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Blood-Brain Barrier Transport of Drugs Across Species with the Emphasis on Health, Disease and Modelling

BY

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Abstract

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The transport of drugs across the blood-brain barrier (BBB) has been investigated in different species using morphine and morphine-6-glucuronide (M6G) as model compounds. The influence of probenecid on the BBB transport of morphine and M6G was investigated, and the consequences of meningitis and severe brain injury on the concentrations of morphine in the brain were examined. All data were obtained by microdialysis, and data analysis using mathematical models was emphasised.

Morphine is exposed to active efflux at the BBB in rats, pigs and humans. In addition, the half-life of morphine is longer in the brain than in blood in these species. These interspecies similarities show the predictive potential of the two animal models for the BBB transport of morphine in humans. In the pig the exposure of the brain to morphine was higher in the presence of meningitis than when healthy. This was interpreted as a decrease in the active efflux and an increase in the passive diffusion over the injured BBB. In contrast, there was no significant difference in the concentrations of morphine in the "better" (uninjured) or the "worse" (injured) brain tissue in brain trauma patients. The extent of the transport across the BBB is similar for morphine and M6G. However, co-administration of probenecid only increased the brain concentrations of morphine, demonstrating that morphine and M6G are substrates for different efflux transporters at the BBB. An integrated model for the analysis of data obtained by microdialysis was developed. This model makes fewer assumptions about the recovery, the protein binding and the time of the dialysate observation than a previous model and traditional non-compartmental analysis and should, therefore, yield more reliable parameter estimates.

Knowledge of the consequences of efflux transporters and disease on the brain concentrations of a drug can be useful for individualising the dosing regimen in patients.

Keywords: Blood-brain barrier, Disease, Microdialysis, Modelling, Transport, Pharmacokinetics, NONMEM

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Till mamma och pappa

Papers discussed

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text.

I *Tunblad K., Jonsson E.N. and Hammarlund-Udenaes M.*
Morphine blood-brain barrier transport is influenced by probenecid co-administration.
Pharmaceutical Research, 20 (4); 618-623 (2003).

II *Tunblad K., Hammarlund-Udenaes M. and Jonsson E.N.*
Influence of probenecid on the delivery of morphine-6-glucuronide to the brain. *In manuscript.*

III *Tunblad K, Ederoth P., Gärdenfors A., Hammarlund-Udenaes M. and Nordström C.-H.* Altered brain exposure of morphine in experimental meningitis studied with microdialysis.
In press, Acta Anaesthesiologica Scandinavica (2004).

IV *Ederoth P., Tunblad K,. Bouw R., Lundberg J., Ungerstedt U., Nordström C.-H. and Hammarlund-Udenaes M.*
Blood-brain barrier transport of morphine in patients with severe brain trauma.
In press, British Journal of Clinical Pharmacology (2004).

V *Tunblad K., Hammarlund-Udenaes M. and Jonsson E.N.*
An integrated model for the analysis of pharmacokinetic data from microdialysis experiments. *In progress.*

Contents

Abbreviations

ABC	ATP-binding cassette
A_{brain}	Total amount of drug in the brain
AUC	Area under the concentration-time curve
AUC_u	Area under the unbound concentration-time curve
ATP	Adenosine triphosphate
BB	"Better" brain tissue
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BEI	Brain efflux index
BUI	Brain uptake index
C_{blood}	Concentration in the blood
$C_{u, brain}$	Unbound concentration in the brain
CDs	Candidate drugs
CL	Clearance
CL_{in}	Influx clearance from the blood to the brain
CL_{out}	Efflux clearance from the brain to the blood
CNS	Central nervous system
$C_{u,ss,blood}$	Unbound steady state concentration in the blood
$C_{u,ss,brain}$	Unbound steady state concentration in the brain
ECF	Extracellular fluid
f_u	Fraction unbound
ICP	Intracranial pressure
IIV	Interindividual variability
i.v.	Intravenous
k_{in}	Rate constant from the blood to the brain
k_{out}	Rate constant out of the brain
k_{av}	Rate constant from arterial to venous blood
k_{va}	Rate constant from venous to arterial blood
k_{10}	Rate constant out of the blood
k_{14}	Rate constant from central to peripheral compartment

k_{41}	Rate constant from peripheral to central compartment
K_{in}	Transfer rate constant
LPS	Lipopolysaccharide
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
MRP	Multidrug resistance associated protein
NCA	Non-compartmental analysis
N.S.	Not significant
Oat	Organic anion transporter
Oatp	Organic anion transporting polypeptide
OFV	Objective function value
PS	Permeability surface area product
P-gp	P-glycoprotein
PL	Plasma
RSE	Relative standard error
Q	Intercompartmental clearance
Q_{av}	Intercompartmental clearance in the blood
SC	Subcutaneous adipose tissue
SD	Standard deviation
SDS	Sodium dodecyl phosphate
$t_{½}$	Terminal half-life
THF	Tetrahydrofuran
TIN	Time of the collection interval for the dialysate fractions
T_{max}	The time to reach the maximum concentration
V_1	Unbound of volume of distribution in the central blood compartment
V_{blood}	Volume of blood in the brain
V_d	Volume of distribution
V_{br}	Volume of distribution in the brain
V_{brain} and $V_{u,brain}$	Unbound volume of distribution in the brain
V_4	Unbound volume of distribution in the peripheral compartment
WB	“Worse” brain tissue

Introduction

The importance of understanding the influence of active transporters on the cerebral pharmacokinetics of drugs has been recognised during the last few years (Abbott *et al.*, 1996; Kusuhara *et al.*, 2001; Pardridge, 1998; Tamai *et al.*, 2000). The transporters act as pumps that transfer drugs across cell membranes against or along a drug concentration gradient. Efflux pumps such as P-glycoprotein (P-gp) (Cordon-Cardo *et al.*, 1989), multidrug resistance associated proteins (MRPs) (Huai-Yun *et al.*, 1998), organic anion transporters (Oats) (Kusuhara *et al.*, 1999) and organic anion transporting polypeptides (Oatps) (Hagenbuch *et al.*, 2002; Sugiyama *et al.*, 2001) are expressed at the blood-brain barrier (BBB). Most of the transporters expressed at the BBB transfer molecules from the brain back to the blood. Consequently, the brain concentrations of drugs that are substrates for any of these transporters will be low in comparison to the blood. For drugs, which exert their pharmacological effects in the central nervous system (CNS), the consequence of the efflux pumps on the brain concentrations of the drugs should be recognised. In addition, by identifying the specific transporters involved in the efflux of a certain drug, drug interactions can be predicted and handled to attain the desired pharmacological effect and to prevent toxicity in patients.

Little is known about how diseases associated with the brain affect the distribution of drugs to the brain. In pharmacokinetic studies, drug concentrations are usually measured in the blood. However, the concentrations in the blood may well differ from those in the brain, both under healthy conditions and in the presence of disease, owing to the properties of the BBB. Therefore the blood concentrations may not be relevant for the individualisation of the dosing regimen to achieving the desired therapeutic drug concentration for each patient. However, measuring the brain concentrations of a drug is more complicated. Consequently, making dosing adjustments in the presence of disease that affect the BBB is difficult.

This thesis focuses on the exposure of drugs to the brain including the transport of drugs across the BBB. With the aim of investigating this transport the cerebral distribution of morphine, a drug which is commonly

used in the treatment of severe pain, and its active metabolite morphine-6-glucuronide (M6G) were used as model compounds. The involvement of active efflux mechanisms at the BBB was investigated for these compounds. In addition, interspecies differences between and similarities in the BBB transport of morphine were studied, including the effect of diseases related to the brain on morphine brain concentrations. Microdialysis was used in all investigations, and since these types of experiments generate data that differ from the data obtained by regular blood sampling, emphasis was given to develop a model for the data analysis.

Drug development

The primary goal of pharmaceutical companies is to develop new drugs for the treatment of medical disorders. These drugs should be safe and effective, and, ideally, put on the market quickly and without huge development costs being incurred. To decrease the time to market, and thereby the development costs, one requirement in drug development is that the majority of the compounds that are selected as candidate drugs (CDs) in the discovery phase will be successfully developed into drugs. This is particularly challenging for drugs that should act in the CNS, and consequently there are few brain diseases that are efficiently treated with medicines (Pardridge, 2002). For the major CNS related diseases, including Alzheimer's disease, multiple sclerosis, Parkinson's disease and brain trauma, the progress has been slow in developing drugs (Pardridge, 2002).

At present, many of the potential CDs that have been selected for their pharmacological effect in the CNS do not reach therapeutic concentrations at the site of action (Lundquist *et al.*, 2003). Thus more than 98% of the candidate CNS-targeting drugs fail to be developed into drugs (Pardridge, 2002). To increase the number of CDs that are successfully developed into CNS active drugs, it is essential to use experimental methods that are predictive of the *in vivo* situation in humans.

The ability of a drug to enter the brain is dependent on the physico-chemical properties of the drug (i.e., its lipophilicity, size, charge and shape) and whether the drug is a substrate for any active processes at the BBB. In general it is difficult for hydrophilic compounds to reach therapeutic concentrations in the brain. This could be explained by slow transport across the BBB of hydrophilic molecules owing to the close alignment of the cells that form the BBB. Furthermore, many lipophilic substances are excluded from the brain because these compounds are often substrates for active efflux at the BBB. For successful drug delivery to the CNS it is essential to

identify active mechanisms at the BBB, since these will affect the target site concentrations.

The blood-brain barrier

Endothelial cells lining the brain capillaries form the BBB (Figure 1). The function of the BBB is to regulate the microenvironment of the brain, and to protect the brain from toxic compounds. The cells that form the BBB are joined to each other by tight junctions. These endothelial cells are also characterised by the absence of fenestrations, and the presence of few pinocytotic vesicles. Consequently, only very small hydrophilic molecules can enter the brain paracellularly via the tight junctions (van Bree *et al.*, 1988). All other molecules must either diffuse through the endothelial cells, or enter the brain by active influx mechanisms (Pardridge, 1995).

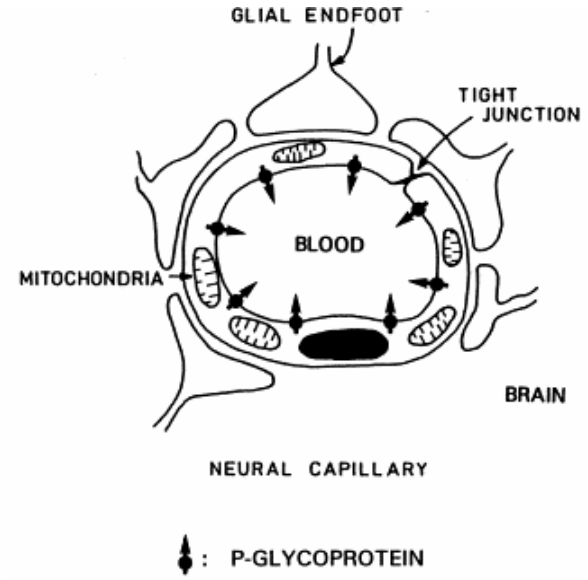


Figure 1. *A schematic representation of the blood-brain barrier. The endothelial cells, which are connected by tight junctions, are characterised by the presence of active transporters, such as P-glycoprotein, and richness in mitochondria (van Bree et al., 1992).*

Another feature of the BBB is the expression of efflux pumps, which are proteins that transfer molecules out of the brain (Cordon-Cardo *et al.*, 1989; Huai-Yun *et al.*, 1998; Tsuji *et al.*, 1992) (Figure 1). By means of this expression, compounds that are substrates for any of these pumps will be excluded from the brain to a certain extent. The combination of the properties of the BBB makes it difficult for the majority of compounds to enter the brain, despite the diversity of their physico-chemical characteristics.

The consequences of the BBB on the brain concentrations of drugs are of particular interest for drugs which exert their effect on the CNS. For example, morphine (Bouw *et al.*, 2000) and the antiepileptic drug gabapentin (Wang *et al.*, 1996) are drugs that are pharmacologically active in the CNS, and that reach lower unbound concentrations in the brain than in the blood at equilibrium. This discrepancy in the brain and the blood concentrations could be explained by active efflux mechanisms at the BBB, metabolism in the BBB (Ghersi-Egea *et al.*, 1994) or bulk flow of the brain extracellular fluid (ECF) (Szentistvanyi *et al.*, 1984).

