suffer from a low electrical resistance due to incomplete tight intercellular junctions and from a down regulation of the expression of some proteins such as the glucose transporter. Porcine BMEC models of growing popularity were also developed that demonstrate high electrical resistance [18] and allow a screening for interactions of drugs with P-glycoprotein efflux at the BBB [19]. A real improvement of in vitro BBB models was possible using cocultures of BCEC and primary astrocytes which induced or increased many characteristics, including active transport properties of BBB in BCEC [20,21]. These coculture models are hardly used as high throughput assays, nevertheless they represent actual in vitro BBB models next to in vivo and were validated by comparing permeability coefficients from in vitro studies with in vivo data [20]. Attempts have been made to immortalize primary BCECs thereby avoiding the lengthy process of cell isolation [22]. Human BCECs are available [23] that appear to be characterized in terms of BBB permeability properties. However, one of the greatest limitations of currently available immortalized BCEC lines is their insufficient tightness, rending these systems unsuitable for use as BBB permeability screens.

In order to define the contribution of the BCSFB to the brain penetration of drug candidates, some in vitro techniques have been developed in order to study transport

mechanisms at the BCSFB without the interference of the BBB and brain parenchyma. While the isolated choroid plexus (CP) provides a convenient system to study choroidal uptake, this technique is not applicable for transepithelial transfer measurements. Recently, primary rat epithelial cells [24] have been successfully cultured to examine the specific transport properties of the BCSFB. This information is important if one considers that BCSFB and BBB differ largely from each other with respect to efflux mechanisms [4].

2.3. In situ and in vivo measurements of brain penetration

A number of techniques are available for in situ and in vivo measurements of brain uptake. In situ methods include carotid artery single injection technique (Brain Uptake Index), internal carotid artery perfusion technique and brain efflux index method. In vivo techniques include intravenous injection with blood, brain, CSF and choroid plexus sampling, autoradiography, positron emission tomography and in vivo brain microdialysis. All these methods are not suitable for HTS, however they are used to characterize lead substances and early development candidates.

In the Brain Uptake Index (BUI) approach [25], a small bolus containing a radioactive tracer dose of the test compound and a reference standard (a freely permeant compound, such as ^{3}H-water or ^{14}C-butanol) is injected rapidly in the carotid artery. At a fixed time (5–15 s), the animal is decapitated and the brain concentrations of test and reference compounds are measured and related to the injectate concentrations. The ratio is a measure for the brain uptake of the test compound that is expressed as a percentage of the penetration of the reference compound. The BUI method is fast and many new molecules can be evaluated rapidly. However, since the brain extraction is measured over a time period as short as 1-s capillary transit time, it is difficult to study compounds with slow BBB permeability.

The internal carotid artery infusion technique is more sensitive than the BUI because the experimental time period is prolonged from 1 to 60 s; this technique has been applied to mice, rats and guinea pigs [26–28]. The external carotid artery is ligated and the test compound together with a vascular marker are perfused in a retrograde fashion. Following perfusion the animal is decapitated and the brain is collected and analyzed for reference and test compounds. The BBB permeability, i.e., the cerebrovascular permeability surface area PS, can then be determined. A capillary depletion method may be coupled with the brain perfusion study in order to quantify the brain vasculature uptake versus the real brain parenchyma penetration [29]. Although the brain perfusion method requires a complex surgery, it has significant advantages like a very high sensitivity (100 times more than the BUI) and a control of the perfusion system in terms of flow and composition.

Brain-to-blood efflux can be investigated with the brain efflux index (BEI) technique. The test compound and an impermeant tracer are introduced by microinjection directly into the rat brain parenchyma [30]. At varying times post-injection, rats are sacrificed and the amount of test/reference compound remaining in the brain is determined. At each time point, the fraction of the administered test substance remaining (corrected for concentration of the impermeant tracer) is calculated. The rate of decrease of this fraction of dose remaining can be used for calculating the efflux rate from the brain. Since BEI isolates the efflux process directly, it allows the detection of selective efflux transport systems; this method was already used to demonstrate the efflux of IgG molecules from brain to blood

across the BBB [31]. BEI suffers however from minor drawbacks like the substrate solubility or the possible alteration of BBB integrity.

