Prediction of brain target site concentrations on the basis of CSF PK : impact of mechanisms of blood-to-brain transport and within brain distribution

Westerhout, J.

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Author: Westerhout, Joost
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Chapter 1

Preclinical prediction of human brain target site concentrations: Considerations in extrapolating to the clinical setting

J. Westerhout, M. Danhof, E.C.M. de Lange

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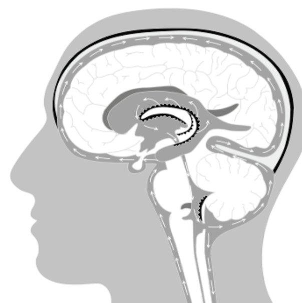

ABSTRACT

The development of drugs for central nervous system (CNS) disorders has encountered high failure rates. In part, this has been due to the sole focus on blood-brain barrier (BBB) permeability of drugs, without taking into account all other processes that determine drug concentrations at the brain target site. This review deals with an overview of the processes that determine the drug distribution into and within the CNS, followed by a description of *in vivo* techniques that can be used to provide information on CNS drug distribution. A plea follows for the need for more mechanistic understanding of the mechanisms involved in brain target site distribution, and the condition-dependent contributions of these mechanisms to ultimate drug effect. As future direction, such can be achieved by performing integrative cross-compare designed studies, in which mechanisms are systematically influenced (e.g. inhibition of an efflux transporter, or induction of pathological state). With the use of advanced mathematical modeling procedures we may dissect contributions of individual mechanisms in animals as links to the human situation.

INTRODUCTION

Central nervous system (CNS) disorders like Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, schizophrenia, migraine, insomnia, depression, and attention-deficit hyperactivity disorder are currently estimated to affect hundreds of millions of people worldwide (World Health Organization, 2007). While established treatments are currently available for most of these disorders, significant unmet medical needs still remain, as currently available drugs are treating symptoms rather than curing the disease (Business Insights, 2010). Therefore, novel treatments or drugs with a different mechanism of action are needed.

In these days, the CNS sector is struggling as the average cost of getting a drug onto the market is ever increasing and now approaching US$1 billion, whereas there is an expected decline in income due to pricing pressure from generics (Business Insights, 2010). Moreover, many potentially therapeutic compounds fail during development because early drug discovery programs are often using the wrong parameters for estimating CNS exposure (Hammarlund-Udenaes *et al.,* 2008).

It is often said that many CNS drug candidates fail because they do not reach the CNS target due to lack of blood-brain barrier (BBB) permeability. Indeed, the BBB effectively isolates the brain from the blood by the presence of tight junction proteins, connecting the endothelial cells of the brain vessels. In addition, specific metabolizing enzymes and efflux pumps, such as P-glycoprotein (P-gp), are located within the endothelial cells, which may actively remove drugs from the brain. It is therefore true that the BBB can play a major role in limiting the delivery of systemically administered drugs to the CNS. However, this is not the sole reason for the high failure rate in CNS drug development. For a proper CNS effect, the unbound drug should have the ability to access the relevant target site within the CNS. Apart from BBB permeability, this also depends upon other factors, such as plasma pharmacokinetics (PK) and within-brain distribution. These factors are controlled by many mechanisms. Each mechanism has its particular influence by its specific rate and extent, and thereby plays a more or less important role in having the drug in the *right place*, at the *right time*, and at the *right concentration*. Moreover, influences of variables like genetics, gender, age, environmental and pathological conditions

have generally been neglected. It is therefore not surprising that most CNS drug candidates finally fail during development.

As the driving force of CNS drug action is the concentration-time profile at the brain target site, it is important for pharmaceutical companies to have effective, cost-efficient tools to measure and predict human brain target site exposure before proceeding to more expensive clinical trials.

For many (potential) CNS drugs, brain target site concentrations are closely linked, or may even be equal, to unbound drug concentrations in the brain extracellular fluid ($brain_{ECF}$) (De Lange *et al.,* 2000; Hammarlund-Udenaes, 2009). However, the possibility of direct measurement of $brain_{ECF}$ concentrations is highly limited in the clinical phase of drug development. Therefore, unbound drug concentrations in human cerebrospinal fluid (CSF) are used as a surrogate for human $brain_{ECF}$ concentrations. However, the usefulness of CSF concentrations as a predictor of brain target site concentrations can be questioned, as a generally applicable relationship between CSF concentrations and $brain_{ECF}$ concentrations does not exist due to qualitative and quantitative differences in processes that govern the PK at these sites (De Lange and Danhof, 2002; Lin, 2008; Shen *et al.,* 2004).

FACTORS THAT GOVERN THE PHARMACOKINETICS IN THE BRAIN

Drug distribution into the brain is governed by many processes, including plasma PK, plasma protein binding, passive and active transport across the BBB or blood-CSF barrier (BCSFB), and once within the brain, bulk flow, diffusion, and passive and active extra-intracellular exchange.

Plasma pharmacokinetics and protein binding

Once drugs are in the systemic circulation, they can bind to different proteins that are present in plasma. Of the many plasma proteins that can interact with drugs, the most important ones are human serum albumin, α_1-acid glycoprotein, and lipoproteins (Peletier *et al.,* 2009). Acidic and neutral drugs are usually bound more extensively to albumin, whereas basic drugs are usually bound

more extensively to α_1-acid glycoprotein and lipoproteins (Peletier *et al.,* 2009). As protein-bound drugs cannot cross the BBB or BCSFB, unbound plasma concentrations, rather than total plasma concentrations, are considered to be the main determinant for the rate and extent of drug entry into the brain (Mayer *et al.,* 1959). However, it must be noted that information about the level of protein binding by itself is not sufficient for predicting drug distribution into the brain (Pardridge, 1995). As the association and dissociation of drugs to plasma proteins is a dynamic process, it indicates that extensively protein-bound drugs can still enter the brain in sufficient amounts, provided that the rate of dissociation and permeability of the BBB and BCSFB is high enough (Mandula *et al.,* 2006; Morgan and Huang, 1993; Tanaka and Mizojiri, 1999). This implicates that information on the kinetics of plasma protein binding is also essential for accurate prediction of the rate and extent of drug entry into the brain.

Transport across the blood-brain barriers

The barriers between blood and brain are the BBB and the BCSFB. These barriers have many similarities but also important differences, as will be discussed below.

The blood-brain barrier - The BBB is formed by the brain capillary endothelial cells, which are interconnected by tight junction proteins that restrict paracellular diffusion of small hydrophilic molecules from blood to the brain. In addition to these tight junctions, numerous active transport systems are present at the BBB that protect the brain from neurotoxic substances, but also help to maintain the homeostasis of the brain by influx of essential substrates such as electrolytes, nucleosides, amino acids, and glucose. These processes are regulated by interactions with adjacent pericytes, astrocytes, and neuronal cells (Abbott *et al.,* 2006; Bernacki *et al.,* 2008; Davson and Oldendorf, 1967). However, in certain specialized regions in the brain, comprising the choroid plexuses and the circumventricular organs, the capillary endothelial cells are fenestrated and therefore highly permeable (Abbott, 2004). Thus, compounds can cross the capillary walls more or less freely in these specialized regions, but may be restricted in entering the rest of the brain by the BCSFB.

The blood-cerebrospinal fluid barrier - The BCSFB is located at the choroid plexuses in the lateral, third and fourth ventricles of the brain, which are responsible for the production of CSF. The barrier function of the BCSFB is provided by the tight junctions between the epithelial cells of the choroid plexus at the apical site, which contacts the CSF. Like the BBB, several different active transport systems are located at the BCSFB to limit the entrance into the brain of compounds that can easily permeate the choroid plexus' capillaries (Davson and Oldendorf, 1967; Wolburg and Paulus, 2010).