Transport mechanisms at the blood-brain barrier

Those drugs able to cross the BBB do so via passive diffusion or active processes (Figure 2). Gaining a better understanding of the mechanisms involved in the BBB transport of a drug would help in predicting the consequences changes in the properties of the BBB would have on the cerebral concentrations of a drug. Knowing the rate of transport across the BBB would enable the dosing regimen to be optimised, thereby increasing the fraction of the dose that reaches the brain. For example, it would be advantageous to administer a drug that is transported across the BBB slowly as a constant intravenous (i.v.) infusion or in an oral slow-release formula to increase the time over which the drug could be distributed to the brain. In addition, by identifying the active transporters that are involved in the brain efflux of a drug, local drug-drug interactions at the BBB could be foreseen. This would mean that in each patient, the dosing regimen of a drug could be selected to avoid undesired effects including toxicity whilst maximising the pharmacological impact.

Passive diffusion

Passive diffusion is the energy independent transport of unbound molecules across a cell membrane. This process involves the movement of molecules

along a concentration gradient. Thus, there will be a net transport from tissues containing high concentrations to tissues with low concentrations until equilibrium is reached. Passive diffusion can be either trans-cellular, taking place through the cells, or para-cellular, occurring between the cells, and the route or rate of diffusion depend on the physico-chemical properties of the molecules. Discrepancies in unbound tissue concentrations at equilibrium could be explained by the involvement of active mechanisms (Hammarlund-Udenaes *et al.*, 1997).

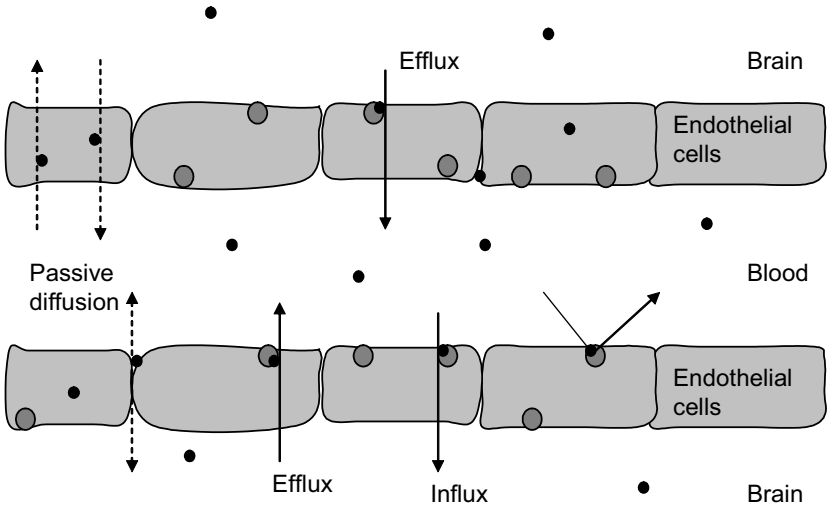


Figure 2. *The transport mechanisms at the blood-brain barrier. The arrows indicate the direction of the transport. Passive diffusion can be trans-cellular or para-cellular. The transporters involved in the active processes are situated on the luminal and/or the basolateral sides of the blood-brain barrier (i.e. facing the blood or the brain respectively).*

Active transport

Active transport is the energy dependent transfer of molecules across a cell membrane, sometimes against the concentration gradient. At the BBB, both transporters for active influx and active efflux are expressed (Figure 2), and therefore, differences in unbound steady state concentrations of a substance in brain and blood indicate that the substance is a substrate for active transporters. Active influx, i.e. transport from the blood to the brain, is demonstrated by the exposure of the brain to the substance being higher than the exposure of the blood to the substance. Also, if the brain concentrations

of a substance decrease when a transporter is inhibited, then it can be deduced that the transporter is involved in the influx of the substance. For example, Bourasset and co-workers recently suggested that M6G is a substrate for the glucose transporter GLUT-1, which is a bi-directional transporter at the BBB (Bourasset *et al.*, 2003).

In contrast, the exposure to a substance is lower in the brain than in the blood if the substance undergoes active efflux, i.e., transport from the brain to the blood. There are many examples of drugs that are substrates for efflux transporters in the CNS. Some of these are the anticancer drug methotrexate (de Lange *et al.*, 1995), the HIV drug zidovudine (Sawchuk *et al.*, 1990), the antiepileptic drug carbamazepine (Potschka *et al.*, 2001a) and morphine (Xie *et al.*, 1999). The effect of an active transporter on the brain concentration of a drug depends on the affinity of the drug to the transporter, the quantity of the relevant transporter expressed at the BBB and the capacity of the transporter. However it is possible that lower unbound concentrations are observed in the brain ECF than in the blood, even if the drug is not a substrate for any efflux pumps. This could be explained by the bulk flow, which is a continuous flow of brain ECF, mainly secreted at the choroid plexus and drained into the venous blood (Szentistvanyi *et al.*, 1984). The effect of bulk flow on the brain concentrations of a drug may be substantial for drugs that are slowly transported out of the brain. For example, the contribution of the bulk flow to the overall elimination of morphine-3-glucuronide (M3G) from the brain was estimated as 16-25 % in the rat (Xie *et al.*, 2000).

Over the last years the emphasis of research has been on identifying efflux pumps at the BBB and the blood-cerebrospinal fluid barrier, to better understand the mechanisms behind the low exposure of many drugs to the brain. Some of the efflux transporters that have been identified are P-gp, MRPs and Oats, all of which belong to the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of transporters. In addition the Oatps, which also belong to the ABC transporters, have been identified as bi-directional transporters. Thus the Oatps may increase or decrease the brain concentration of drugs. For example 17beta-estradiol-D-17beta-glucuronide is effluxed by Oatp2 (rat) (Sugiyama *et al.*, 2001), while the opioid peptides D-penicillamin enkephaline (DPDPE) and deltorphine II are actively influxed by OATP-A (human) (Gao *et al.*, 2000). At present seven subtypes of transporters in the MRP family are known (Kusuhara *et al.*, 2002; Loscher *et al.*, 2002), and seven and eleven subtypes of the Oats (Kusuhara *et al.*, 2002) and Oatps (Hagenbuch *et al.*, 2003) respectively, have been identified.

To characterise the transporters that are involved in the efflux of a certain drug, cell lines that lack the expression of a specific transporter could be

used. However, for many drugs several transporters are involved in the brain efflux and, when using an *in vitro* system, it is difficult to quantify the contribution of each transporter to the overall efflux *in vivo*. In addition, endogenous substances might interact with the transporters *in vivo*, and thereby the effect of the transporter on the efflux of a drug might change. For example, it has been demonstrated that corticosterone and aldosterone are substrates for P-gp, and that progesterone inhibits the same transporter (Ueda *et al.*, 1992). This means that if the concentration of a drug that is a substrate for P-gp is high, there is a risk that the transporter will become saturated. As a result of this it is likely that the effect of P-gp on the exposure of the brain to the drug is different *in vivo* than *in vitro*.

By using knock-out mice, the role of a certain transporter on the brain efflux of a drug can be quantified *in vivo*. This has been demonstrated for several drugs including morphine (Xie *et al.*, 1999), quinidine (Kusuhara *et al.*, 1997) and vinblastine (Schinkel *et al.*, 1994). In addition, transport inhibitors have been developed for use in the clinical situation. Unfortunately most of these inhibitors lack specificity, and, because of this it is difficult to assess the contribution of a specific transporter to the overall efflux. For example, probenecid has been used as an inhibitor of MRP (Kim *et al.*, 2001; Potschka *et al.*, 2001b). However, up till now seven subtypes of MRP have been identified, and it is not yet clear which of the subtypes are affected by probenecid. It has also been shown that probenecid inhibits Oatp1, Oatp2, Oat1 and Oat3 (Sugiyama *et al.*, 2001). This indicates that probenecid cannot be regarded as a specific inhibitor. Similarly Cvetkovic and co-workers have shown that PSC833 (Valspodar), a cyclosporine analogue that was formly considered to be a specific P-gp inhibitor, also affects Oatp1 and Oatp2 (Cvetkovic *et al.*, 1999). Yet another example is GF120918, a compound that modulates not only P-gp but also the breast cancer resistance protein (BCRP) (Allen *et al.*, 1999; Kruijtzer *et al.*, 2002). At present, LY335979 would be the compound to choose to modulate P-gp, since it is still regarded as a specific inhibitor of that transporter (Shepard *et al.*, 2003).

Methods for studying blood-brain barrier transport

There are *in vitro* models of the BBB that can be used to predict the penetration of drugs into the brain. These models, which use primary cultures of brain capillary endothelial cells either in the absence or presence of astrocytes, can be used to identify the specific transporters that act on a drug. However as many of these transporters are expressed at multiple sites within the CNS, and in the peripheral tissues, *in vivo* methods are preferable for quantitative measurements (Golden *et al.*, 2003; Lundquist *et al.*, 2002;

Pardridge, 1999). One quantitative *in vivo* method that can be used for this purpose is microdialysis. Other *in vivo* methods that are commonly used are the intravenous injection technique, the brain uptake index (BUI) method, the brain efflux index (BEI) method and the *in situ* brain perfusion method. A brief description of the principles behind these methods is given below.

Microdialysis

Microdialysis measures unbound drug concentrations over time at multiple sites in an individual. Thereby complete concentration-time curves are obtained for the unbound drug in both the brain and blood in each individual. Consequently, both parameters describing the BBB transport, i.e. the influx clearance (CL_{in}) and the efflux clearance (CL_{out}), can be estimated using this technique. The microdialysis method is further discussed in the section entitled "The Principles of microdialysis".

The brain uptake index method

The BUI method was developed by Oldendorf (Oldendorf, 1970). This is a single pass method where the animal is decapitated 15 seconds after administration of the drug and a reference compound into the carotid artery. The reference should be a highly diffusible and flow-limited compound. The uptake of the drug by the brain is calculated according to:

$$\mathrm{BUI}\,(\%) = \frac{C_{\mathrm{drug,\,brain}} / C_{\mathrm{reference,\,brain}}}{C_{\mathrm{drug,\,injected}} / C_{\mathrm{reference,\,injected}}} \cdot 100 \qquad (1)$$

$C_{drug,\,brain}$ and $C_{reference,\,brain}$ are the concentrations of the drug and the reference compound respectively in the brain and $C_{drug,\,injected}$ and $C_{reference,\,injected}$ represent the concentrations of the drug and reference compound in the injected solution. With this method, the transfer rate constant, K_{in}, which is comparable to CL_{in}, and the permeability surface area product (PS) can be calculated. An advantage of this method is that it is quickly and easily performed. However the BUI method will underestimate the brain uptake of drugs that are slowly transported across the BBB (Bickel *et al.*, 1996).

The intravenous injection technique

The i.v. injection technique is a multiple pass method where the drug is either administered as a bolus injection or by constant infusion until steady state concentrations are attained. The animals are decapitated at different time-points after dosing and K_{in} is calculated from the total amount of drug in the brain tissue (A_{brain}) and the area under the concentration-time curve (AUC) for plasma (Equation 2) (Ohno *et al.*, 1978).

$$K_{in} = A_{brain} / AUC_{plasma} \tag{2}$$

This technique has been used to measure the brain uptake of morphine (Wu *et al.*, 1997) and M6G (Bickel *et al.*, 1996; Wu *et al.*, 1997). An assumption that is made with this method is that the transport across the BBB is unidirectional, implying that there is no significant efflux of the drug from the brain back to the blood during the experimental period. For drugs that cross the BBB slowly, this method is preferable to the BUI method. If, however, the drug is subjected to significant levels of active efflux, the i.v. injection technique is likely to underestimate the K_{in} value.

The brain efflux index method

The brain efflux index method (BEI), which is used to characterise drug efflux from the brain to the blood was presented by Kakee and co-workers (Kakee *et al.*, 1996). In short, the drug and an impermeable reference compound are injected into the brain, and whereupon the animals are sacrificed at different points in time. The BEI is obtained as follows:

$$BEI\,(\%) = 1 - \frac{A_{drug,\,brain} / A_{reference,\,brain}}{A_{drug,\,injected} / A_{reference,\,injected}} \cdot 100 \tag{3}$$

$A_{drug,\ brain}$ and $A_{reference,\ brain}$ represent the amounts of the drug and the reference compound in the brain, respectively, and $A_{drug,\ injected}$ and $A_{reference,\ injected}$ represent the doses administered. With this method the apparent elimination rate constant, k_{out}, can be calculated. This parameter can be transformed to a CL_{out} value according to:

$$CL_{out} = k_{out} \cdot V_{br} \quad (4)$$

where V_{br} is the volume of distribution in the brain. This method is easy to perform and it can be used to examine the effect of inhibitors on the efflux rate of a compound by co-administration or pre-administration of an inhibitor.