The most straightforward technique for examining CNS uptake is direct sampling from blood, brain, CSF and the choroid plexus at different times following the intravenous injection of the test compound to animals. The analysis of blood concentrations allows a quantification of the animal exposure to the tested compound and consequently of its delivery to the brain. The presence of active transport at the blood-brain interfaces may be identified by simultaneous analysis of blood and brain samples at steady-state conditions: a dose-dependent increase or decrease in the brain:blood partition coefficients are suggestive of asymmetric transport at the BBB and/or BCSFB with efflux or influx processes. Another effective approach is to compare brain tissue concentrations in transgenic animals that do not express a specific efflux transport in the brain with transport-competent animals [32]. Alternatively, the brain:blood distribution ratio can be determined in the presence of a specific transport inhibitor [33]. The strength of the blood/brain pharmacokinetic studies is in their simplicity; in addition, this pharmacokinetic approach retains the physical integrity of the blood–brain interfaces. However, this approach is animal consumptive, relatively unspecific and brain samples are contaminated by vascular blood. The concentration time profiles of the test compound in the CSF of rats can be obtained by serial sampling via a permanent canula in the cisterna magna [34]. The crucial argument for CSF as an appropriate CNS sampling site has been relied on the assumption that CSF concentration represents the unbound concentration in the brain at steady-state and the possibility of sampling in clinical studies. However CSF concentrations may be of limited value since different active transport processes at BBB and BCSFB lead to complex relationships between CSF, brain and blood concentrations and consequently to difficult interpretations [35,36]. Specific and quantitative distribution of test compounds to the choroid plexus may be also observed [37] that can be attributed to specific transport systems at the BCSFB; such findings highlight the importance of the BCSFB in controlling drug entry into the CNS.

Quantitative autoradiography [38] permits quantification of radioactivity in small regions of the brain, i.e., in the pharmacokinetically important choroid plexus, following intravenous injection of ^{3}H- or ^{14}C-labelled compounds to small animals. This technique determines spatial distribution of drug candidates into the brain, however no time resolution can be achieved within one animal and no differentiation is made between parent drug and radioactive drug-related metabolites.

Brain penetration of drug candidates may be investigated by positron emission tomography (PET). This method involves the intravenous administration of positron emitting isotopes, i.e., ^{18}F or ^{11}C [39]. PET scanning is a very powerful method for selective brain kinetics at multiple sites and represents the only non invasive method to quantify brain penetration in human. P-gP functionality at the BBB has been investigated with ^{11}C-verapamil in mdr1a (+/+) and (−/−) mice by mean of micro-PET studies [40]. However, in addition to its high cost, PET studies do not allow a distinction between parent compound and drug-related metabolites.

Microdialysis has become a relatively common technique for assessing drug candidate concentration in brain and CSF of rats and mice [41,42]. There are several advantages associated with microdialysis: free drug concentrations may be quantified simultaneously

in selected brain areas and in the blood of one animal, parent compound may be distinguished from metabolites. However brain microdialysis may be limited by the sensitivity of the analytical method, the dialysis probe efficiency with lipophilic compounds and the possibility of BBB disruption at the probe implantation site.

3. Strategies for brain drug delivery

From an industrial point of view, two approaches to achieve the desired brain concentrations of drugs have to be considered: structural modifications of existing drugs in order to increase brain penetration and/or various approaches to enhance drug delivery. The purposes and uses of these approaches will be discussed in this chapter. The existing strategies for brain drug delivery may be classified as being invasive or neurosurgically based, pharmaceutically based or biologically based [8,43].

3.1. Invasive delivery strategies

A drug may be introduced intraparenchimaly by direct injection or infusion, or by implantation of polymer matrix preloaded with the drug [44]; this type of administration yields the highest degree of targeting, it is however limited by invasiveness and diffusion restrictions. Intracerebroventricular and intrathecal administrations deliver the drug directly into the CSF compartment, either in the lateral ventricle or in the subarachnoid space; these routes of administration are less invasive than a direct intracerebral injection and allow access to a much wider area of the CNS through CSF circulation pathways. However, diffusional and cellular barriers for penetration into surrounding tissues and significant clearance of CSF into the venous and lymphatic circulation are also limiting factors [45].

Two routes have been proposed for the direct passage of drugs from the nose to the brain: an intraneuronal and an extraneuronal pathway [46]; evidence from animal models [47] and human studies [48] suggests that even large molecules like peptides can be transported from the nasal cavity to the CNS. The main advantage of intranasal administration is the minimal invasiveness, however variable systemic absorption and CNS drug delivery will limit the utility of the intranasal strategy to compounds with large therapeutic indices.