BBB versus BCSFB - It has been assumed that the surface area of the BBB in humans, which is estimated to be ~20 m^2 (Pardridge, 2002), is at least a hundred-fold larger than that of the BCSFB, which is reported to be only 0.2 m^2 (Dohrmann, 1970). This implicates that the BCSFB only plays a minor role in the control of the brain environment (Hammarlund-Udenaes *et al.,* 2008; Lee and Bendayan, 2004; Lee *et al.,* 2001; Roberts *et al.,* 2008). However, this calculation ignores the large surface area provided by the apical microvilli on the epithelial cells. When taking these into account, the total surface area of all choroid plexuses in the rat is estimated to be ~75 cm^2 (Keep and Jones, 1990a). This is only two-fold smaller than the surface area of the BBB, which is estimated to be ~150 cm^2 (Gjedde, 1981; Keep and Jones, 1990b). This implicates that the BCSFB plays a more important role in drug transport between blood and brain than originally assumed. However, because the BBB and BCSFB are anatomically and physiologically different, their relative contributions in the exchange of compounds between blood and brain does not necessarily correspond to the ratio of their respective surfaces. BCSFB transport will (initially) influence the periventricular spaces and tissues, while BBB transport will more affect the total brain.

Active transport across the BBB and BCSFB can be either by carrier-mediated (facilitated) transport, or ATP-dependent transport. Facilitated transport across the BBB and BCSFB is carried out by members of the solute carrier (SLC) family, which include organic cation transporters (OCT), carnitine/organic cation transporters (OCTN), organic anion transporters (OAT), organic anion-transporting polypeptide (OATP), and glucose transporter (GLUT). The different ATP-dependent transport systems at the BBB and BCSFB are all members of the ATP-binding cassette (ABC) transporter

superfamily and include P-gp, breast cancer resistance protein (BCRP), and the multidrug resistance-associated protein (MRP) family (Graff and Pollack, 2004). The direction of flux and subcellular localization of the different transporter systems at the human BBB and BCSFB are depicted in figure 1. Due to inconsistencies in the literature, the direction of flux and subcellular localization of the different transporters are classified as either ‘known’, ‘likely’, or ‘unknown’. However, it can be concluded that there are some differences between the BBB and BCSFB.

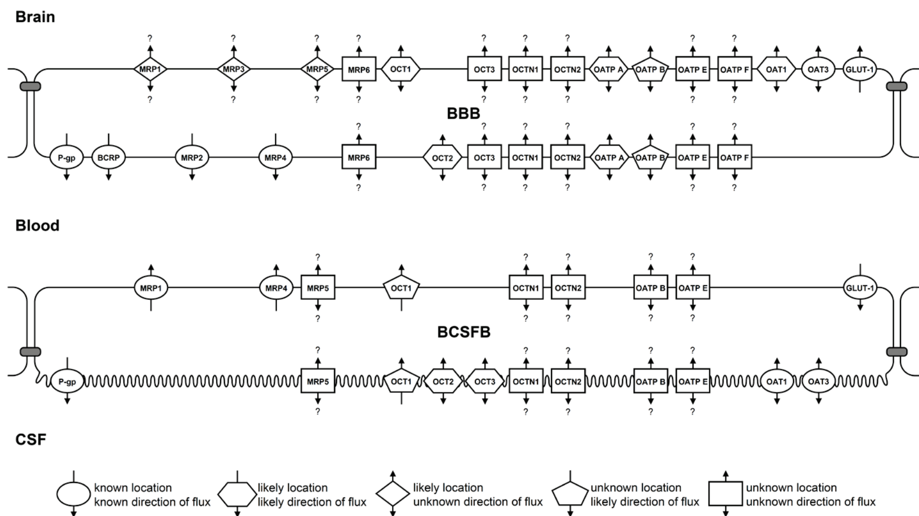

Figure 1. Cartoon of the different active transport systems that are located on the human BBB and BCSFB

It has been well established that P-gp functions as an efflux transporter at the BBB (Schinkel, 1999). However, there has been some evidence that P-gp functions as an influx transporter at the BCSFB (Kassem *et al.,* 2007; Rao *et al.,* 1999). However, previous work by Gazzin and colleagues has indicated that P-gp levels at the BBB are over 200-fold higher than at the BCSFB (Gazzin *et al.* 2008). In contrast, MRP1 has a much higher expression at the choroid plexuses compared to the brain microvessels. This indicates that there are major differences in the mechanisms by which the BBB and BCSFB protect the brain, which could result in significant differences between concentrations at the brain target site and in CSF (Gazzin *et al.*, 2008).

Altogether, this indicates that the impact of active transport on the predictability of brain target site concentrations from CSF concentrations needs to be considered and further investigated.

Enzymatic activity

Several drug metabolizing enzymes, like cytochromes P-450, monoamine oxidases, and UDP-glucuronosyltransferases, have been found at different extracellular and intracellular sites in the brain (Ghersi-Egea *et al.,* 1993; Ghersi-Egea *et al.,* 1994). The activities of some drug-metabolizing enzymes appeared to be several times higher in the small brain microvessels and choroid plexus epithelial cells compared to the cortical parenchymal cells. Thus, the BBB and BCSFB also form an enzymatic barrier to limit the exposure of the brain to drugs (Ghersi-Egea *et al.,* 1993; Ghersi-Egea *et al.,* 1994).

The metabolism that takes place within the brain could result in the conversion of molecules and/or prodrugs to pharmacologically active metabolites. This needs to be taken into account as well during drug development.

$\text{Brain}_{\text{ECF}}$ bulk flow and CSF turnover

In rats, approximately 80% of the CSF is generated by the choroid plexuses of the lateral, third and fourth ventricles, whereas the remaining 20% comes directly from the $\text{brain}_{\text{ECF}}$ (Cserr, 1965). In humans, the contribution of $\text{brain}_{\text{ECF}}$ is estimated to be about 50% (Kimelberg, 2004). After formation, CSF flows from the lateral ventricle through the third and into the fourth ventricle via the aqueduct of Sylvius. CSF then leaves the brain to enter the basal cisterns and subarachnoid spaces, where it is absorbed in the venous system (figure 2) (Miyan *et al.,* 2003; Proescholdt *et al.,* 2000; Segal, 1993).

In humans, CSF is produced at a rate of about 0.4 ml/min (Nilsson *et al.,* 1992). With a total CSF volume of approximately 140 ml (Kohn *et al.,* 1991), this indicates that the total volume of CSF is replaced every 6 hours. In rats, the relative rate of CSF turnover is much higher. With a rate of production of 2.2 µl/min (Cserr, 1965), and a total CSF volume of 250 µl (Bass and Lundborg,

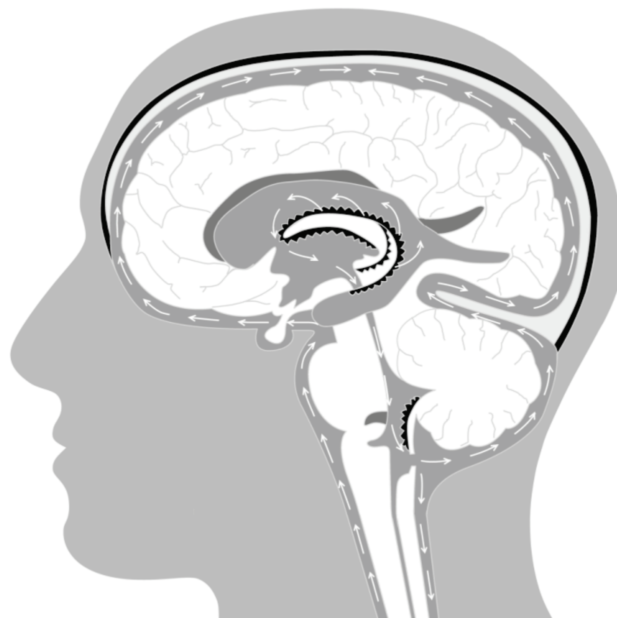

Figure 2. Schematic representation of the CSF flow path

1973), this indicates that the total volume of CSF in the rat is replaced every 2 hours.