The *in situ* brain perfusion method

This technique was developed by Takasato and co-workers to investigate the rate with which the brain takes up compounds that penetrate the BBB slowly (Takasato *et al.*, 1984). With this method the blood that is directed to the brain is substituted with the perfusate, which is infused into one of the major vessels leading to the brain. The perfusate contains the drug of interest and an intravascular marker such as inulin. The animals are decapitated directly after stopping the perfusion, and the amount of drug in the brain is determined. K_{in} is subsequently calculated from the total amount of drug in the brain, the concentration of the drug in the perfusate ($C_{perfusate}$) and the time of the infusion (T) according to:

$$K_{in} = A_{brain} / (C_{perfusate} \cdot T) \quad (5)$$

In a manner similar to that adopted in the i.v. injection method the *in situ* brain perfusion technique assumes that no brain efflux occurs during the experiment.

The principles of microdialysis

The most common way to measure drug concentrations in the body is by regular blood sampling. However the concentrations in blood are not necessarily equivalent to the concentrations in ECF in the organ of interest. When taking blood samples, both the bound and the unbound drug molecules are measured. Since it is the unbound drug at the site of action that is related to the pharmacological effect, measuring the total drug concentration may be unrepresentative for those drugs that are highly bound to plasma proteins and/or tissue components. Ideally, therefore, the unbound drug concentrations at the site of action would be measured. This makes

microdialysis a very attractive method as it monitors the unbound local tissue concentrations.

The microdialysis probe is composed of a semi-permeable membrane with a certain cut-off that only allows solutes that are smaller than the pores of the membrane to pass (Figure 3). The probe is implanted into the tissue of interest, and, during the experiment, it is continuously perfused with an artificial ECF solution, called the perfusate. A concentration gradient is created along the microdialysis probe as a consequence of the continuous flow of the perfusate through the probe. Thus, depending on the direction of the concentration gradient, substances are either recovered from, or delivered to, the ECF surrounding the probe. The fluid leaving the probe, the dialysate, is collected in fractions at predetermined intervals.

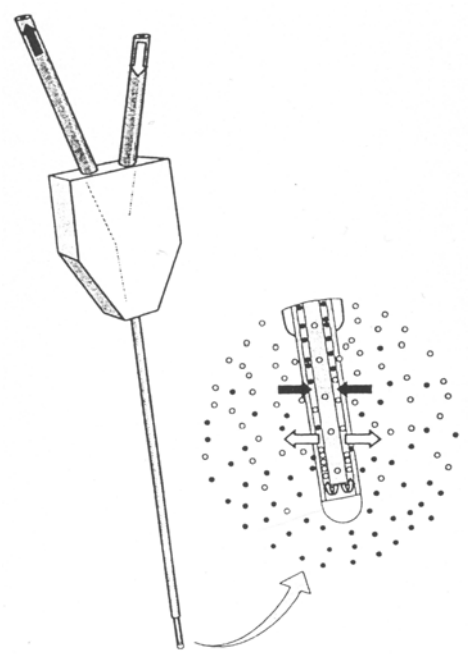


Figure 3. *The principle of microdialysis. The perfusate passes through the inner cannula to the tip of the probe. Thereafter it flows between the inner cannula and the dialysate membrane, where the exchange of solutes takes place. The dialysate is collected in fractions. (Ungerstedt, 1991).*

The dialysate concentration will reflect, but not be identical to the unbound concentration in the ECF. This is a consequence of the perfusion of the microdialysis probe. However, the true unbound tissue concentration can be calculated if the fraction of the tissue concentration recovered in the dialysate is known. This fraction is referred to as the relative recovery or simply the recovery.

The advantages and drawbacks of using microdialysis in pharmacokinetic studies are summarised in Table 1.

Table 1. The advantages and drawbacks of using microdialysis.

Advantages	Drawbacks
Can be used to measure unbound tissue concentrations of drugs and endogenous substances, or for local drug administration	The recovery issue must be considered
Measures the unbound concentrations in the extracellular fluid	The risk of tissue damage should be recognised
Can be performed locally in most organs	A sensitive analytical method is often required as the sample volume is small and the unbound concentration is usually low
Repeated sampling is possible even in small animals	The experiments are time consuming
Pharmacokinetic profiles can be generated in different tissues simultaneously	The experiments are expensive to perform
Cross-over studies can be performed in rats and mice	
Can be used in conscious animals	
Can be used to identify active transport systems	
No fluid is lost during sampling	
No sample preparation is needed	

Methodological considerations for intracerebral microdialysis

Several aspects need to be considered before performing a microdialysis experiment to obtain quantitative measurements in the brain. The key factors are related to the recovery of the drug, the probe characteristics, the perfusate, and tissue damage.

To obtain true tissue concentrations of a drug, the observed dialysate concentrations must be corrected for the recovery of the drug in each probe. According to the theory of quantitative microdialysis, the recovery will depend on the resistances of the dialysate, the membrane and the external medium (Bungay *et al.*, 1990). The resistance of the external medium is usually the most important contributor to the overall resistance for a solute (Bungay *et al.*, 1990). The rate of diffusion through the brain parenchyma is in general slow, compared to diffusion in water, and the speed depends on the properties of the tissue, i.e., the tortuosity, including active processes such as uptake into cells and active efflux mechanisms. Because of this the *in vivo* recovery is superior to the *in vitro* recovery, as the *in vitro* recovery underpredicts the tissue concentrations in most cases.

There are several methods used to estimate the *in vivo* recovery. In the studies in this thesis, the method of retrodialysis by drug (Bouw *et al.*, 1998) or by calibrator (Wong *et al.*, 1992) was applied. Another method that is commonly used is the no net flux method (Lönnroth *et al.*, 1987). The assumption underlying these methods is that the recovery is the same in both directions across the probe membrane (Amberg *et al.*, 1989). Thus, in an *in vivo* experiment, the loss of drug from the perfusate to the surrounding tissue during retrodialysis is equal to the gain of drug from the tissue to the dialysate after systemic drug administration.

The composition of the perfusate should resemble the extracellular environment to mimic loss or gain of fluid across the probe membrane. Using acetaminophen and atenolol as model drugs representing a moderately lipophilic and a moderately hydrophilic drug, respectively, it was demonstrated that the intracerebral concentrations were affected by the composition and the temperature of the perfusate (de Lange *et al.*, 1994). The intracerebral concentrations of atenolol increased approximately four fold when using a hypotonic perfusion solution instead of an isotonic solution. The exposure of the brain to acetaminophen was mainly affected when the perfusate was hypotonic and had a temperature of 24°C (de Lange *et al.*, 1994). Thus the impact of the perfusate on the brain concentrations depends on the physico-chemical properties of the drug under investigation.

Some tissue damage is unavoidable when inserting a microdialysis probe into the brain tissue. However, by understanding the time-course of the changes in the tissue properties the experiment could be performed under optimal conditions. In a study by Tossman and co-workers it was demonstrated that the integrity of the BBB was re-established approximately 30 minutes after the probe implantation (Tossman *et al.*, 1986). Moreover, the cellular reactions were minimal within one or two days following the brain probe insertion (Benveniste *et al.*, 1987). In contrast, the passage of inulin from the blood to the brain and extravasation of Evans Blue to the brain was demonstrated up to 24 hours after probe implantation (Westergren *et al.*, 1995). These results indicate that the function of the BBB is disturbed in the area around the microdialysis probe during the 24-hour period following probe implantation (Westergren *et al.*, 1995).

Disease

Diseases related to the brain may affect the distribution of drugs to the brain. Thus the exposure of a drug, and consequently the pharmacological effect of that drug, may be altered. For drugs with small therapeutic concentration ranges, changes in the brain exposure to a drug may lead to toxicity or to a lack of effect. For example, morphine is widely used in the clinic in the treatment of severe pain. Since the pharmacological effect of morphine is mediated via the μ-receptors, which are widely distributed within the CNS, it would be of clinical interest to understand how diseases affect the brain's exposure to this drug. With this knowledge, the morphine dosing regimens could be adjusted appropriately in patients suffering from diseases that affect the properties of the BBB.

During clinical conditions such as bacterial meningitis (Boje, 1995; Quagliarello *et al.*, 1992) and traumatic brain injury (Holmin *et al.*, 1998) the permeability of the BBB is increased as a result of an inflammatory response. This inflammation causes extravasation of granulocytes and monocytes/macrophages across the BBB, thereby increasing the water content of the brain. Consequently, the intracranial pressure (ICP) is increased, a condition which is associated with an increased probability of mortality (Ståhl *et al.*, 2001b). Thus it has been recognised that the BBB is impaired during bacterial meningitis and brain trauma. However it is not well understood how these conditions affect either passive diffusion or the active transport of drugs across the BBB.

Microdialysis has been used to study the cerebral energy metabolism in brain trauma patients (Ståhl *et al.*, 2001b). At Lund University Hospital brain

concentrations of glucose, glutamate, lactate and pyruvate measured at the bedside of these patients are used for clinical decision making (Ståhl *et al.*, 2001a). These metabolic markers have also been measured in a porcine model of meningitis, which was developed and confirmed by Gärdenfors and co-workers (Gärdenfors *et al.*, 2002). In that model, lipopolysaccharide (LPS), which is released from the bacterial cell walls and mediates the inflammation, was injected into the cisterna magna in the brain to induce meningitis. As the cerebral pharmacokinetics of drugs had not been studied in meningitis or brain trauma patients, the distribution of morphine was investigated in these models in Papers III and IV respectively.

Modelling

Pharmacokinetic data could be evaluated by traditional non-compartmental analysis (NCA), which would yield individual estimates of the pharmacokinetic parameters. Another approach is to fit a model to the data. By applying non-linear mixed effects modelling, both the fixed and the random effects could be estimated. The clearance (CL) and the volume of distribution (V_d) are examples of fixed effects parameters. The random effects include the interindividual variability (IIV) and the residual error. The IIV accounts for the difference between the individual parameter estimate and the typical estimate of the parameter in the population studied. The individual estimate of CL can be described by an additive model as in Equation 6.

$$CL_i = CL_{typ} + \eta_i \quad (6)$$

CL_i denotes the individual estimate, CL_{typ} the typical value of the parameter and η_i the random effect. It is assumed that the η_i values are symmetrically distributed with a mean of zero and variance ω^2.

If CL_i is known, the drug concentration in that individual could be predicted at any time-point. However it is likely that the predicted concentration at a certain time-point will differ from the observed concentration at that point in time. This difference, the residual error, is a consequence of errors associated with the chemical assay, the dosing and sampling time and model misspecification. In Equation 7 an additive model is used to account for the residual error.

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \quad (7)$$

$C_{obs,ij}$ is the *j*:th observation in the *i*:th individual, $C_{pred,ij}$ is the predicted concentration and ε_{ij} is the residual error. It is assumed that the ε is a symmetrically distributed zero mean variable with a variance of σ^2.

Non-compartmental analysis versus modelling

The NCA is a descriptive method that is easy to perform. Building a mathematical model to describe the data is more time-consuming and computer intensive. However there are several advantages with the modelling approach.

By population modelling using non-linear mixed effects models, all data are analysed simultaneously, and therefore the information from all individuals is shared. Consequently individual parameter estimates can also be obtained for individuals where too few data points are available for adequate estimations to be made using the NCA. In addition, a model could be used for simulations, which would be useful for study design optimisation during drug development. Nowadays population modelling is used during drug development to evaluate data from clinical studies (van Kesteren *et al.*, 2002; Vozeh *et al.*, 1996; Zingmark *et al.*, 2003). In addition, the use of the modelling approach is encouraged by the U.S. Food and Drug Administration (U.S. Department of Health and Human Services, 1999).

In studies of the BBB transport of drugs, both the rate and the extent of transport need to be considered. The rate of transport is related to the physico-chemical properties of the drug, while the extent of transport is dependent on the involvement of active processes at the BBB. Using the modelling approach, both the rate and the extent of transport can be estimated, whereas the NCA only measures the extent of BBB transport. Therefore, modelling is an attractive approach for studies of the BBB transport.

Models

The system we want to describe includes the distribution of unbound drug between the arterial, venous and brain compartments (Figure 4). Devising a model for this system is challenging since the observed dialysate concentrations and the total concentrations obtained from the regular blood sampling are different from the concentrations that describe the drug distribution. In addition to this, it should be considered that the dialysates are collected in fractions, in contrast to the regular blood samples which are drawn at specific time points.