Another invasive method relies on reversible BBB disruption. Intracarotid injection of an inert hypertonic solution such as mannitol has been employed to initiate endothelial cell shrinkage and opening of BBB tight junctions for a period of a few hours, and allowing the delivery of antineoplastic agents to the human brain [49]. In contrast to osmotic disruption methods, a biochemical opening approach uses bradykinin receptor stimulation as a means to transiently increase BBB permeability [50]. Although from a quantitative point of view these approaches look attractive, the risk exists of disrupting the BBB, even for brief periods.

3.2. Pharmaceutical strategies

The improvement of passive penetration of the brain can be achieved by developing lipophilic pro-drugs that are taken up into the brain and metabolized to release active parent compounds [51]. The use of this lipidization strategy is however limited since drug

lipidization also increases its penetration in other tissues in the body and decreases systemic exposure.

Another possibility to increase the passive diffusion of drugs through the BBB is the inclusion of the compound into small liposomes or nanoparticles [52]. Although the mechanism is not fully understood [53], nanoparticle delivery to the brain may be an interesting strategy for the treatment of brain tumors.

3.3. Biological strategies

A prominent strategy is based on the use of endogenous transporters that are potential conduits to the brain for small or large molecules [54]. The endogenous BBB transport systems may be classified as carrier-mediated transport, receptor-mediated transcytosis, adsorptive-mediated transcytosis and active efflux transporters.

Carrier mediated systems include the large neutral amino acid transporter (LAT)-1, this transporter mediates the brain uptake of various drugs like L-DOPA, melphalan and baclofen [55]; carrier-mediated transport may be considered by drug development focusing on structural requirements that enable BBB transport via a carrier-mediated system. The uptake of drugs by the brain can improved by conjugation to an endogenous compound, which uses receptor-mediated transcytosis. Examples of endogenous receptor-mediated transcytosis include the BBB insulin receptor or the BBB transferring receptor. A highly studied receptor-mediated transport vector is the OX 26 monoclonal antibody to transferrin receptor; conjugation of this transport vector being facilitated with avidin/biodin technology [56]. A promising approach also consisted in incorporating daunomycin in OX 26 immunoliposomes thus increasing largely the transport capacity of OX 26 [57]. Cationized proteins, i.e., cationized albumin and immunoglobin G, across the BBB by adsorption-mediated transcytosis that becomes saturated at higher concentrations than receptor-mediated transcytosis [58]; doxorubicin coupled to a small peptide vector, Syn B, appears to cross the BBB by adsorption-mediated endocytosis [59]. The advantage of using brain influx transport systems is the specificity; however only limited amounts of drugs can be delivered by this way since the brain penetration is limited by the number and the carrying capacity of the transporters.

Finally, in the search of strategies for increasing cerebral delivery of drugs, one has to now consider the development of specific inhibitors to reduce drug efflux at the BBB and eventually at the BCSFB. Considering their broad substrate profiles and their demonstrated expression at both BBB and BCSFB, it is clear that these transporters represent an attractive target for brain delivery [60]. Modulation of these efflux transporters by design of inhibitors and/or design of compounds having minimal affinity for these transporters may improve the treatment of CNS disorders. Important issues in the search for efflux inhibitors remain their ability to interact exclusively at the levels of BBB and BCSFB as well as the existence of multiple efflux transport systems at these barriers.

4. Discussion

As described above, various in silico, in vitro, in situ and in vivo methods have been developed to estimate drug transport to the brain. Each technique has its own application with specific advantages and limitations; ideally the strategy used by pharmaceutical

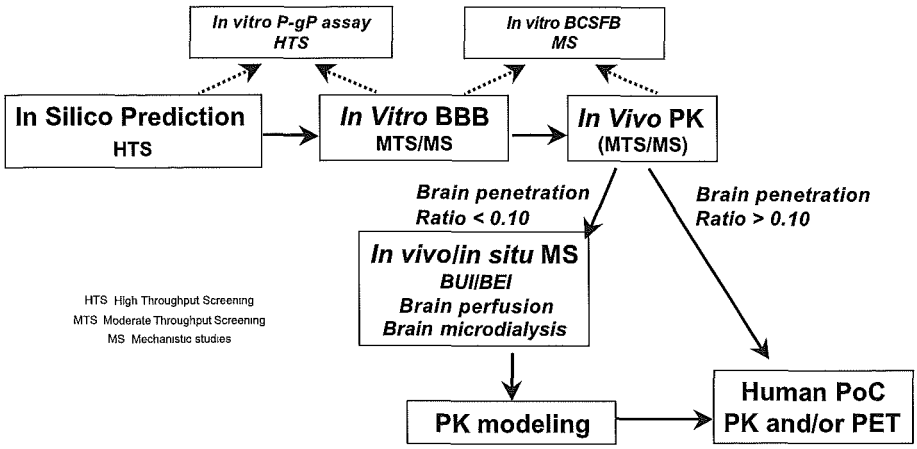

Fig. 1. Sequences of brain penetration studies in research and development: the ideal perspective.

research should be a combination of different models (Fig. 1) in order to obtain the best predictability to the clinical situation.