It is often stated that drugs equilibrate readily between $brain_{ECF}$ and CSF as the ependymal epithelium that separates $brain_{ECF}$ from CSF does not present a significant barrier to drug movement (Lee *et al.,* 2001). However, compounds do not only diffuse from $brain_{ECF}$ into CSF. It has been shown that there is also bulk flow of $brain_{ECF}$ (Abbott, 2004; Cserr *et al.,* 1981). Due to the bulk flow of $brain_{ECF}$ to CSF, the transport of drugs from $brain_{ECF}$ into CSF is more efficient than the diffusion of drugs from CSF into the $brain_{ECF}$. As a result, the continual drainage of CSF can act as a 'sink' for brain tissue, indicating an active elimination from the brain.

In humans, the rate of $brain_{ECF}$ production is estimated to be 0.15-0.2 ml/min (Begley, 2000; Kimelberg 2004) which is calculated from the estimation that 50% of the CSF production comes from $brain_{ECF}$. With a total $brain_{ECF}$ volume of approximately 240 ml (Begley, 2000), this indicates the total $brain_{ECF}$ volume is replaced every 20-27 hours. At a rate of 0.2-0.5 µl/min in rat brain (Abbott, 2004; Cserr *et al.,* 1981), the rate of $brain_{ECF}$ production is about 10-20% of that of CSF, which corresponds well to the 20% of CSF production from $brain_{ECF}$

(Cserr, 1965). With a total $brain_{ECF}$ volume of 290 µl (Cserr *et al.,* 1981), this indicates that the total $brain_{ECF}$ volume in rats is replaced every 10-24 hours.

It has been suggested that the source of the $brain_{ECF}$ is from a combination of ions and water that come from the bloodstream and from CSF that flows back into the brain along perivascular channels from the ventral surface in the subarachnoid space (Abbott, 2004). This indicates that there is a fraction of CSF that takes a longer route from its site of origin in the choroid plexuses back into the systemic circulation. This also indicates that drug concentrations in $brain_{ECF}$ and CSF are only in part directly related.

Extra-intracellular exchange

Once a drug is in the $brain_{ECF}$ or CSF, it can bind to brain tissue or distribute into brain cells, depending on its physicochemical properties. Hydrophilic drugs are more likely to remain dissolved in the $brain_{ECF}$ and CSF, whereas lipophilic drugs are more likely to bind to brain tissue, or distribute into brain cells (De Lange and Danhof, 2002). However, active transport between $brain_{ECF}$ and the cell is possible as well (Lee *et al.,* 2001). The effect of $brain_{ECF}$-parenchymal exchange has been clearly demonstrated by the study on valproate by Scism *et al.* (2000). Co-administration of probenecid increased the intracellular concentrations without affecting $brain_{ECF}$ concentrations, indicating the presence of a probenecid-sensitive efflux transporter at the brain parenchymal cells.

While most CNS drug targets are facing the $brain_{ECF}$, intracellular targets also exist (Lee *et al.,* 2001). This has to be considered when aiming to determine brain target site concentrations. For targets like membrane receptors, extracellular enzymes, and transporters, $brain_{ECF}$ concentrations may provide the most useful information (De Lange and Danhof, 2002).

TECHNIQUES

In the preclinical setting there are several *in vitro*, *ex vivo* and *in vivo* techniques that provide information on brain target site exposure. Such information can be either direct or indirect, on bound or unbound concentrations, with or without

temporal resolution, and with or without spatial resolution (De Lange *et al.,* 1997; Hammarlund-Udenaes *et al.,* 2009).

A typical parameter that is often used to describe the level of brain exposure is the brain-to-plasma partition coefficient (K_p), which is defined as the ratio of the total drug concentration in the brain versus the total drug concentration in plasma. To compensate for differences in plasma protein binding, as that may restrict brain entry, the total brain-to-unbound plasma partition coefficient ($K_{p,u}$) can be calculated. However, as it is pharmacologically more relevant to use unbound brain target site concentrations rather than total brain concentrations, the unbound brain-to-unbound plasma partition coefficient ($K_{p,uu}$) is considered to be a better parameter for brain target site exposure (Gupta *et al.*, 2006).

All the processes discussed in the previous section affect the rate and/or extent of drug distribution to the brain, being two distinct parameters (Hammarlund-Udenaes *et al.,* 2008). The rate of transport into the brain is dependent on the speed at which a drug molecule can pass the BBB and BCSFB. This is often expressed as permeability (distance/time) or clearance (volume/time). The rate of transport across the BBB and BCSFB is often limited to the permeability rate of the BBB and BCSFB, which is dependent on their condition-dependent characteristics and the physicochemical properties of the drugs. As classical paradigm, BBB and BCSFB transport is governed by lipophilicity. Indeed, if passive transport (diffusion) is the only transport mechanism involved, small lipophilic drugs cross the BBB and BCSFB more readily than large hydrophilic drugs (De Lange and Danhof, 2002; Levin, 1980). For highly permeable compounds, however, the rate of transport across the BBB and BCSFB is limited by the cerebral blood flow (Dagenais *et al.,* 2000). Furthermore, active transport processes into or out of the brain may increase or reduce the permeability rate.

The extent of drug distribution to the brain can be calculated as the ratio of unbound drug concentrations in the brain compared to unbound drug concentrations in plasma at steady state, or by the ratio of unbound AUC in brain relative to unbound AUC in plasma. For compounds that freely diffuse into and out of the brain, thus irrespective of what time that may take, this ratio should be equal to 1. If the ratio is < 1, this indicates that elimination processes (bulk flow, metabolism, active transport out of the brain) play an additional role. For a ratio > 1, active transport into the brain occurs, but may also be the

consequence of tissue binding. Alternatively, if ionization plays a role, ratios < or > 1 may as well result from pH differences between one site and another.

Generally, the extent of drug distribution into the brain is considered of most importance in relation to brain target site exposure. This means that drug discovery and development studies have focused on measuring drug concentrations in brain and plasma under (assumed) steady-state conditions. However, for drugs with a desired rapid onset of action (e.g. analgesics or anesthetics), the rate of entry into the brain is also relevant. Finally, it may be questioned whether fluctuations in brain concentrations, due to fluctuations in plasma concentrations following multiple dosing regimens, may have an impact on the pharmacodynamics (PD) of the drug.

This all implies the need for mechanistic investigations on the contribution of the different processes that govern the brain target site exposure. The techniques that are used to provide information on CNS drug distribution, in the perspective of extrapolation to the clinical setting and prediction of the effect, are discussed below.

Brain perfusion technique

Mayer and colleagues were one of the first to study the rate and extent of drug penetration into the brain (Mayer *et al.,* 1959). During the experiments, plasma concentrations were maintained at steady state by a bolus dose, followed by a constant rate infusion. By measuring brain homogenate concentrations at certain time points, distribution ratios could be calculated. Based on the time needed to reach a distribution ratio of 1, compounds were classified as having a fast (<5 min), intermediate (1.5-2 h), and very slow (>3 h) rate of entry.

Over time, this technique has been adapted so the initial rate of drug transport into the brain could be studied in more detail. Among the adapted techniques are the indicator diffusion technique (Crone, 1965), the brain uptake index technique (Oldendorf, 1970), the intravenous injection technique (Ohno *et al.,* 1978), and the in situ brain perfusion technique (Takasato *et al.,* 1984). In short, radiotracer quantities of a test substance and a diffusible reference such as $^{3}H_2O$ and/or a non-diffusible reference such as [^{14}C]inulin are injected intracarotidly or intravenously and the animal is decapitated 5-30 s later. Total

brain concentrations of the test substance and reference are then compared to concentrations in the injected solution or plasma.