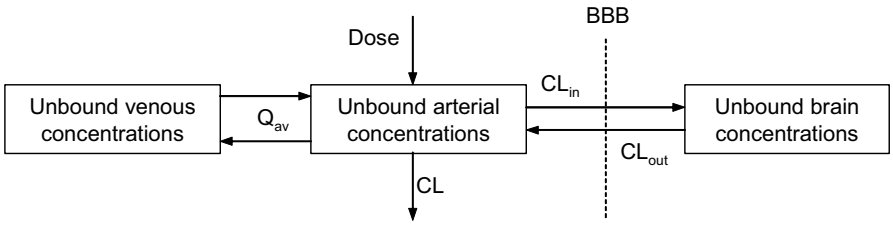


Figure 4. *The system that describes the distribution of unbound drug between the two blood compartments and across the blood-brain barrier (BBB). Abbreviations: CL, clearance;* Q_{av}*, intercompartmental clearance in the blood;* CL_{in}*, clearance from the blood into the brain and* CL_{out}*, clearance from the brain back to the blood.*

In previous studies on the BBB transport of opioids, the arterial concentrations, measured by regular blood sampling and corrected for protein binding, were used to determine the brain concentrations (Figure 5) (Bouw *et al.*, 2000; Bouw *et al.*, 2001; Xie *et al.*, 2000). In these studies the dialysate concentrations had been corrected for the individual estimate of the recovery prior to the data analysis, and the mid point in the collection interval was taken as the time of the observation. This approach, which will be referred to as the restricted or the previous model, assumes that the recovery and the protein binding are exact values with no uncertainty, assumptions which rarely are true. Also linear pharmacokinetics and small changes in the tissue concentrations within each collection interval are assumed (Patsalos *et al.*, 1995; Ståhle, 1992). Thus the validity of this approach depends on the length of the collection interval and the pharmacokinetic properties of the drug at the probe site.

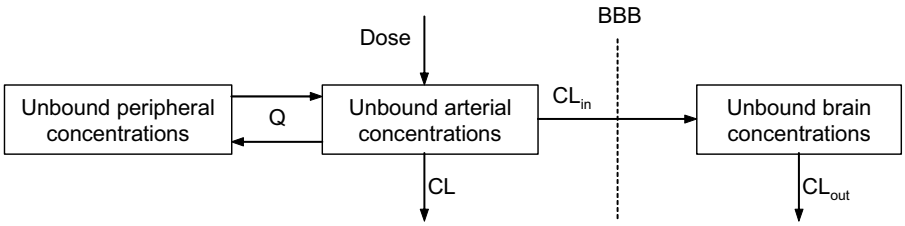


Figure 5. *The restricted model that has been used to describe the distribution of drugs across the blood-brain barrier (BBB). Prior to the data analysis the brain dialysate concentrations were corrected for the recovery, and the observed arterial concentrations were corrected for the protein binding. Abbreviations: CL, clearance; Q, intercompartmental clearance; CL_{in}, clearance from the blood into the brain and CL_{out}, clearance out of the brain.*

An alternative to the restricted model would be to include all the data as it was observed. This includes the total arterial concentrations, the brain and blood recovery measurements obtained for each probe from the retrodialysis period and the dialysate concentrations in the brain and in venous blood. In this way, both the recovery and the protein binding could be estimated within the model. Thereby the uncertainty in the recovery value and the protein binding would be handled correctly, to give more reliable estimates of the pharmacokinetic parameters. In Paper V a new integrated model is presented that takes these issues into account. The integrated model was evaluated by comparing it to the restricted model and the NCA in terms of parameter estimates, model structures, mechanistic insight and practical aspects. The integrated model was also applied in the data analysis in Papers I and II.

Aims of the thesis

The primary objectives of this thesis were to investigate the transport properties of morphine and its active metabolite M6G at the BBB, and to examine how disease related to the brain might affect the exposure of morphine to the brain in comparison to healthy conditions. The predictive capacity of animal studies for the BBB transport in humans was investigated by studying the exposure of the brain to morphine in different species.

A secondary objective was to develop microdialysis as a tool for the study of the BBB transport of drugs, emphasising the analysis of the data obtained from this type of experiment.

The specific aims were:

- To study the involvement of the probenecid-sensitive transporters on the BBB transport of morphine and M6G
- To investigate the ability of animal studies in rats and pigs to predict the transport across the BBB in humans using morphine as a model compound
- To study the influence of meningitis and severe brain trauma on morphine transport across the BBB
- To develop an integrated model for the analysis of data obtained from microdialysis experiments that does not make assumptions about error free recovery or protein binding, and compare this model to the restricted model and the NCA

Material and methods

Animals

Male Sprague-Dawley rats (Møllegaard, Denmark) weighing 262-318 g were used in Papers I and II. The rats were acclimatised for at least seven days prior to surgery. They were group housed at 22 °C under a 12-hour light-dark cycle with free access to food and water. The Animal Ethics Committee of Uppsala University approved the study protocols (C 197/97 and C 144/99).

In Paper III pigs (19.6-22.5 kg) of mixed Swedish domestic breeds were used. The animals were deprived of food for 24 hours prior to the experiment, but had free access to water. The Ethical Committee for Laboratory Animal Experiments at the Medical Faculty of Lund University approved the protocol (M 289-00).

Surgical procedure

The rats in Papers I and II were anaesthetised by inhalation of 2.5 % Enfluran® balanced with 1.5 L/min oxygen and 1.5 L/min nitrous oxide. PE-50 cannulae fused with PE-10 were inserted into the femoral vein for drug administration and in the femoral artery for blood sampling. A CMA/20 (CMA, Stockholm, Sweden) microdialysis probe was placed into the right jugular vein and sutured to the pectoral muscle. A stereotactic instrument (David Kopf Instruments, Tujunga, USA) was used for the insertion of a CMA/10 probe into the striatum in the brain. A piece of PE-50 tubing was looped subcutaneously on the back of the rat to the posterior surface of the neck, to let the perfusion solution adjust to body temperature before entering the brain probe. The ends of all cannulae were passed subcutaneously and gathered in a plastic cup that was sutured to the posterior surface of the neck. The rats were placed in individual CMA/120 cages, where they recovered for 24 hours prior to the experiment.

In Paper III the pigs were kept under anaesthesia by intravenous administration of fentanyl (Leptanal 50 μg/mL, Janssen-Cilag, Sollentuna, Sweden) and thiopental (Pentothal Natrium, Abbot Laboratories, Chicago, Illinois, USA). A pressure transducer (Honeywell Microtransducer 9815 155 00201) was positioned in the brain tissue for measurements of the ICP. In addition, microdialysis probes (CMA/70) were inserted in the occipital, right frontal and left frontal brain cortex. One probe was also placed in the left internal jugular vein. The right internal jugular vein was catheterised for drug administration. Another catheter was inserted into the femoral artery for blood sampling and to enable blood pressure recordings to be made.

Patients

Seven patients with severe brain trauma were included in the study (Paper IV) after consent from relatives to each patient. The Ethics Committee at Lund University Hospital approved the study (LU 289-98).

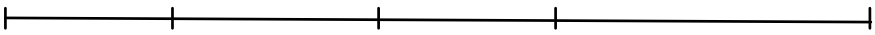
Surgery

Craniotomy was performed for evacuation of focal mass lesions in five of the patients. In these patients a CMA/70 microdialysis catheter was inserted in the penumbra zone surrounding the injured brain tissue. This is referred to as the “worse” position. Contralaterally, another catheter was inserted. The brain tissue in this area had normal appearance on the computer tomography scan, and is therefore referred to as the “better” position. The catheters in the two patients with only one catheter were categorised as the “better” position. A pressure transducer was inserted in the uninjured brain tissue for ICP measurements. A CMA/60 catheter was placed in the abdominal subcutaneous fat. The patients were sedated with midazolam and fentanyl during controlled ventilation prior to the study. Fifteen minutes before drug administration the infusion of fentanyl was stopped.

Experimental procedures (Papers I-IV)

Study designs

The experimental setting used in Papers I, II and IV is shown in Figure 6. First the probes were perfused with a blank Ringer solution containing 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 0.2 mM ascorbic acid in 2 mM phosphate buffer with pH 7.4 (Papers I and II) or with Ringer's solution (Perfusion Fluid, CMA Microdialysis, Sweden) (Paper IV) for one hour to stabilise the system, and to obtain blank samples for the chemical analysis. The rats in Papers I and II were studied on two consecutive days. On the first day the animals only received the drug under investigation and on the second day that drug was co-administered with probenecid, which was administered as an i.v. bolus dose followed by a constant infusion (Table 2). The infusion of a blank buffer solution (Day 1) or probenecid dissolved in the buffer solution (Day 2) was started during the stabilisation period.



Stabilisation period	Probe calibration	Wash-out period	Morphine administration
			4-hour exponential i.v. infusion (I-II)
			10-min i.v. infusion (IV)
60 min	75 min (I-II) 120 min (IV)	75 min (I-II) 120 min (IV)	Sampling for 6 hours (I-II) Sampling for 3 hours (IV)

Figure 6. *A schematic representation of the experimental design and time-planning used to study the influence of probenecid on the blood-brain barrier transport of morphine in rats (Paper I) and morphine-6-glucuronide in rats (Paper II). In Paper IV the blood-brain barrier transport of morphine was studied in brain trauma patients.*

For the *in vivo* calibration of the probes the perfusate was changed to a Ringer's solution spiked with a low concentration of the drug being studied (Papers I, II and IV). Prior to drug administration, a wash-out period was utilised to clean the system (Papers I, II and IV). The studied drugs and the doses that were administered in the different studies are shown in Table 2. Dialysate fractions were collected during drug administration and two to three hours post-infusion (Figure 6). Once the drug administration had been completed on the second experimental day, approximately half of the rats included in the studies were decapitated (Papers I and II). Their brains were removed and divided into the right and left hemispheres, weighed frozen and immediately at -20 ° C pending analysis.

Table 2. Drugs and doses

Paper	Drug	Dose	Time of infusion
I	Morphine	Exp. inf.[a] aiming at 1800 ng/mL	4 h
I	Morphine + Probenecid[b]	20 mg/kg (bolus) + 20 mg/kg/h	4 h + 9.5 h
II	M6G[c]	Exp. inf.[a] aiming at 3000 ng/mL	4 h
II	M6G + Probenecid[d]	20 mg/kg (bolus) + 20 mg/kg/h	4 h + 7.5 h
III	Morphine	1 mg/kg[e]	10 min
IV	Morphine	10 mg[f]	10 min

[a] Exponential infusion; [b] Co-administration of probenecid and morphine on Day 2; [c]Morphine-6-glucuronide; [d]Co-administration of probenecid and M6G on Day 2; [e]Administered as 1 mg/kg of morphine hydrochloride; [f] Administered as 10 mg of morphine hydrochloride

In Paper III two pigs were included in the recovery experiments to confirm the validity of using nalorphine as a calibrator for morphine recovery. Six pigs were subsequently included in the pharmacokinetic study. The study design is shown schematically in Figure 7. The Ringer's solution used in the recovery experiments contained both morphine (100 ng/mL) (Pharmacia, Stockholm, Sweden) and nalorphine (250 ng/mL) (Sigma Chemicals, St Louis, USA), while the perfusate used in the pharmacokinetic experiment contained nalorphine only.

In Paper III dialysates were collected over the three hours following the first morphine infusion (Table 2). The experimental meningitis was subsequently induced by an injection of 200 µg of LPS from E. Coli (Serotype 0111:B4.

Sigma, Stenheim, Germany) directly into the cisterna magna (Gärdenfors *et al.*, 2002). A consecutive morphine infusion was administered when the meningitis was established, and dialysates were collected over three to five hours. The experiment was terminated and the animals were sacrificed by an injection of pentobarbital in ethanol.

In the clinical study (Paper IV) the catheters were perfused at a flow-rate of 0.3 µL/min during the clinical monitoring prior to the morphine study. During the experiments the perfusate was delivered at a flow-rate of 1.0 µL/min in all studies, and the dialysate was collected in 5-15 minute intervals. The dialysate fractions were frozen at -20 °C pending analysis. In all studies arterial blood samples were collected according to a pre-defined schedule. The plasma was separated by centrifugation, and the samples were frozen until analysis.