While there is probably no single universal system for delivering drugs to the brain, the approaches described above promise to provide practical methods for the delivery of new drug candidates. Much studies have to be done in the field of brain transport biology in order to improve brain drug delivery. Genomic programs are still used in the discovery of novel brain transporters [61], this could lead to new pathways for drug targeting to the CNS. The influence of brain diseases on the physiology of the BBB and BCSFB should also be integrated into the development of brain delivery strategies.

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Discussion

Fricker
I have one question regarding the role of the choroid plexus. I have the feeling that the role of the choroid plexus is totally underestimated. When we have requests from the pharmaceutical industry to test compounds for permeability, people always ask for permeability across the blood–brain barrier, but nobody asks to look at the choroid plexus. You can probably confirm this. How is this in your company? Do you regularly look at the choroid plexus?

Lemaire
You are right, this question is very important. For many years the concept was that the blood–brain barrier was about 4000 times larger than the choroid plexus in terms of exchange area. Apparently, it is no longer the case; recently a ratio of 10 was proposed, this means that the blood–brain barrier is only ten times more important in terms of exchange area than the choroid plexus, i.e., the blood–CSF barrier. For this reason and also because of some examples of choroid plexus accumulation of test compounds, we are now considering the use of an in vitro model of the choroid plexus as an additional screening tool.

Van Tellingen
Concerning the cerebrospinal fluid and also the lecture of Joan Abbott, a philosophical question would be: if we consider the cerebrospinal fluid as the sink, what purpose would it serve to put our drugs in the sink?

Lemaire
The problem is the kinetics of the compound once it is introduced into the CSF reservoir. Of course, if the test compound is rapidly exported by CSF flow to the blood, the possibility for exchange with the brain compartment is low. The situation is different when the site of action in the brain is contiguous with the CSF or when the test compound has a high affinity for brain constituents, in this case it is likely that the diffusion of the compound to the brain will be more extensive than its export to the blood.

Van Tellingen
Also when it has to go against the fluid projection, which is towards the cerebrospinal fluid.

Lemaire
This is correct, however we also have to be aware that more and more transporters from blood to CSF are now identified that could compensate for the expected clearance through CSF turnover.

Abbott
Can I just comment on that point? I think there is a big difference between the rat and the human, partly because of the geometry. In the rat, the distances are small enough so that when injecting into CSF, then maybe as much as 10% of the CSF flow could circulate back along the perivascular spaces to reach the parenchyma (Abbott 2004, Neurochem. Int. 5: 545–552). There are some studies showing this is possible. In human brain we don't know the equivalent numbers, but my guess is that because everything is so much bigger it is going to be much harder for one to influence the neuronal function by CSF manipulation. However, the choroid plexus does secrete into the CSF a number of growth factors which the brain needs. Now whether they are just to nourish the periventricular cells or whether they have some more widespread role in the rest of the brain (implying that that they reach brain parenchyma), we really don't know. So I think it's going to be necessary to use the kind of brain imaging you mentioned, to try and understand some of these issues.

Lemaire
Absolutely. I think that PET is presumably the best approach to explore the human situation.

De Boer
You showed many methods that were applicable to small molecules, but if you consider bigger molecules, like proteins, enzymes and even genes, what do you think about that?

Lemaire
Actually, I think the choice is very limited. I'm speaking from approaches that deliver drug behind the BBB or disrupt the BBB; unfortunately all these approaches suffer from many disadvantages. The use of transporter systems for bringing large molecules in the brain should be the approach of the future.

Scherrmann
You said that the brain efflux inhibition is very promising using inhibitors of P-gP, but as P-gP is also expressed in the kidney and mediate drug excretion, are you not worried about drug toxicity issued from the blockade of drug elimination, for example? Not looking only at the brain, you may also have side effects in other parts of the body.

Lemaire
Absolutely, you are right. P-gP is not only at the blood–brain barrier, or at the blood–CSF interface, but also in many excretory organs, including the kidney and this represents a main problem. We're just at the beginning of discovering new transporters at the blood–brain barrier or at the blood–CSF barrier and we can just hope that specific transporters to the brain will be discovered that should not be present in other tissues or elimination interfaces.