These techniques are very suitable to study the rate and extent of exposure of the brain to the radioactivity, reflecting the compound of interest. However, the radioactivity potentially also includes metabolites. The major disadvantage of the different brain perfusion techniques is that they all use brain homogenate, so they cannot distinguish between intracellular space (ICS), $brain_{ECF}$ or CSF, and bound or unbound concentrations (Liu *et al.,* 2009). Modifications to the in situ brain perfusion technique have allowed distinguishing between accumulation in brain endothelial cells and uptake into brain parenchyma (Preston *et al.,* 1995), as well as uptake into the choroid plexus (Deane *et al.,* 2004). However, none of the brain perfusion techniques can distinguish between the different routes of entry to the brain, which could be by crossing the BBB or BCSFB. Furthermore, it is expensive and time-consuming to synthesize radioactively labeled drugs, making this technique less suited for early drug discovery (Liu and Jia, 2007). Alternatively, non-labeled compounds can be used as well. However, this requires the development of adequate analytical methods, which is evenly time consuming.

Brain homogenate free fraction method

Different methods have been developed to be able to distinguish between unbound and bound concentrations. Lin and colleagues used an equilibrium dialysis setup to determine the unbound fractions in plasma and brain homogenate (Lin *et al.,* 1982). Using the equilibrium dialysis setup, calculation of unbound brain concentrations from total brain concentrations improves the prediction of receptor occupancy compared to the use of just total brain concentrations (Watson *et al.,* 2009). Kalvass and Maurer (2002) adapted the equilibrium dialysis technique to a 96-wells plate that allowed for more high throughput screening. When the fraction unbound in plasma and brain is known, the $K_{p,uu}$ can be calculated from the K_p. Also, the unbound volume of distribution in the brain, which relates the total brain concentration to the unbound $brain_{ECF}$ concentration, can be determined (Fridén *et al.*, 2007).

Even though the brain homogenate free fraction method allows for higher throughput screening during early drug discovery compared to the brain

perfusion technique, the disadvantages remain of using brain homogenate. Because the brain tissue is homogenized, cell structures are destroyed and binding sites that are normally not accessible to a drug *in vivo* may be unmasked (Fridén *et al.,* 2007; Liu *et al.,* 2009). This could result in an erroneous estimation of the unbound fraction in brain tissue. As an alternative, the brain slice technique was developed.

Brain slice technique

The brain slice technique was developed by Newman *et al.* (1991). However, it was Kakee *et al.* (1996) that first applied the brain slice technique to determine unbound drug concentrations in brain tissue. In short, a section of a drug-naïve brain is cut into 300 μm slices, which are then incubated at 37°C in buffer containing the drug. After a certain time, assuming a steady state between buffer and brain slice ECF, the slices are homogenized and drug concentrations in the homogenate and buffer analyzed. In contrast to the brain homogenization method, the cellular structure of the brain tissue remains intact with the brain slice technique, thus it allows to distinguish between intracellular and extracellular as well as bound and unbound concentrations (Fridén *et al.,* 2009a).

Kakee *et al.* (1996) applied the brain slice technique to determine the unbound volume of distribution, which they used for the calculation of the brain efflux clearance rather than brain target site exposure. Fridén and colleagues compared the brain slice technique to the brain homogenate free fraction method in their ability to predict unbound volumes of distribution, as determined by the intracerebral microdialysis technique (see below). They concluded that the brain slice technique was better than the brain homogenate free fraction method for predicting unbound volumes of distribution (Fridén *et al.,* 2007).

All in all, the brain slice technique appears to be better than the brain homogenate free fraction method for the prediction of unbound brain target site concentrations. However, as the brain slice technique is more labor intensive and lower throughput compared to the brain homogenate free fraction method, the latter is still preferred during early CNS drug discovery (Read and Braggio, 2010). Even though recent developments have improved the throughput of the

brain slice technique (Fridén *et al.,* 2009a), it remains to be seen if the brain slice technique will replace the brain homogenate free fraction method during early CNS drug discovery.

CSF sampling

Back in 1959, Mayer and colleagues already recognized that the unbound plasma concentrations, rather than total plasma concentrations, are the main determinant for the rate and extent of drug entry into the brain, as protein-bound drugs cannot cross the BBB or BCSFB (Mayer *et al.,* 1959). However, due to the lack of suitable methods, it took nearly 25 years to acknowledge that the free drug hypothesis is also applicable for pharmacological activity at the brain target site. Danhof and Levy (1984) were one of the first to suggest the use of unbound CSF concentrations rather than total brain concentrations for relating pharmacological activity. They showed that, for phenobarbital, not plasma, nor total brain, but CSF concentrations were constant at the onset of anesthesia (the minimal effective concentration).

The most common method for collecting a CSF sample from humans is by a lumbar puncture (Hill *et al.,* 1999). However, for continuous CSF sampling, a cannula could also be implanted in the lumbar region (Bruce and Oldfield, 1988). In animals, CSF can be obtained relatively easy by a single puncture or the implantation of a cannula in the lateral ventricle or cisterna magna (Bouman and van Wimersma Greidanus, 1979; Cserr, 1965; Cserr, 1971; Nielsen *et al.,* 1980; van Bree *et al.,* 1989). Also, a few examples in literature exist on a lumbar puncture taken from in rats (De La Calle and Paíno, 2002; Wang *et al.,* 2005). As a concentration gradient could exist along the CSF flow path due to its high flow, the location of CSF sampling is very important for interpretation of data and extrapolation to the clinical setting (Summerfield and Jeffrey, 2006).

Mayer and colleagues combined the brain perfusion technique with CSF sampling from the cisterna magna in rabbits to compare the rate and extent of drug penetration between CSF and specific areas of the brain (Mayer *et al.,* 1959). They concluded that these parameters are determined by the lipophilicity of the compound. However, small differences in the rate of entry between CSF and the brain were observed for antipyrine and barbital, whereas their extent of brain entry was similar. Also, differences in both the rate and extent of

penetration of salicylic acid between CSF and brain were observed (Mayer *et al.,* 1959). As salicylic acid is known to be transported by an organic anion transport system (Lorenzo and Spector, 1973), the difference in the rate and extent of penetration may in part result from differences in the activity of such transport systems at the BBB and BCSFB. However, the difference in the rate and extent of penetration may also in part result from differences in physiology between the BBB and BCSFB.

Kalvass and Maurer (2002), Maurer *et al.* (2005), Liu *et al.* (2006), and Fridén *et al.* (2009b), all used single time point CSF samples from the cisterna magna of rats or mice in combination with the brain perfusion technique to compare steady state CSF concentrations to steady state unbound brain concentrations of over one hundred structurally diverse compounds. They all concluded that steady state CSF concentrations were comparable to steady state unbound brain concentrations within a 3-fold error range for compounds that freely diffuse across the BBB and BCSFB. Liu *et al.* (2009) also applied CSF sampling from the cisterna magna of rats in combination with the intracerebral microdialysis technique and showed that steady state CSF concentrations predicted steady state unbound brain concentrations very well within the 3-fold error range for the selected compounds, which also include some P-gp substrates. Possibly, CSF and $brain_{ECF}$ concentrations have had the time to reach equilibrium at steady state. However, for compounds with differences between CSF and $brain_{ECF}$ concentrations beyond the 3-fold error range, qualitative and quantitative differences in transporter activity between BBB and BCSFB may play a role.

The use of CSF as a surrogate for unbound brain target site concentrations has been discussed previously by De Lange and Danhof (2002), Shen *et al.* (2004), and Lin (2008). In short, they concluded that the use of CSF offers one significant advantage over other methods, in that this fluid is accessible both in animals as well as in humans, and provides information on the unbound concentration. However, CSF sampling generally lacks the possibility to obtain concentration-time profiles, at least in humans, whereas in animals, taking a CSF sample significantly affects the CSF volume, of which the impact is not yet known (De Lange *et al.,* 1997).

Intracerebral microdialysis

The intracerebral microdialysis technique is an *in vivo* technique that permits monitoring of local concentrations of drugs and metabolites by implantation of a microdialysis probe in a specific site in the brain (De Lange *et al.,* 1994; Morrison *et al.,* 1991; Nicolaysen *et al.,* 1988; Sabol and Freed, 1988; Ståhle *et al.,* 1991; Terasaki *et al.,* 1991; Wang *et al.,* 1995; Wong *et al.,* 1992). The probe contains a semipermeable membrane that is continuously perfused with a physiological solution that matches the ionic composition of the (extracellular) fluid surrounding the probe. Small enough molecules that are able to pass the membrane will diffuse into or out of the perfusate, down their concentration gradients. The solution that exits the probe, the dialysate, is then collected for analysis. However, because of the continuous flow of the perfusing solution, the concentration in the dialysate will be different from that in the surrounding fluid. The term 'concentration recovery' is used to describe this relationship and should always be determined for quantification of microdialysis data (De Lange *et al.,* 2000).