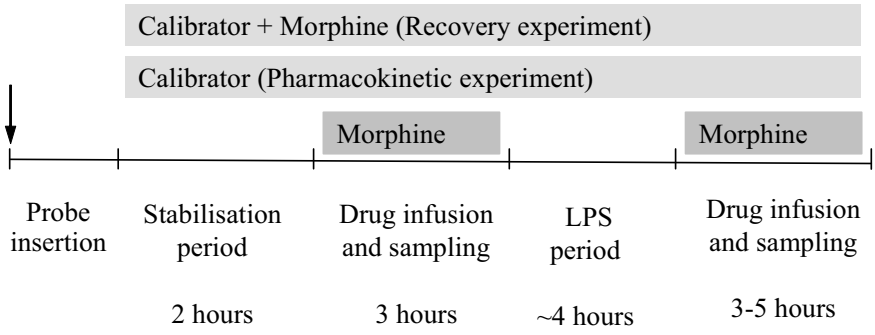


Figure 7. *The study design used to investigate the blood-brain barrier transport of morphine in a control situation and during experimentally induced meningitis using a pig model. Abbreviation: LPS, lipopolysaccharide.*

Recovery measurements

Each microdialysis probe was calibrated *in vivo* using the method of retrodialysis by drug (Bouw *et al.*, 1998) (Papers I, II and IV). This method assumes that the loss of drug from the perfusate to the tissue into which the probe is implanted, is equal to the drug gain from the surrounding tissue when the drug is administered systemically. Since microdialysis measures a fraction of the tissue concentration it is possible to estimate the true unbound

concentration by correcting for the relative recovery. This was calculated according to Equation 8.

$$\text{Recovery}_{in\ vivo} = \frac{\sum_{i=1}^{x} \frac{C_{in} - C_{out,i}}{C_{in}}}{x} \quad (8)$$

C_{in} is the drug concentration entering the probe and $C_{out,i}$ is the concentration in the *i*:th fraction leaving the probe and x is the number of recovery measurements. The samples collected during the drug administration and post-infusion were corrected by the value of the individual recovery.

In Paper III the relative recovery of morphine was estimated using nalorphine as a calibrator for morphine recovery. This method assumes that the calibrator and the drug to be studied have similar physico-chemical properties, and that they behave similarly *in vivo*. This was confirmed in the recovery experiment in which the perfusate contained both morphine and nalorphine, thereby enabling the relative *in vivo* recoveries of both compounds to be calculated and compared. In the pharmacokinetic experiment, the *in vivo* morphine recovery was calculated by measuring the loss of nalorphine from the perfusate during the consecutive morphine infusions according to:

$$\text{Recovery}_{\text{morphine}} = \frac{C_{\text{in,nalorphine}} - C_{\text{out,nalorphine}}}{C_{\text{in,nalorphine}}} \cdot \left(\frac{\text{Recovery}_{\text{morphine}}}{\text{Recovery}_{\text{nalorphine}}} \right)_{recovery\ exp.} \quad (9)$$

An average recovery value for each infusion was used to correct the dialysate concentrations of morphine to the corresponding infusion. However, if there was a trend in the nalorphine recovery, then linear regression was used to describe the trend. The equation obtained by the linear regression was subsequently used to calculate the morphine recovery for each dialysate fraction collected. Then the recovery value corresponding to each sample was used to calculate the true tissue concentration of morphine.

Protein binding

Equilibrium dialysis was used to assess the individual protein binding of morphine in human plasma (Paper IV). Five hundred µL plasma, adjusted to physiologic pH, was dialysed against the same volume of phosphate buffer (pH 7.4) in an atmosphere of 5 % CO_2 in air at 37° C. After seven hours the dialysis was stopped, and samples were collected from both chambers. The samples were dialysed in triplicate.

Blood gas status

In the rat studies the respiratory parameters (pH, pO_2, pCO_2 and O_2-saturation) were checked throughout the experiments using an AVL Compact II blood gas analyser (AVL Medical Nordic, Stockholm, Sweden). In the pig study pO_2, pCO_2, pH, glucose and the body temperature were monitored.

Chemical analysis

The morphine content of the microdialysis samples was analysed (Papers I, III and IV), as were M6G (Paper II) and nalorphine (Paper III). An HPLC system with electrochemical detection (Coulochem II, ESA Inc., USA) with a guard cell (ESA 5020, ESA Inc., USA) and an analytical cell (ESA 5011, ESA Inc., USA) was used for this concentration analysis. The samples were injected (Triathlon, Spark Holland, the Netherlands) into the system, and separation was achieved using a Nucleosil C18 column (150 x 4.6 mm i.d. and 5 µm particles, Chrompack, Sweden). The mobile phase used to analyse morphine in Paper I consisted of 620 mL (brain) or 580 mL (blood) 0.01 M phosphate buffer (pH 2.1) containing 0.4 mM of sodium dodecyl phosphate (SDS), 380 mL (brain) or 420 mL (blood) methanol and 20 mL tetrahydrofuran (THF). In Paper IV the mobile phase consisted of 600 mL phosphate buffer with 144 mg SDS, 400 mL methanol and 20 mL THF for the analysis of the dialysates collected in the pigs. The mobile phase was somewhat changed (670 mL phosphate buffer containing 0.2 mM SDS, 330 mL methanol and 20 mL THF) for the analysis of the dialysates collected in the pig. After some modification this mobile phase was used to analyse M6G in Paper II. The mobile phase was delivered at 1 µL/min and the peak height was used to quantify the drug content in each sample.

The plasma samples were pre-treated using a slightly modified method described by Joel and co-workers (Joel *et al.*, 1988). The samples were eluted with 3 mL of methanol and evaporated under a flow of nitrogen at 45° C. The residue was dissolved in 150 µL of mobile phase (Papers I, III and IV) or in phosphate buffer (0.01 M) containing 0.2 mM SDS (Paper II), and 55 µL was injected into the column. The chromatographic system described above was used for the analysis of morphine (Papers I, III and IV) and M6G (Paper II). The mobile phase used for the analysis of morphine was composed of 670 mL phosphate buffer containing 0.2 mM of SDS, 330 mL methanol and 20 mL THF. The mobile phase used to measure the M6G concentrations in plasma contained 680 mL of phosphate buffer (0.2 mM SDS), 320 mL methanol and 25 mL THF. The concentrations of M3G (Papers I and IV) were analysed by fluorescence detection (Jasco 821-FP, Japan).

The brain tissue (Papers I and II) was homogenised with a 5-fold larger volume of 0.1 M perchloric acid. After centrifugation the supernatant was pre-treated and analysed for the total drug concentrations in the same way as the plasma samples.

Data analysis

Non-compartmental methodology

The terminal half-life ($t_{½}$) of morphine in brain, subcutaneous fat and blood was determined from the terminal rate constant, which was itself obtained from log-linear regression of the terminal phase of the concentration-time curve (Papers I, III and IV). The dialysate concentrations were corrected for the recovery corresponding to each microdialysis probe to estimate the unbound tissue concentrations of morphine (Papers I, III and IV). The extent of the transport of morphine across the BBB was calculated from the unbound steady state concentration ratio between brain and venous blood ($C_{u,ss,brain}/C_{u,ss,blood}$) (Paper I).

In Paper III the exposure of morphine in venous blood and in the different brain regions was determined by calculating the unbound area under the concentration-time curve (AUC_u) corresponding to each probe, from the start of each morphine infusion to infinity. The ratio of the AUC_u at each location in the brain to that in venous blood was used to estimate the extent of morphine BBB transport.

In Paper IV the unbound concentrations of morphine in plasma were calculated for each patient by correcting the total concentrations for the individual estimate of the protein binding. The AUC_u of morphine in injured ("worse") and uninjured ("better") brain tissue, in subcutaneous fat and in plasma was used to estimate the exposure of morphine. The AUC_u ratio between the tissue concentrations and plasma was used as a measure of the extent of morphine transport.

Statistics

A two-sided Student's paired t-test was used for comparisons of the systemic pharmacokinetic parameters and the parameters describing the BBB transport between the two experimental days (Paper I). The same statistical test was used to compare the systemic pharmacokinetic parameters and the blood glucose level between the control and meningitis periods in Paper III. In addition a two-way ANOVA for repeated measurements on the factors probe location and treatment was used to differentiate between the AUC_u ratio between brain and blood and the physiological data during the control and meningitis periods (Paper III).

In Paper IV a Wilcoxon Signed Rank Test was used to evaluate the data obtained from the different microdialysis catheter positions in the brain. The data from the "better" brain tissue, subcutaneous adipose tissue and plasma was obtained in all seven patients, while the data in the "worse" brain tissue was acquired in the five patients with two or more intracranial microdialysis catheters.

In all statistical evaluations the null hypothesis was rejected at a 5 % significance level.

Modelling

The data in Papers I, II and V were analysed by nonlinear mixed effects modelling in the computer program NONMEM version VIß (Beal *et al.*, 1994). All final models were confirmed using NONMEM version V. The first order conditional estimation method with interaction (FOCE INTER) was used for all analysis, and the model selection was based on the objective function value (OFV) from the NONMEM output and graphical analysis using Xpose version 3.1 (Jonsson *et al.*, 1999). To discriminate between two nested models a drop in the OFV of 3.84, which corresponds to $p<0.05$, was required. The same criterion was applied for the inclusion of probenecid as a categorical covariate (Papers I and II).

An exponential model was used to describe the interindividual variability in the parameters.

$$P_i = P \cdot \exp^{\eta_i} \quad (10)$$

P_i denotes the *i*:th individual's parameter value, P the typical value of the parameter and η_i the individual random effect that accounts for the difference between the typical parameter and the individual estimate. The residual error, which accounts for the unexplained difference between the individual observation and the individual prediction, was described by the additive, the proportional or the slope-intercept models (Beal *et al.*, 1994).

The model building was carried out using either the restricted model (Papers I and V) or the integrated model (Papers II and V). In addition, the data presented in Paper I has been reanalysed after publication using the integrated model. The data obtained from the modelling are presented as typical values [Relative standard errors (%)].

The restricted model

In this model the arterial concentrations were modelled after corrections for the individual estimate of the protein binding, which was obtained from the ratio between the AUC_u in venous blood and the AUC in arterial blood. The unbound population parameter estimates from the analysis of the arterial concentrations were subsequently fixed in the analysis of the brain data. The unbound brain concentrations were obtained by correcting the brain dialysate concentrations for the recovery calculated for each probe according to Equation 8. The mid time point in the collection interval of the microdialysis data was used as the time of the observation.

The BBB transport was parameterised in terms of volumes of distribution and clearances. The influx clearance (CL_{in}) represents the transport from the blood compartment into the brain, the efflux clearance (CL_{out}) corresponds to the transport out of the brain and the CL_{in}/CL_{out} ratio measures the extent of BBB equilibration. The unbound volume of distribution in the brain (V_{brain} or $V_{u,brain}$) was used as a fixed parameter and calculated from the individuals that were decapitated on the second experimental day according to:

$$V_{brain} = \frac{A_{brain} - V_{blood} \cdot C_{blood}}{C_{u,brain}} \quad (11)$$

A_{brain} is the total amount of drug in the brain, V_{blood} is the volume of blood in the brain, C_{blood} is the total concentration in blood and $C_{u,brain}$ is the unbound brain concentration. The amount of morphine in the brain was assumed to be small relative to the rest of the body. Thus mass transport from brain back to blood was neglected. Models comprising one or several compartments in brain and blood respectively were considered. With this model the unbound estimates of the parameters were assessed.

The integrated model

Using this approach all data collected, i.e. the total arterial concentrations, the retrodialysis data from the blood and brain probes and the blood and brain dialysate concentrations, was included in the model to obtain the parameter estimates in brain and blood. The microdialysis data, collected as fractions, was modelled using an output compartment, and the dialysate concentrations were predicted from the model by integrating the concentration-time profile in each dialysate collection interval. To describe the recovery of the blood and the brain probes both interindividual variability (between probes) and inter occasion variability (between experimental days) were considered.

Models consisting of one more compartments in blood, with or without a lag-time in the drug distribution between the arterial and venous compartments, were considered. Similarly to the restricted model the BBB transport was described by influx and efflux clearances. By analysing both the blood and the brain data simultaneously bi-directional transport across the BBB could also be investigated. Similarly to the restricted model the parameters obtained from the integrated model were the unbound parameters.

Results and discussion

Morphine transport over the blood-brain barrier

The exposure to unbound morphine was less in the brain than in the blood in the three species that were studied, indicating that morphine is a substrate for active efflux transporters at the BBB in rats (Paper I), pigs (Paper III) and humans (Paper IV) (Table 3). It had previously been shown that morphine is a substrate for P-gp in rats (Letrent *et al.*, 1999; Xie *et al.*, 1999) and in humans (Sadeque *et al.*, 2000; Sisodiya *et al.*, 2002). Earlier, Childs and co-workers had demonstrated that the pig capillary endothelial cells express P-gp (Childs *et al.*, 1996).

Table 3. Transport of morphine over the blood-brain barrier in three different species under control conditions and in the presence of disease or with probenecid co-administration. The values are presented as average ± SD.