Whittle
I was interested in P-gP inhibitors and the increase in paclitaxel.In terms of absolute increase in dose, it was only from only about 100–500 units, but in terms of biological activity if you are treating a tumour, often you need several log folds in increased concentration to get one log kill. So although you may have a statistically significant increase in drug-delivery, one wonders if it is that going to translate into a biological effect.

Lemaire
This is an important point, but frankly, it's difficult for me to answer this question related to Paclitaxel.

Smith
I was wondering if the pharmaceutical industry is more interested in broad-based drug efflux transport inhibitors because they may act upon a greater variety of efflux carriers induced in cancer resistance, or is the predominant interest in highly selective inhibitors that could surgically go in and strike only a single specific system?

Lemaire
Of course, the main interest for industry would be to use selective inhibitors.

Scherrmann
In the field of drug selection for the CNS profile, you said that in Novartis the cut off level is 10%. You know that in other companies it's 5%, so what is the rationale to say, if there is less than 5% in the compound it is a not a good CNS candidate, and if you have more than 10% it could be a good CNS drug candidate? What is the rationale in a big pharmaceutical company to say what is a good cut off level?

Lemaire
Frankly, this is a difficult question to answer and this is a permanent matter of discussion with pharmacologists.

Van Tellingen
I am always a bit puzzled when I look at those in vitro models, especially if it comes to P-gP. We now know that P-gP is a major disadvantage for a drug to be used in the brain and then I see people trying to develop in vitro models, very sophisticated co-culture models, etc, whereas there are much more simple models for determining whether a compound is a P-glycoprotein substrate? What are you thoughts about this?

Lemaire
This is really a good question for research and development in the industry. There are two schools in my company: the minimalist school saying, why to consider sophisticated blood–brain barrier or blood-CSF models, just take a simple model like the Caco-2 cells since Caco-2 cells also express P-gP? At the contrary the maximalist school assumes that

although Caco-2 may be a P-gP model, other transporters exist at the blood–brain barrier that are not expressed in Caco-2 cells and consequently more specific in vitro blood–brain barrier or blood–CSF models should be used. The debate is still open and is mainly influenced the high throughput characteristics of the various in vitro models proposed.

Van Tellingen
Of course, besides Caco-2 there are also other cell systems available which you may use to test the other transporters as well.

Lemaire
It is clear that specific transporters for blood–brain barrier or for blood–CSF barrier that are not present in the Caco-2 are still present in other in vitro systems.

Tsuji
What do you think about this discrepancy in P-gP, which is not so important in the intestine, but is very important in the BBB?

Lemaire
I think that the respective concentrations of the drug in the blood and in the intestine are one explanation. In addition we have to consider the free concentrations in the blood which are of course quite low compared to the very high concentration observed in the stomach and in the intestine.

Van Tellingen
The gastro-intestinal barrier is certainly something where we could have a second meeting on. We did quite a lot of work on pacletaxel and indeed we showed increased uptake of paclitaxel when administered with blockers, and intriguingly, we found that a very similar compound, docetaxel, is taken up quite well in the gut of the wild type animal. And we found that its biological availability is so low because of first pass metabolism. These compounds are very alike and we gave them in similar doses. So it's not only the concentration in the gut which is important, and I have a feeling, I have not scientific evidence for this, that in some cases some of those compounds may lift in on some inward transporters, and perhaps docetaxel is more sensitive to one of those. The gut is a tissue that is essentially made to take up compounds, whereas the blood–brain barrier also has to take up compounds, but it is also built to keep it out, perhaps maybe that is some of the difference.

Scherrmann
I think to an additional factor which is the differential expression of the P-gP according to the level of the gut and according to the window of your drug absorption. So you probably have the same level of P-gP at the BBB, but not in the gut. Early work on cyclosporine showed that you have no P-gP in the stomach and a lot of P-gP in the last part of the gut, so I think that this effect could be very important in managing the influence of P-gP in drug absorption.

Lemaire
Additionally there is still a debate concerning the role of P-gP at the choroid plexus. Has P-gP a CSF input or a CSF output function at the blood–CSF barrier?

Whittle
Getting back to what Professor Abbott was talking about earlier, once you put the compound into the CSF by an intracerebroventricular infusion, there is this potential efflux mechanism across the choroid plexus. But what about transependimal penetration of the drug? What influences that?

Lemaire
As far as I know there is no real barrier and no transporters between CSF and brain parenchyma. Therefore I can imagine that this diffusion from the CSF compartment to the brain compartment is mainly linked to the physico-chemical properties of the drug.