The microdialysis technique has a number of advantages: (1) Sampling can be performed continuously without fluid loss. (2) High resolution concentration-time profiles can be obtained from distinct brain regions in freely moving individual animals. (3) This reduces the number of animals needed for PK investigations. (4) The samples obtained are protein free. (5) *Ex vivo* analysis of the dialysate samples permits measurement of drug concentrations by any suitable analytical technique. However, the microdialysis also has its disadvantages: (1) The microdialysis technique is labor intensive, expensive, and low throughput. (2) Due to the invasive nature of implantation of the microdialysis probe, this technique only has a limited applicability in the human situation. (3) Due to the diluting effect of the dialysis, sensitive analytical methods are required to detect low concentrations. (4) The microdialysis technique is not always suitable for lipophilic compounds as they tend to adsorb to the microdialysis equipment (De Lange *et al.,* 1997; De Lange *et al.,* 2000; Sun and Stenken, 2003). However, the composition of the microdialysis perfusion fluid may be altered to improve the recovery, for example by addition of albumin or cyclodextrins to the perfusion fluid (Sun and Stenken, 2003).

Despite the very important advantage of being able to measure unbound brain concentrations as a function of time, the microdialysis technique is not often applied during early drug discovery because of the low throughput. However, it may provide information on the rate and extent of BBB and BCSFB transport, and modulations thereof. Furthermore, the microdialysis technique can be used to further distinguish the possible contribution of many dynamically regulated passive and active transport mechanisms.

One particular case is the investigation of the relationship between CSF and $brain_{ECF}$ concentrations, for the interpretation of human CSF data (De Lange and Danhof, 2002; Lin, 2008; Shen *et al.,* 2004). Several studies have been performed in which two microdialysis probes were implanted in a single animal; one in a selected brain region for measuring drug concentrations in the $brain_{ECF}$, and one in the lateral ventricle or cisterna magna for measuring drug concentrations in the CSF (Malhotra *et al.,* 1994; Matos *et al.,* 1992; Wong *et al.,* 1992).

Wong *et al.* (1992) found ventricular CSF concentrations being twice as high compared to rabbit $brain_{ECF}$ concentrations for zidovudine. Matos *et al.* (1992) measured morphine in CSF from the rat lateral ventricle, third ventricle, cisterna magna and spinal cord in the rat, and found morphine evenly distributed in all selected brain regions and CSF, except for cisternal CSF. Interestingly, cisternal CSF concentrations were about 5-fold higher than ventricular and lumbar CSF concentrations. This is counterintuitive, considering the CSF flow path. However, the concentration recovery was not addressed adequately. Finally, Malhotra *et al,* (1994) found that rat ventricular CSF concentrations were ~3-fold higher compared to $brain_{ECF}$ concentrations at steady state for EAB 515.

PET and SPECT

Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are very powerful techniques to study brain kinetics at multiple sites (Dresel *et al.,* 1998; Erlandsson *et al.,* 2005; Mamo *et al.,* 2004; Remington *et al.,* 2006; van Waarde, 2000). In short, small amounts of radiolabeled compounds are injected into the bloodstream, after which the total brain uptake can be estimated by calculating the ratio of the total brain radioactivity divided by the injected radioactivity.

The main difference between PET and SPECT is the type of radiation that is used. PET, as the name suggests, uses a positron emitter (usually ^{11}C or ^{18}F) for imaging, whereas SPECT uses a gamma emitter (often ^{123}I) (Erlandsson *et al.,* 2005; Haubner, 2010; Kouris, 1984). PET provides higher resolution images than SPECT. The major advantages of PET and SPECT are that these techniques are noninvasive and applicable to both animals and humans (van Waarde, 2000). As disadvantages, however, (1) PET and SPECT require radioactively labeled compounds, which is expensive and time-consuming in producing (Liu and Jia, 2007). Moreover, especially for PET radionuclides, the duration of a scan is limited by the decay of the radioactive label. For ^{11}C, which has a half-life of only 20 min, this means that synthesis of the radiolabeled compound should be performed on-site, right before the experiment. (2) While ^{18}F (half-life of 110 min) and ^{123}I (half-life of 13.2 h) can provide more flexibility in time, the labeling with these nuclei may change the compounds' properties. (3) Also, for animals, anesthesia is required, which may affect the physiological status of the animal, and may further limit the comparison to humans (Claassen, 1994). (4) Another disadvantage is that PET and SPECT signals do not distinguish between compounds and their metabolites, (5) nor between bound and unbound concentrations, as they can only localize the level of radioactivity (Neuwelt *et al.,* 2008).

CONSIDERATIONS IN ANIMAL-TO-HUMAN EXTRAPOLATION / PREDICTION

All vertebrates share the same mechanisms of blood-brain transport (Cserr and Bundgaard, 1984). However, the rate and extent of these mechanisms depend on conditions such as species, gender, tissue, genetic background, diet, disease, drug use, etc. As a result, observations in a particular setting are not necessarily predictive of what would be observed in another setting. In the following section, we will discuss the possible use of the different techniques in the clinical setting or for the prediction of human brain target site concentrations, as well as how different conditions may affect brain processes and therewith drug distribution into the brain.

Application of the different techniques in the extrapolation to the clinical setting

The brain perfusion technique, the brain homogenate free fraction method and the brain slice technique can all be applied to determine the rate and extent of brain target site exposure. This is often done during early CNS drug discovery, as these techniques have relatively high throughput and allow for good drug candidate selection (Read and Braggio 2010). However, these techniques have very limited applicability in the extrapolation to the clinical setting. The techniques that can be applied both in animals as well as in humans are the CSF sampling technique, the intracerebral microdialysis technique, PET, and SPECT. Of these techniques, PET and SPECT are the most promising, as they are very powerful techniques to study brain kinetics at multiple sites (Dresel *et al.,* 1998; Erlandsson *et al.,* 2005; Mamo *et al.,* 2004; Remington *et al.,* 2006; van Waarde, 2000). However, the time needed for the development of appropriate tracers and high costs of PET and SPECT scanning currently limit the use of these techniques during drug development.

So far, the CSF sampling technique is the technique that is most often used during drug development, as CSF sampling is relatively simple, straightforward, and cheap, compared to intracerebral microdialysis, PET, and SPECT (Lin, 2008). Moreover, this can be applied both to humans and animals. However, the use of CSF concentrations as a surrogate marker for brain target site concentrations is not that simple and straightforward, as a generally applicable relationship between CSF concentrations and brain target site concentrations does not exist (De Lange and Danhof, 2002; Lin, 2008; Shen *et al.,* 2004). Therefore, it is questionable whether brain target site concentrations can be predicted on the basis of CSF concentrations.

A number of studies have indicated that for most compounds there is not much difference between apparent steady-state concentrations in CSF and $brain_{ECF}$ in the rat (concentration ratios are < 3) (Fridén *et al.,* 2009b; Kalvass and Maurer, 2002; Liu *et al.,* 2006; Maurer *et al.,* 2005). However, the following questions still remain:

- Are such ratios predictive for the human situation?
- How does disease state influence such ratios?

- And, most importantly: if human CSF concentrations would have been predictive than why hasn't it proven itself as such?

The only technique that allows the measurement of unbound drug concentrations at the brain target site as well as in CSF *in vivo* is the intracerebral microdialysis technique. In the preclinical setting, the intracerebral microdialysis technique could very well be used to investigate the relationship between CSF concentrations and brain target site concentrations (Malhotra *et al.,* 1994; Matos *et al.,* 1992; Wong *et al.,* 1992). All in all, each of the different techniques has its advantages and disadvantages for extrapolating to the clinical setting.