Species	Condition	$C_{u,ss,brain}/C_{u,ss,blood}$ $AUC_{u,brain}/AUC_{u,blood}$	$t_{½}$ blood (min)	$t_{½}$ brain (min)
Rat	Control	0.29 ± 0.07	37 ± 5	58 ± 9[b]
Rat	Probenecid-treated	0.39 ± 0.04[a]	52 ± 15[c]	115 ± 25[b,c]
Pig	Control	0.47 ± 0.19	59 ± 13	94 ± 14[b]
Pig	Meningitis	0.95 ± 0.20[a]	51 ± 9	103 ± 18[b]
Human	“Better” brain tissue	0.64 ± 0.25	69 ± 7	139 ± 21[b]
Human	”Worse” brain tissue	0.78 ± 0.28	69 ± 7	161 ± 42[b]

[a] Different exposure of the brain from that in the control condition; [b] Different half-life in the brain than in the blood and [c] Different half-life upon probenecid co-administration.

However, transporters other than P-gp might also affect the brain concentrations of morphine in these species. For example, it was shown that transporters belonging to the OATP (human)/Oatp (rat) family are expressed at the BBB both in humans and in rats (Gao *et al.*, 2000), and the expression of MRPs has been demonstrated in endothelial cells in the porcine brain (Gutmann *et al.*, 1999). Consequently, it is possible that several transporters are involved in the efflux of morphine at the BBB.

In the rat morphine transport across the BBB was slow, and equilibrium was attained approximately two hours after the steady state concentration was reached in blood (Figure 8). Similarly, the maximum concentration of morphine in the human brain appeared later than that in the blood (Figure 9). In contrast, the maximum concentrations in the pig were reached at approximately the same time in the blood and in all three regions of the brain investigated (Figure 10).

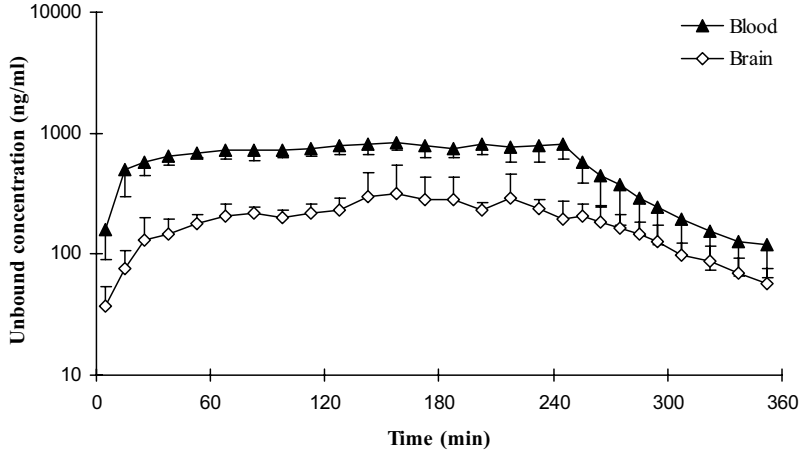


Figure 8. *Unbound morphine concentrations (average ± SD) in the brain and in the blood during and after a 4-hour exponential i.v. infusion of morphine to rats. All data were obtained by microdialysis.*

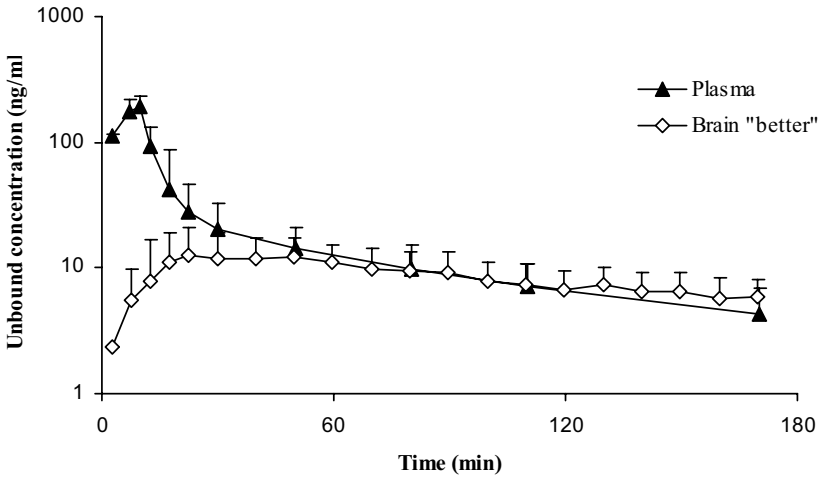


Figure 9. *Unbound morphine concentrations (average ± SD) in the human brain and in plasma during and after a 10-minute i.v. infusion of 10 mg of morphine hydrochloride. The brain data were obtained using microdialysis and the plasma data were obtained by regular blood sampling.*

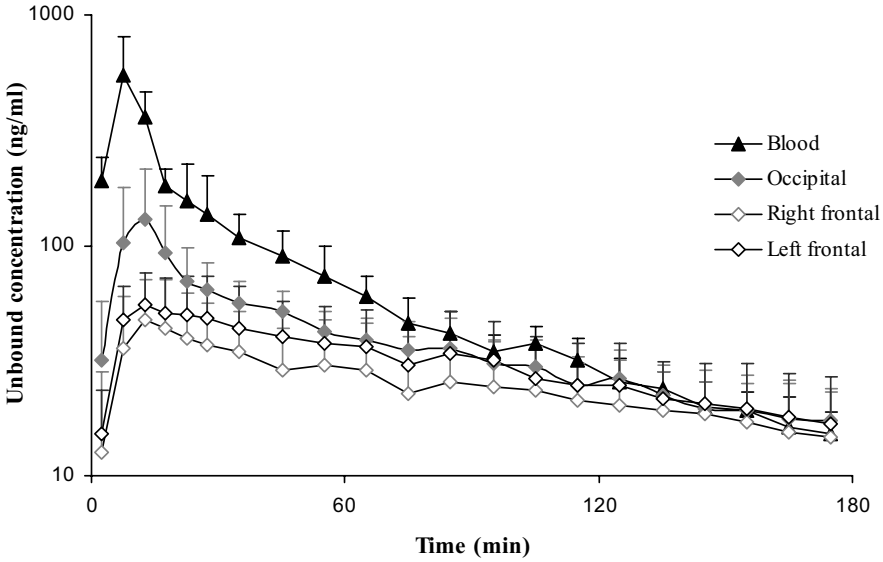


Figure 10. *Unbound morphine concentrations (average ± SD) in the blood and at the three brain locations during and after a 10-minute i.v. infusion of 1 mg/kg of morphine hydrochloride to the pig. The data were collected by microdialysis.*

In all species the morphine half-life was longer in the brain than in the blood, indicating that the local cerebral pharmacokinetics of morphine differs from the systemic pharmacokinetics (Table 3). These interspecies similarities demonstrate that animal studies should prove useful to predict the BBB transport of morphine in humans. However to be able to draw more general conclusions about the suitability of animal models for predictions of the BBB transport in humans, more drugs, ideally with a variety of physico-chemical properties, need to be investigated.

Morphine and probenecid co-administration increased the amount of morphine being transported to the brain, indicating that morphine is a substrate for the probenecid-sensitive transporters at the BBB (Table 3) (Paper I). Still, the ratio of unbound drug concentrations in the brain to those in the blood was below unity, which indicates that the probenecid-sensitive transporters are not the only transporters involved in the brain efflux of morphine. In studies using P-gp knockout mice, (Xie *et al.*, 1999) or the P-gp inhibitor GF120918 (Letrent *et al.*, 1999), it was shown that morphine is also a P-gp substrate. Thus the $C_{u,ss,brain}/C_{u,ss,blood}$ ratio of morphine was expected to be below unity despite being co-administered with probenecid.

The bulk flow of the brain ECF may also contribute to the lower concentrations of the drug in the brain than in the blood at equilibrium. The influence of the bulk flow on the elimination of drugs from the CNS depends on the magnitude of the efflux clearance in relation to that of the bulk flow. In the rat, the bulk flow has been estimated as 0.18-0.29 μL/min per gram brain (Szentistvanyi *et al.*, 1984). In Paper I the typical value [RSE (%)] of the morphine efflux clearance was estimated as 42 (10) μL/min per gram brain using the restricted model. Thus, the bulk flow is not of major importance for the elimination of morphine from the brain.

In the rat the V_{brain} was calculated to be 2.8 ± 0.4 mL, i.e., approximately 1.8 mL per gram brain. Since V_{brain} exceeds the volume of the rat brain, it is likely that morphine is distributed into the cells in the brain, and/or that it binds to tissue components in the brain. The theory of intracellular distribution or binding of morphine to cell components is supported by the need for two compartments in the brain, to adequately describe the cerebral concentrations of morphine using the restricted model.

The half-life of morphine in the brain increased upon probenecid co-administration (Table 3). This suggests that the transport of morphine across the BBB is rate-limiting for morphine elimination from the brain.

Blood-brain barrier transport of M6G (Paper II)

The unbound concentrations of M6G in the brain and in the venous blood, corrected for the estimated recovery obtained in every probe on each occasion, are shown in Figure 11. As expected from the exponential infusion, equilibrium was reached quickly in the blood compartment. However, the distribution of M6G into the brain was slow, and steady state concentrations were only reached after approximately two hours (Figure 11).

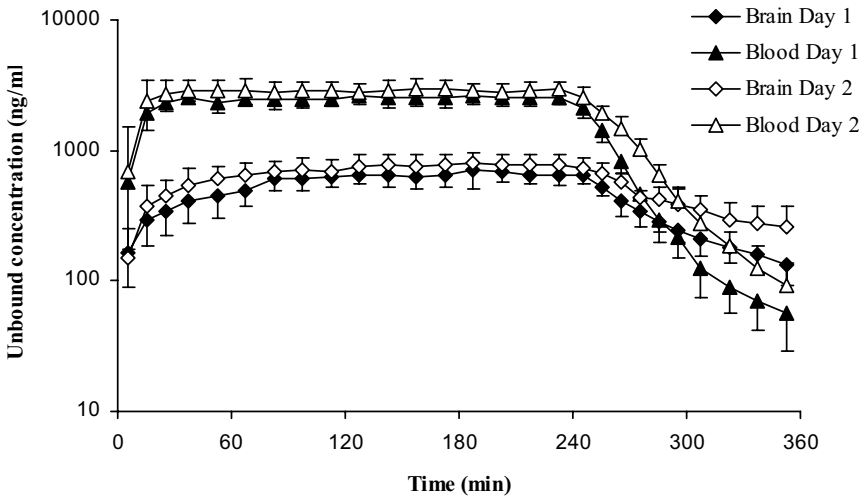


Figure 11. *Unbound blood and brain concentrations (average ± SD) of morphine-6-glucuronide (M6G) in the rat. M6G was administered as a 4-hour exponential i.v. infusion with the addition of probenecid on Day 2.The microdialysate concentrations were adjusted according to the individual estimate of the recovery that was obtained in each probe from the modelling.*

The extent of the BBB equilibration of M6G, given by CL_{in}/CL_{out}, was estimated as 0.29 on both experimental days. Thus M6G must be a substrate for active efflux at the BBB, even though the probenecid-sensitive transporters are not involved in this efflux. Although probenecid is a non-specific modulator of organic anion transporters it is known to inhibit Oatp1, Oatp2, Oat1 and Oat3 (Sugiyama *et al.*, 2001). Thus the present study indicates that M6G is not a substrate for any of these transporters, or any other transporters that might be inhibited by probenecid. These results are

consistent with the findings of Lötsch and co-workers, who concluded that the concentration of M6G in the spinal cord was unaffected by probenecid co-administration (Lötsch *et al.*, 2002). The contribution of other transporters to the limited exposure of M6G to the brain has been investigated and, recently, it was recently demonstrated that M6G is a substrate for digoxin-sensitive transporters other than P-gp (Bourasset *et al.*, 2003).

As for morphine, the half-life of M6G was longer in the brain than in the blood. The individual half-lives of M6G in the brain were 65.9 ± 18 min on Day 1 and 80.3 ± 28 min on Day 2 respectively, both values being longer than in blood (17.1 ± 1.7 min on Day 1 and 20.5 ± 2.6 min on Day 2) on both experimental days ($p<0.05$).

The V_{brain} of M6G was calculated as 0.19 mL per gram brain. This was in agreement with previous results in the rat (Bouw *et al.*, 2001). Since this value is similar to the volume of the ECF in the rat brain (Rosenberg *et al.*, 1980), it is likely that intracellular distribution and binding of M6G to tissue components in the brain are negligible.

The typical value [RSE (%)] of the efflux clearance of M6G was estimated as 5.66 (16) μL/min (approximately 3.7 μL/min per gram brain). This was about ten times lower than the estimated value for morphine in Paper I. Nevertheless, the contribution of the bulk flow to the elimination of M6G from the brain is probably small, considering the low flow rate of the brain ECF (Szentistvanyi *et al.*, 1984).