Condition-dependent factors

In the drug development setting we would like to use animal data as best as possible for prediction of the human situation. This means that we have to consider sources of variation from controlled preclinical situations to clinical practice. One of the major issues in extrapolating from animal data to the human situation is that the vast majority of studies are being done in healthy animals, whereas there is increasing evidence that dysfunctional BBB mechanisms are at the core of CNS diseases (Jeffrey and Summerfield, 2010). However, for certain CNS disorders, there are simply no appropriate animal models available. In that case, animal experiments can improve our understanding of the biological system, which is essential to be able to understand the processes that underlie the disease. Then, for accurate prediction of CNS drug concentrations and effect in humans on the basis of animal data, one should take into account the possible influences of CNS diseases and genetic differences between species on the processes that govern CNS exposure (Syvänen *et al.,* 2009).

Inflammatory and oxidative stress, which are cofactors in nearly every CNS disease, can acutely disrupt the BBB and BCSFB at the level of the tight junctions (Miller, 2010). It has also been shown that a bacterial or viral infection of the CNS can cause a disruption in the integrity of the BBB and BCSFB (Paul *et al.,* 1998). So far, only limited information is available on the impact of CNS diseases on the expression levels of the different active transport systems that are located on the BBB and BCSFB. Of the different transport systems, most information is available on the regulation of expression levels of

P-gp. However, there is some evidence that also BCRP, MRP1, MRP2, and MRP4 expression is altered after exposure to certain xenobiotics, which include therapeutic agents and environmental toxicants (Miller, 2010). Some of these xenobiotics have also been found to increase expression of drug metabolizing enzymes in brain capillary endothelial cells. Increased expression levels of the different transport systems and drug metabolizing enzymes can make it very difficult to get sufficient amounts of drugs to their brain target site. Despite the success of specific transport inhibitors in improving drug delivery to the brain in animal studies, the results have not been translated to the clinic (Miller, 2010). It has been suggested that blocking of P-gp could improve drug delivery across the BBB. However, effective doses of P-gp inhibitors, such as cyclosporine and verapamil, are often toxic (Neuwelt, 2004).

The effect of inflammation on the activity and expression levels of transport systems is rather complex, as the direction and degree of change in expression levels is found to be dependent on the inflammatory signal and time after exposure. For instance, it has been found that P-gp activity is decreased after short-term exposure to proinflammatory signals, whereas there is no change in P-gp expression (Roberts and Goralski, 2008). Following a more prolonged exposure, the activity and expression of P-gp are both increased (Roberts and Goralski, 2008). However, rather than being an effect of CNS diseases, the change in transporter expression can also be the cause of CNS diseases. There have been some reports that a decrease in P-gp expression causes an increase in amyloid β protein levels in the brain, which is involved in the pathogenesis of Alzheimer's disease (Cirrito *et al.,* 2005; Vogelgesang *et al.,* 2002; Vogelgesang *et al.,* 2004). Reduced expression or activity of P-gp has also been associated with Creutzfeld-Jakob disease (Vogelgesang *et al.,* 2005), Parkinson's disease (Vautier *et al.,* 2009), HIV infection (Langford *et al.,* 2004), and normal aging (Bauer *et al.,* 2009). Furthermore, increased expression or activity of P-gp, but also BCRP, MRP1, and MRP2 is associated with epileptic seizures (Löscher and Potschka, 2005a).

Of all the different transporter systems, P-gp is considered to be the most clinically relevant, as it has a broad substrate specificity and plays an important role in drug disposition and response. Besides being expressed at the BBB and BCSFB, P-gp can also be found in the liver, kidneys, and intestine (Thiebaut *et al.,* 1987). As a result, a variable expression or activity of P-gp will alter the

extent of absorption, tissue distribution, and excretion of compounds that are substrates for P-gp (Marzolini *et al.,* 2004). The variable expression or activity could be caused by single-nucleotide polymorphisms (SNPs) that have been reported for the gene encoding P-gp (Marzolini *et al.,* 2004). While the effect of most SNPs on the expression or activity of P-gp still remains unclear, it has been reported that the absorption by the gut is altered by a specific polymorphism in exon 26 (C3435T), which results in a lower expression level of P-gp (Hoffmayer *et al.,* 2000). The same SNP has also been reported to be involved in drug-resistant epilepsy, where epileptic patients that have the specific SNP are more likely to respond to antiepileptic drugs (Siddiqui *et al.,* 2003). This is probably the result of a decreased expression and activity of P-gp at the BBB.

Even though most of the different transport systems at the BBB and BCSFB are expressed both in humans as well as in rats, their expression levels and activities might differ greatly between species (table 1) (Begley, 2004; Choudhuri *et al.,* 2003; Graff and Pollack, 2004; Hagenbuch and Meier, 2004; Hoshi *et al.,* 2013; Kusuhara and Sugiyama, 2004; Kusuhara and Sugiyama, 2005; Löscher and Potschka, 2005b; Perrière *et al.,* 2007; Syvänen *et al.,* 2009; Uchida *et al.,* 2011; Vannucci, 1994). This is probably the result of the genetic differences between humans and rats. The most profound differences can be seen for P-gp and the OATP family.

In humans, P-gp is encoded by the MDR1 gene, whereas in rodents it is encoded by two genes; mdr1a and mdr1b. Both mdr1a and mdr1b are present in rodent brain, but only mdr1a is located at the BBB, while mdr1b is present in brain parenchyma (Demeule *et al.,* 2002). The substrate specificity of mdr1a and mdra1b P-gp is largely overlapping, although there are some differences (Schinkel, 1999). As the tissue distribution of mdr1a and mdr1b P-gp is different, but partly overlapping, this suggests that the mdr1a and mdr1b P-gp in rodents functions the same as the MDR1 P-gp in humans (Schinkel, 1999). For the OATP family, the differences between species are more profound (Hagenbuch and Meier, 2004). However, because the OATP family has a broad and partially overlapping substrate specificity, it is likely that the functions of the different subtypes are comparable between rodents and humans (Hagenbuch and Meier, 2004).

Table 1. Overview of the different active transport systems that are located at the human and rat BBB and BCSFB

Transporter protein	Human BBB	Rat BBB	Human BCSFB	Rat BCSFB
P-gp	+ [1]	+ [5,a]	+ [2]	+ [2,a]
BCRP	+ [1]	+ [5]	-	-
MRP1	+ [2]	+ [6]	+ [2]	+ [11]
MRP2	+ [2]	+ [7]	-	+ [11]
MRP3	+ [2]	+ [6]	-	+ [11]
MRP4	+ [1]	+ [5]	+ [8]	+ [11]
MRP5	+ [2]	+ [6]	+ [8]	+ [11]
MRP6	+ [2]	-	-	+ [11]
OCT1	+ [2]	-	+ [2]	+ [11]
OCT2	+ [2]	+ [8]	+ [2]	+ [11]
OCT3	+ [2]	+ [8]	+ [2]	+ [8]
OCTN1	+ [2]	+ [8]	+ [8]	+ [11]
OCTN2	+ [2]	+ [8]	+ [8]	+ [11]
OATP A	+ [3]	-	-	-
OATP B	+ [3]	+ [8,b]	+ [8]	+ [11,b]
OATP E	+ [3]	+ [8,c]	+ [8]	+ [11,c]
OATP F	+ [3]	+ [8,d]	-	+ [11,d]
Oatp1	-	+ [3]	-	+ [2]
Oatp2	-	+ [3]	-	+ [2]
Oatp3	-	+ [3]	-	+ [2]
OAT1	+ [2]	+ [2]	+ [10]	+ [8]
OAT2	-	-	-	+ [8]
OAT3	+ [4]	+ [5]	+ [10]	+ [8]
GLUT-1	+ [1]	+ [5]	+ [2]	+ [9]

Notes to table 1: [1] Uchida et al., 2011; [2] Graff and Pollack, 2004; [3] Hagenbuch and Meier, 2004; [4] Löscher and Potschka, 2005b; [5] Hoshi et al., 2013; [6] Perrière et al., 2007; [7] Begley, 2004; [8] Kusuhara and Sugiyama, 2004; [9] Vannucci, 1994; [10] Kusuhara and Sugiyama, 2005; [11] Choudhuri et al., 2003.

[a] Human P-gp encoded by MDR1, rat P-gp encoded by mdr1a/mdr1b; [b] The rat ortholog of OATP B is Oatp9; [c] The rat ortholog of OATP E is Oatp12; [d] The rat ortholog of OATP F is Oatp14.

Another important difference between species is the relative rate of CSF production, which is estimated to be about 0.88 %/min in rats, compared to 0.29 %/min in humans (table 2). Thus, there is a higher CSF turnover in rats compared to humans. This could be important for hydrophilic compounds, as they can be taken along with the CSF and the relatively high flow of CSF could thereby prevent the diffusion to the brain target site. As for lipophilic compounds, even though the protein concentration in human CSF is reported to be about 400 times less than in human blood, the CSF protein concentration in the rat is 5-10 times higher compared to humans (Maurer, 2010). It has also been reported that CSF protein concentrations increase along the flow path. Therefore, a concentration gradient could exist along the CSF flow path for both hydrophilic as well as lipophilic compounds. This indicates that the location of CSF sampling is very important (Summerfield and Jeffrey, 2006).

These are all examples illustrative of the fact that we need a more thorough understanding of the processes that occur in the brain. To do so, we must gather and combine mechanistic information on the contribution of processes on the causal chain from drug administration, to drug brain target site PK, to ultimate CNS drug effects, including the variability in these contributions that will exist between conditions. Such will pave the way for adequate prediction of human drug effects. Consequently, a lot of data needs to be integrated, which is beyond individual intellectual capabilities. Therefore, we need to make use of mathematical modeling.

Table 2. Differences between rat and human physiological parameters

Parameter	Human value	Rat value
$Brain_{ECF}$ volume	240 ml [1]	290 µl [6]
$Brain_{ECF}$ production rate	0.15-0.2 ml/min (0.0625-0.083 %/min) [1,2]	0.2-0.5 µl/min (0.069-0.17 %/min) [6,7]
CSF volume	140 ml [3]	250 µl [8]
CSF production rate	0.4 ml/min (0.29 %/min) [4]	2.2 µl/min (0.88 %/min) [9]
Cerebral blood flow	700 ml/min (14% of cardiac output) [5]	1.1 ml/min (2.5% of cardiac output) [10]

[1] Begley, 2000; [2] Kimelberg, 2004; [3] Kohn et al., 1991; [4] Nilsson et al., 1992; [5] Ito et al., 2006; [6] Cserr et al., 1981; [7] Abbott, 2004; [8] Bass and Lundborg, 1973; [9] Cserr, 1965; [10] Harashima et al., 1985.

Mathematical modeling

We can learn more on the inter-relationship between plasma PK, BBB transport and intra-brain distribution, by performing integrative cross-compare designed studies in animals in which variables are systematically varied (e.g. inhibition of an efflux transporter, or induction of pathological state). This allows us to dissect contributions of individual mechanisms of blood-brain transport in animals, which provides links to the human situation. Humans have the same mechanisms as animals, but these may have a different rate and extent and therefore have different contributions to the dose-effect relationships (Cserr and Bundgaard, 1984). As many variables are in play, we need to organize and integrate all these, and further condense and store such knowledge in mathematical models.

Compartmental modeling - In order to be able to predict human brain target site concentrations and ultimate drug effect on the basis of preclinical data, different mathematical modeling techniques can be applied (Danhof *et al.,* 2008). The most commonly applied mathematical modeling technique is the relatively simple compartmental model analysis, viewing the body as a series of virtual and interconnected compartments (Fleishaker and Smith, 1987). Using mass balance differential equations, concentration-time profiles in different compartments can be described. These equations can also be used to calculate PK parameters like volume of distribution, systemic clearance, and elimination half-life.

Allometric scaling - Extrapolation of animal PK parameters to the human situation is necessary for designing first in human trials, which can sometimes be done reasonably well by allometric scaling (Bonati *et al.,* 1984; Lavé *et al.,* 1999; Mahmood and Balian, 1999; Obach *et al.,* 1997; Yassen *et al.,* 2007; Zuideveld *et al.,* 2007). Allometric scaling uses bodyweight or body surface area as the main determinant of PK parameters, assuming that there are anatomical, physiological and biochemical similarities among different species (Boxenbaum, 1982; Dedrick, 1973; Mordenti, 1986). However, for predicting human PK parameters that are involved in more complex systems on the basis of animal data, a physiologically-based (PB) PK model is more appropriate.

PBPK modeling - PBPK models integrate drug-dependent, physiological, and biological parameters as they vary in between species, subjects, or with age and disease state (Colburn, 1988a; Espié *et al.,* 2009; Ings, 1990). In a PBPK model the tissues of interest are viewed as body compartments and are arranged in anatomical order, based on blood circulation, to form an integrated physiological model (Colburn, 1988a; Espié *et al.,* 2009; Ings, 1990; Rowland *et al.,* 2004). Once a suitable model has been developed, the concentration-time profiles of the drug in each of the body compartments can be calculated based on a mass balance. The distribution of the drug can then be linked to the physicochemical properties of the drug and the type of tissue involved. Distribution of the drug into tissues can further be classified as either perfusion rate limited or permeability rate limited (Colburn, 1988a; Ings, 1990; Jones *et al.,* 2009).

A typical PBPK model consists of non-eliminating (adipose, bone, brain, gut, heart, lung, muscle, skin, spleen) and eliminating tissues (kidney and liver) (figure 3A) (Jones *et al.,* 2006; Jones *et al.,* 2009; Rowland *et al.,* 2004). However, to be able to properly predict brain target site concentrations, the brain compartment in the PBPK model should describe the complexity of the CNS (De Lange and Danhof, 2002; Ooie *et al.,* 1997; Shen *et al.,* 2004). Figure 3B shows the complexity of the CNS in the brain compartment of a PBPK model.

Even though the PBPK approach may be more suitable for complex systems, it also results in complex mathematical models that are expensive and time consuming. Therefore, PBPK models are often only used in the later stages of drug development (Espié *et al.,* 2009; Ings, 1990; Jones *et al.,* 2006; Jones *et al.,* 2009; Rowland *et al.,* 2004).

Modeling of drug effect - Ultimately, the goal of mathematical modeling is to be able to predict drug efficacy and safety in humans on the basis of animal data (Danhof *et al.,* 2008). To link drug concentration-time data to the physiologic response, PK-PD modeling is often applied. The primary objective of PK-PD modeling is prediction of the time course of the drug effect *in vivo* in health and disease (Breimer and Danhof, 1997). Depending on the concentration-effect relationship, as well as the type of response (inhibition or stimulation), different PK-PD models can be used (Csajka and Verotta, 2006; Swinghammer and