Comparing the transport properties of some selected opioids

Transport across the blood-brain barrier

The way that opioids behave at the BBB depends upon the characteristics of the drug. In Papers I and II it was shown that both morphine and M6G are substrates for active transporters at the BBB, and that the extent (i.e., the exposure of the drug to the brain compared to the blood) of BBB transport was similar for the two compounds. However, the transporters that cause the efflux of morphine and M6G are not the same. This was clearly shown in Papers I and II when the two drugs were co-administered with probenecid. In

addition, morphine is a substrate for P-gp (King *et al.*, 2001; Xie *et al.*, 1999), while M6G does not seem to be affected by P-gp (Bourasset *et al.*, 2003). However, it should be recognised that the results presented by Bourasset and co-workers are inconsistent with a previous report using an *in vitro* system of porcine brain capillary endothelial cells (Huwyler *et al.*, 1996). The extent of the BBB transport, measured as $C_{u,ss,brain}/C_{u,ss,blood}$, of M3G is lower than that of morphine and M6G, and it has been estimated as 0.10 (Xie *et al.*, 2000). It has also been demonstrated that M3G is a substrate for the probenecid-sensitive transporters at the BBB (Xie *et al.*, 2000), and that the brain concentrations of M3G are unaffected by P-gp modulation (Letrent *et al.*, 1999). In contrast, codeine does not seem to be a substrate for any efflux pumps at the BBB, as the unbound brain/blood concentration ratio is unity (Xie *et al.*, 1998).

Since the only difference between M3G and M6G is the position of the glucuronide, it was anticipated that these metabolites would have similar transport properties. But the structure of M6G makes it possible for the molecules to fold and thereby cover the hydrophilic glucuronide. In this way the molecules are able to attain more lipophilic properties. In contrast, M3G has a rigid structure, so the molecules are unable to fold (Carrupt *et al.*, 1991). Consequently, M3G and M6G may have different properties *in vivo*. Since the extent of the BBB transport depends on the affinity to active transporters, which is associated with the physico-chemical properties of a substance, the ability of M6G to fold could explain the difference in the transport properties of these metabolites.

The time taken for the brain to attain steady state concentrations of morphine, M6G and M3G (Xie *et al.*, 2000) was longer than in the blood, as a consequence of the limited permeability of the BBB to these compounds. Similarly, the half-lives of these compounds (Xie *et al.*, 2000) were longer in the brain than in the blood. In contrast, codeine was quickly distributed into the brain and equal concentrations of codeine were reached immediately in the brain and in the blood (Xie *et al.*, 1998). In addition, there was no difference in the half-life of codeine in the brain and in the blood (Xie *et al.*, 1998). Thus, for morphine, M6G and M3G, the BBB seems to have an impact on both the amount of drug that enters the brain and the time-course of the brain concentrations of these compounds. For codeine, the opposite was observed because neither the amount of drug entering the brain nor the time-course of the brain concentrations were different from the values for the blood (Xie *et al.*, 1998).

The similar values of the V_{brain} of M3G (0.23 mL per gram brain) (Xie *et al.*, 2000) and M6G (0.19 mL per gram brain) indicate that the distribution within the brain was similar for the two metabolites, and that they are mainly

allocated extracellularly in the brain. In contrast, morphine is also distributed into the cells in the brain. Thus the metabolites seem to have similar distributional properties once inside the brain, while morphine behaves differently.

From the modelling, CL_{in} was estimated as 0.17 (M3G) (Xie *et al.*, 2000), 1.1 [M6G, (Paper II)] and 11.4 [morphine (Paper I)] μL/min per gram brain. The rate of BBB transport of M6G, expressed as PS values, were reported as 0.11 (Bickel *et al.*, 1996) and 0.14 μL/min per gram brain (Wu *et al.*, 1997). These PS values were determined by the i.v. injection technique, and in these studies the rats were decapitated 60 minutes after the administration of M6G. Using the same experimental set-up, the PS value for morphine was reported as 8.0 μL/min per gram brain (Wu *et al.*, 1997). An explanation for the rate of BBB transport being lower when obtained by the i.v. injection technique than the values obtained by modelling could be that the brain efflux is neglected with the i.v. injection technique. Hence, if there is substantial brain efflux of a compound, the PS value is likely to be underestimated. From the modelling, the efflux clearance was estimated as 3.7 (M6G) and 42 (morphine) μL/min per gram brain. Since these values exceed the CL_{in} values, the brain efflux can not be neglected in the determination of the brain parameters. For comparison, the efflux clearance of M3G was estimated as 1.15 μL/min brain per gram brain (Xie *et al.*, 2000). Altogether, these results suggest that the rate of transport of both metabolites is slow in comparison to that of morphine.

Systemic elimination

In the rat morphine is predominantly eliminated by metabolism to M3G (Kuo *et al.*, 1991), which is mainly eliminated via the kidneys (Van Crugten *et al.*, 1991). M6G is eliminated both through the kidneys (Van Crugten *et al.*, 1991) and via the bile (Stain-Texier *et al.*, 1998). Codeine is eliminated as unchanged drug, and also metabolised to norcodeine and to morphine, which is further metabolised to M3G (Oguri *et al.*, 1990; Xu *et al.*, 1995).

Probenecid co-administration had no effect on the systemic elimination of M3G (Xie *et al.*, 2000). Conversely, the systemic elimination of morphine and M6G were affected to the same degree by probenecid (21 % and 22 %, respectively). However, as the routes of elimination are not the same for these compounds, the mechanisms behind the interactions must be different. The decrease in the clearance of morphine was explained by slower formation of M3G. Since M6G is eliminated through the kidneys and via the bile it seems as if the transporters that act on M6G and that are also inhibited by probenecid, might be located in the kidneys and/or in the liver. Thus

M6G is a substrate for the probenecid-sensitive transporters, although not at the BBB.

In summary, these results demonstrate that morphine, M3G and M6G are differently affected by probenecid at the BBB and systemically. Clearly this indicates that probenecid affects a range of different transporters, and that the expression of these transporters varies within the body.

Disease

Bacterial meningitis (Gärdenfors *et al.*, 2002) and severe brain trauma (Ståhl *et al.*, 2001a) cause changes in the physiology and the biochemistry of the brain. These changes have been attributed to an inflammatory response that causes alterations in the permeability of the BBB (Holmin *et al.*, 1998; Quagliarello *et al.*, 1992). In the porcine model used in Paper III, lipopolysaccharide was directly injected into the cisterna magna in the brain to induce meningitis (Gärdenfors *et al.*, 2002). The meningitis, defined as an increase in the intracranial pressure to a stable level above 10 mm Hg (Gärdenfors *et al.*, 2002), was established after 3.6 ± 0.6 hours, and it remained stable for the duration of the study (Figure 12).

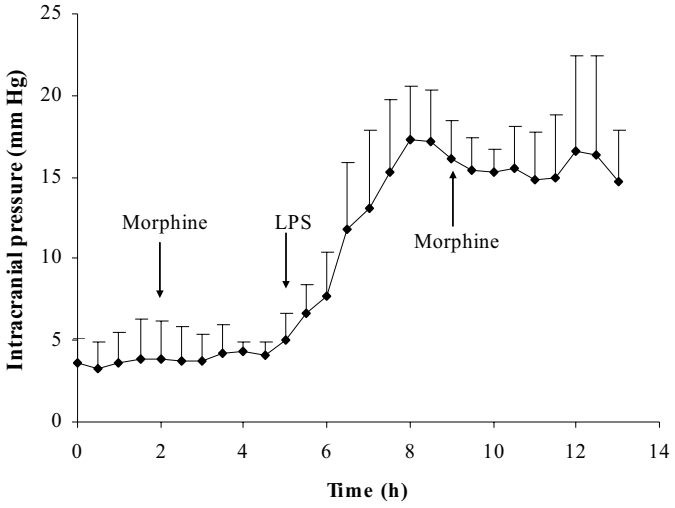


Figure 12. *The intracranial pressure over time (average ± SD) in all pigs. The first two hours represent the baseline period, and the following three hours represent the control period, when the first 10-minute infusion of morphine was administered. Lipopolysaccharide (LPS) was injected five hours after the start of the experiment. At about nine hours the morphine administration was repeated.*

In the brain trauma patients (Paper IV), the cerebral concentrations of glucose were lower and the glycerol concentrations were higher in the "worse" brain tissue than in the "better" brain tissue (Table 4). This is indicative of disturbance in the cerebral metabolism in the "worse" brain tissue, as a consequence of the brain trauma (Hillered *et al.*, 1998; Ståhl *et al.*, 2001a; Ståhl *et al.*, 2001b; Ungerstedt *et al.*, 1997). Altogether these results confirm the establishment of meningitis in the pigs and the capability of the microdialysis method to detect severe brain injury in humans.

Meningitis (Paper III)

The shape of the concentration-time profiles of morphine in the pig brain was similar for the two periods (Figure 13). However the concentrations were elevated in the presence of meningitis over those during the control period, with the exception of a single probe, which showed no change in the morphine brain exposure between the two situations (Figure 13). Under healthy conditions the brain/blood AUC_u ratio was 0.47 ± 0.19 for all probes, indicating that morphine is subjected to efflux at the BBB in the pig (Table 3).

In the presence of meningitis the unbound morphine concentrations in the brain increased, even though the concentrations of morphine in the blood were unaffected. Thus the increase in the morphine brain concentrations associated with meningitis was caused by changes in the properties of the BBB. The increase in the average ratio for the brain/blood AUC_u to 0.95 ± 0.20 ($p<0.001$), implies decreased active efflux of morphine at the BBB and/or increased passive diffusion over the injured BBB.

A brain/blood AUC_u ratio close to unity in the presence of meningitis indicates that there is no active efflux of morphine at the BBB, or that passive diffusion occurs to a greater extent than active transport. In this study it was not possible to discriminate between these two mechanisms. However, an increase in the passive diffusion is supported by the extravasation of Evans Blue into the brain, as observed with this porcine model (Gärdenfors *et al.*, 2002). On the other hand, lower concentrations of ATP were found in the cerebral cortex of piglets with meningitis (Park *et al.*, 2000). Thereby it is possible that there is a decrease in the active transport arising from an energy deficiency. Furthermore, Piquette-Miller and co-workers demonstrated that the expression of P-gp in the rat liver was decreased as a result of acute inflammation (Piquette-Miller *et al.*, 1998).

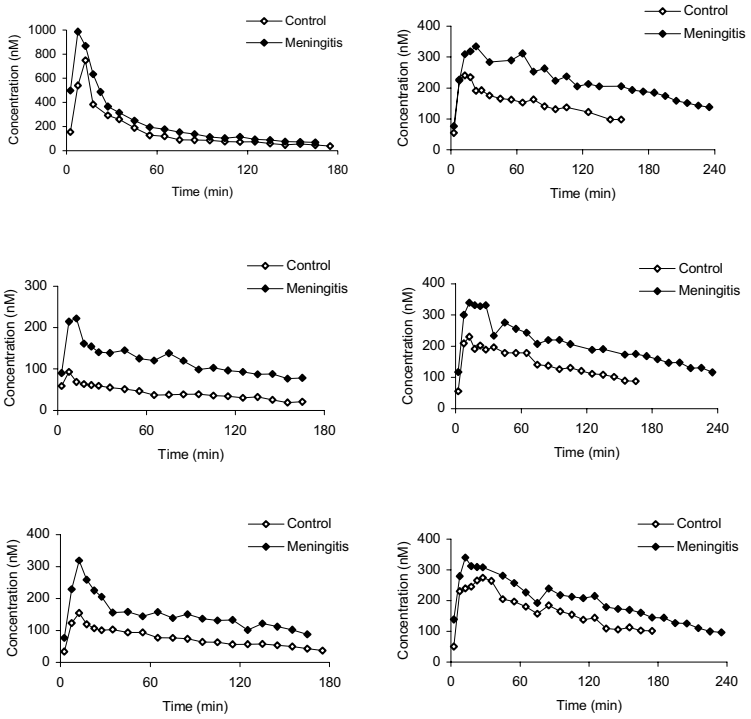


Figure 13. *Brain concentrations of unbound morphine in three microdialysis probes in Pig 2 (left panels) and Pig 7 (right panels) after two consecutive 10 min morphine infusions of 1 mg/kg in a control period and during experimentally induced meningitis. The probes were placed in the left occipital (top panels), the right frontal (middle panels) and the left frontal (bottom panels) cortex in the brain.*

There was a significant difference in the brain/blood AUC_u ratio depending on the probe placement in the brain ($p<0.05$). The AUC_u ratio for the occipital probes was 0.63 ± 0.11, and the ratios were 0.36 ± 0.22 and 0.41 ± 0.18 for the right and the left frontal cortical probes, respectively, in the control period. Once meningitis had been induced, the AUC_u ratios increased to 1.07 ± 0.08, 0.90 ± 0.21 and 0.92 ± 0.25 in the occipital, the right frontal and the left frontal probe positions, respectively. These differences in the exposure of the brain to morphine could be explained by different quantities of active transporters being expressed in different regions of the brain. Spatial differences in brain drug delivery have not been reported previously, however there are examples where no regional differences in the brain concentrations of drugs were observed (Matos *et al.*, 1992; Van Belle *et al.*, 1995).