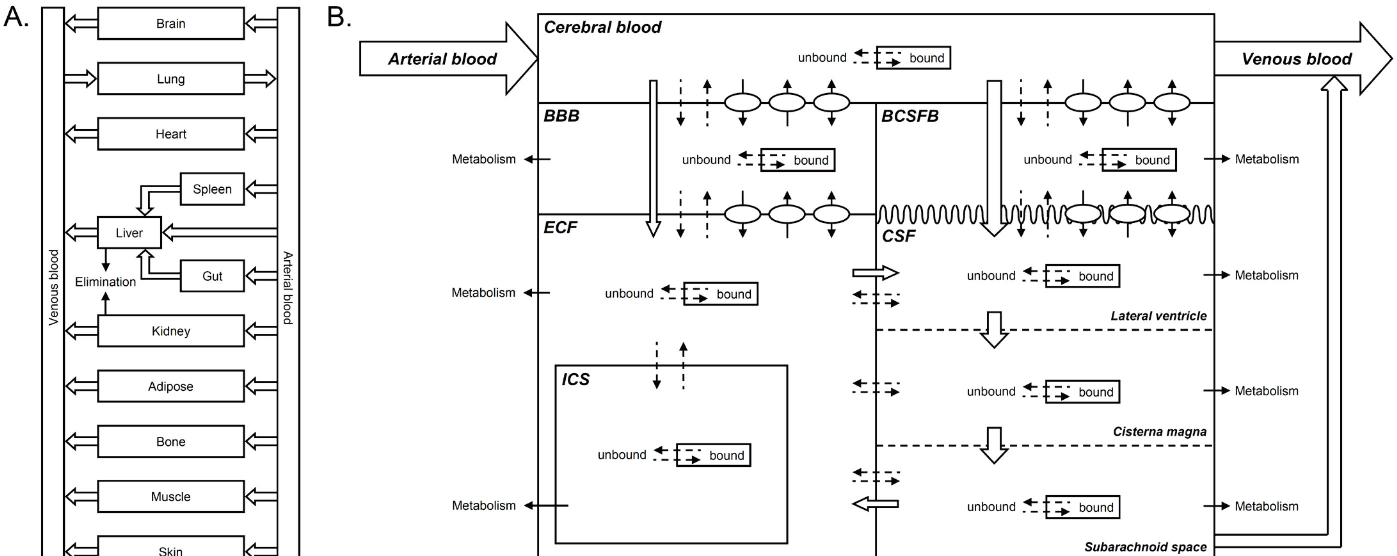

Figure 3. (A) Schematic representation of a typical PBPK model. (B) Schematic representation of the brain compartments on the basis of the information that is presented in this review. Block arrows indicate fluid flow; solid arrows indicate active transport processes; dashed arrows indicate permeability rate-limited transport; solid lines indicate restricted diffusion; dashed lines indicate free diffusion

Kroboth, 1988). When the effect is directly related to plasma concentrations, a PK compartmental model with the addition of an effect compartment could be sufficient (Sheiner *et al.,* 1979). However, as CNS drug effects often have their origin in the brain, an alternate model that relates the effect to drug concentrations in a peripheral compartment is more appropriate (Colburn, 1981; Colburn, 1988b).

Prediction of human drug effect - Extrapolation of animal PK-PD data to predict the time course of the drug effect in humans can sometimes be done by simple allometric scaling of the PK parameters and the PK-PD relationship (Mager *et al.,* 2009). However, for accurate prediction of human drug effect on the basis of animal data, one should also understand the biological processes that underlie the effect. These processes include target site distribution, target binding, target activation, transduction, homeostatic feedback, and disease processes (Danhof *et al.,* 2005; Danhof *et al.,* 2007). Information on these processes is included in mechanism-based (MB) PK-PD modeling. A major advantage of MBPK-PD modeling is the distinction between drug-specific parameters and biological system-specific parameters. Drug-specific parameters are dependent on the physicochemical properties of the drug and include target affinity and target activation, whereas biological system-specific parameters describe the functioning of the biological system (Danhof *et al.,* 2008). This indicates that MBPK-PD modeling can be applied for the extrapolation of animal data to the human situation, but also from one drug to another.

DISCUSSION AND CONCLUSIONS

The development of drugs for central nervous system (CNS) disorders has encountered high failure rates. In part this has been due to the sole focus on BBB permeability of drugs, without taking into account all other processes that determine drug concentrations at the target site. Moreover, conditional dependence of these processes has typically been neglected.

The impact of these processes can be studied in the preclinical setting with several *in vitro*, *ex vivo*, and *in vivo* techniques. However, considering the

animal-to-human extrapolation, the CSF sampling technique and intracerebral microdialysis technique are currently the best available techniques.

In practical terms, of special interest is CSF sampling as it can be performed in animals as well as in humans and provides information on unbound drug concentrations. A number of studies have shown that steady state CSF concentrations can be used very well for the prediction of steady state brain concentrations within a 3-fold error range for compounds that freely diffuse across the BBB and BCSFB, whereas the difference between CSF exposure and brain exposure may be beyond 3-fold for compounds that are substrates for the different active transport systems at the BBB and BCSFB (Fridén *et al.,* 2009b; Kalvass and Maurer, 2002; Liu *et al.,* 2006; Maurer *et al.,* 2005). On that basis it may be questioned:

- How useful are steady state concentration ratios? What is the degree of fluctuation in plasma concentrations and how does this correspond to the degree of variation in concentrations at the brain target site? And, in what extent are the CNS effects sensitive for such fluctuations?
- How useful are single time point CSF or total brain samples? One should consider that the use of a single time point sample as a marker for CSF or total brain exposure tells us nothing about the rate of blood-brain transport, which is relevant for drugs with a desired rapid onset of action.
- What is the impact of the 3-fold error that is allowed for the estimation of CSF-brain$_{ECF}$ concentration ratios? The 3-fold error is considered to be of little pharmacologic or pharmacokinetic consequence for the prediction of unbound brain concentrations on the basis of CSF concentrations (Maurer *et al.,* 2005). However, the 3-fold error is also allowed for the prediction of human CSF exposure on the basis of rat CSF exposure (Fridén *et al.,* 2009b). This results in a 9-fold error that is allowed for the prediction of human unbound brain exposure on the basis of human CSF exposure. This may have significant consequences if a drug has a steep concentration-effect relationship or a narrow therapeutic window.

This implicates that in order to be able to accurately predict CNS drug effect in humans, it is essential that we increase our understanding of the complexity of the CNS and CNS disorders. Given that CSF concentrations are often

considered to be the best available surrogate for brain target site concentrations in humans (Fridén *et al.,* 2009b; Kalvass and Maurer, 2002; Liu *et al.,* 2006; Liu *et al.,* 2009; Maurer *et al.,* 2005), future research should focus on studying the relationship between CSF concentrations and brain target site concentrations.

The only technique that allows the measurement of unbound drug concentrations at the brain target site, provided that the CNS drug target faces the $brain_{ECF}$, as well as in CSF *in vivo* is the intracerebral microdialysis technique. In the preclinical setting, the intracerebral microdialysis technique could very well be used to investigate the relationship between CSF concentrations and extracellular brain target site concentrations (Malhotra *et al.,* 1994; Matos *et al.,* 1992; Wong *et al.,* 1992). When the CNS drug target is located intracellularly, there is not a single technique that allows to study the relationship between CSF concentrations and intracellular concentrations. This indicates that a combination of different techniques should be applied. However, to be able to predict CNS drug effect in humans, it is also essential to study the underlying processes that govern the concentration-effect relationship.

In combination with CSF sampling, brain tissue sampling and serial blood sampling, the intracerebral microdialysis technique provides very useful data to determine the kinetics of transport equilibration across the BBB and BCSFB under a variety of conditions, in the species of choice, such as mice, rats, rabbits, piglets and monkeys, as well as in humans (intensive care patients). Therefore it is of importance to investigate the inter-relationship between plasma PK, BBB transport and intra-brain distribution in integrative cross-compare designed studies. By systematically influencing one (or a subset) of variables, one can decipher the impact of changes at the level of this variable on the blood-brain transport.

Such information on the rate and extent of BBB and BCSFB transport, and modulations thereof, will be useful to further distinguish the possible contribution of many dynamically regulated passive and active transport mechanisms. The knowledge thus provided on BBB and BCSFB transport mechanisms and regulation is critical for the understanding of brain homeostasis, and how disturbances thereof may lead to CNS diseases. Also, it will be critical in ultimately being able to predict the PK-PD relationship of CNS active compounds.

Apart from striving towards reduction of the use of animals and the fact that animal models of CNS diseases will never exactly reflect the disease conditions in human, we will move forward considerably by systematic research on CNS drugs in the preclinical setting, including animal models of CNS diseases that reflect important parts of disease mechanisms. As many variables are in play, we need to organize and integrate all these, and further condense and store such knowledge in mathematical frameworks.

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