The morphine half-life in the brain was unaffected by meningitis (Table 3), even though the exposure of morphine to the brain was increased. It is likely that an increase in the passive diffusion across the BBB would decrease the half-life in the brain, while decreased active efflux would result in a longer half-life. In Paper I it was shown that the half-life of morphine in the brain was prolonged upon probenecid co-administration. Similarly, inhibition of P-gp resulted in an increase in the half-life of morphine in the brain (Letrent *et al.*, 1999). Thus, the unchanged half-life in the present study might be interpreted as arising from both an increase in the passive diffusion and a decrease in the active efflux. As the half-life in the brain was independent of meningitis, the pharmacokinetics of morphine seem to be relatively unaffected by meningitis once the drug is inside the brain.

Severe brain trauma (Paper IV)

The transport across the BBB was slow both in the "better" and in the "worse" brain tissues since it took a longer time for the peak concentrations (T_{max}) of morphine to be attained at both sites in the brain than the i.v. morphine infusion took. Although not significant, there was a tendency for T_{max} being shorter in the "worse" brain tissue than the "better" brain tissue ($p = 0.068$) (Table 4, Figure 14C), indicative of an increased permeability to morphine in injured brain tissue. However, there was no significant difference between the exposure to the "better" brain tissue and the "worse" brain tissue to morphine (Table 4, Figure 14A). This unexpected finding could be a consequence of the number of patients enrolled in the study being so few, and also of the large variability in the pharmacokinetic parameters related to the brain. It could also be questioned whether the "better" brain tissue genuinely represents intact brain tissue, since severe brain trauma is often global, especially in patients with global brain swelling without a focal mass, as in patients Nos. 6 and 7.

No difference was found in the morphine half-life in the brain regions investigated. However, the large interindividual variability in this parameter in the "worse" brain tissue should be noticed (Figure 14B).

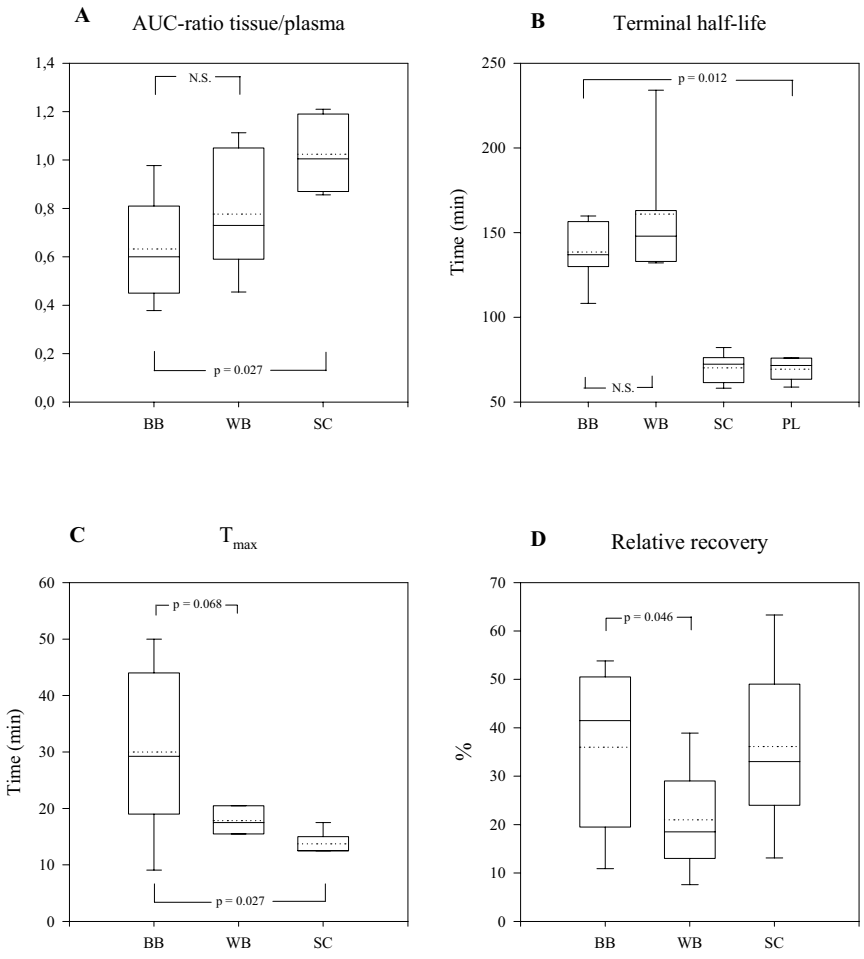


Figure 14. *Box plots demonstrating the median (solid centre line), mean value (dotted centre line), 25th and 75th percentile (lower and upper box limit), and 5th and 95th percentile (error bars) for morphine pharmacokinetics and the relative recovery in patients with traumatic brain injury. Abbreviations: BB, "better" brain tissue; WB, "worse" brain tissue; SC, subcutaneous adipose tissue; PL, plasma and N.S., not significant.*

Table 4. The cerebral concentrations of glucose, glycerol, lactate and lactate/pyruvate ratio, together with cerebral and subcutaneous morphine pharmacokinetics in patients with severe brain trauma. The morphine recovery and the half-life in plasma are also presented.

		Cerebral energy metabolism					Morphine pharmacokinetics		
Patient	Place	Glucose (mmol/L)	Glycerol (μmol/L)	Lactate (mmol/L)	Lactate/Pyruvate ratio	Recovery (%)	$t_{½}$ (min)	AUC-ratio tissue vs. plasma	T_{max} (min)
1	BB	1.5	33.6	1.7	15.6	33.0	156	0.36	17.5
1	WB	0.8	82.6	1.5	18.6	21.0	135	1.05	17.5
1	SC					18.1	83.3	0.87	12.5
1	PL						57,1		
2	BB	4.8	89.7	5.8	21.3	50.4	135	0.60	20.5
2	WB	0.1	469.7	8.9	607.0	7.0	242	0.86	15.5
2	WB	2.9	129.1	5.1	17.8	12.7	132	0.59	20.5
2	SC					32.7	72.4	1.21	12.5
2	PL						76.0		
3	BB	9.4	21.9	7.1	23.2	54.9	161	0.48	38
3	WB	2.1	281.0	3.7	25.0	15.6	163	1.12	15.5
3	SC					40.5	57.9	1.17	12.5
3	PL						64.2		
4	BB	1.8	27.8	2.3	15.1	26.4	101	0.42	50
4	WB	0.7	270.4	8.1	36.3	28.5	133	0.60	17.5
4	SC					10.5	58.7	0.98	17.5
4	PL						62.8		
5	BB	5.0	43.6	3.6	14.4	51.3	139	0.65	50
5	WB	1.2	37.4	8.1	33.6	40.0	161	0.44	20.5
5	SC					65.6	79.6	0.87	17.5
5	PL						72.7		
6	BB	1.8	68.7	1.4	16.0	10.1	125	0.98	5.5
6	SC					56.9	64.2	0.85	12.5
6	PL						76.1		
7	BB	2.5	81.5	3.8	26.1	12.7	157	0.97	38
7	SC					30.0	72.9	1.03	12.5
7	PL						70.4		
Mean ± SD	BB	3.8 ± 2.8 *	52.4 ± 27.3 *	3.7 ± 2.1	18.8 ± 4.7	34.0 ± 18.6 *	139.2 ± 21.3 †	0.64 ± 0.25 ‡	31.4 ± 17.1 ‡
Mean ± SD	WB	1.3 ± 1.1	211.7 ± 160.4	5.9 ± 3.0	123.1 ± 237.2	21.0 ± 11.9	161.0 ± 42.1	0.78 ± 0.28	17.8 ± 2.3
Mean ± SD	SC					36.6 ± 19.8	69.9 ± 9.9	1.00 ± 0.15	13.9 ± 2.4
Mean ± SD	PL						68.5 ± 7.3		

BB = “better” brain tissue, WB = “worse” (injured) brain tissue, SC = subcutaneous adipose tissue, PL = plasma.
* = $p<0.05$ vs. WB, † = $p<0.05$ vs. PL, ‡ = $p<0.05$ vs. SC.

Recovery

Estimating the *in vivo* recovery in each microdialysis probe is essential for quantitative measurements. From the recovery experiments in Paper III it was concluded that nalorphine could be used as a calibrator for morphine recovery in the brain and in the blood both under healthy conditions and in the presence of meningitis in the pig. The recovery data obtained in one of the brain probes is shown in Figure 15.

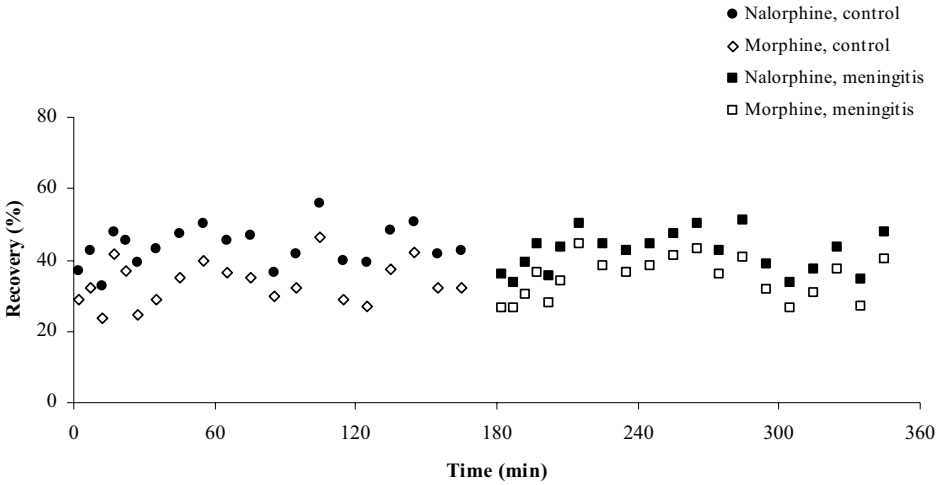


Figure 15. *The in vivo brain probe recovery of morphine and nalorphine in the recovery experiment in Pig 1, probe B.*

The brain probe recovery decreased during the meningitis period from 36 ± 8 % to 29 ± 12 % ($p<0.01$) (Paper III). There were no regional differences in the brain probe recovery. In addition, no change was observed in the blood probe recovery between the two conditions. In Paper IV the recovery of morphine was lower in the "worse" brain tissue than in the "better" brain tissue ($p<0.05$) (Table 4, Figure 14D).

The decrease in the recovery during meningitis and a low recovery in the "worse" brain tissue as compared to the "better" brain tissue is indicative of an increased resistance to mass transfer during disease (Bungay *et al.*, 1990; Chen *et al.*, 2002). Since the microdialysis probes and the flow-rate of the

perfusate were the same in the control period and during meningitis (Paper III), and in both brain regions (Paper IV), it is likely that the difference in the recovery reflects alterations in the brain tissue surrounding the microdialysis membranes. Thus, the results from these two studies imply that the relative recovery *per se* might reflect the degree of tissue trauma.

Modelling

The restricted model

The restricted model was used to analyse the morphine data in Papers I and V. The parameters derived from the model were the unbound parameters. Prior to the model development the recovery in the blood and in the brain probes was calculated according to Equation 8. The typical values of the recovery [RSE (%)] obtained from the data in Paper V were 48.4 (4.6) % and 10.5 (5.4) % for the blood and brain probes, respectively. Similar values were obtained on both of the experimental days investigated in Paper I.

The morphine data obtained from the study in rats (Paper I) was adequately described by a two-compartment model both in the brain and in the blood (Papers I and V). The parameters describing the transport across the BBB of morphine were derived from the differential equations that characterised the drug distribution between the brain and the blood compartments (Equations 12 and 13).

$$CL_{in} = k_{in} \cdot V_1 \tag{12}$$

$$CL_{out} = k_{out} \cdot V_{brain,1} \tag{13}$$

V_1 and $V_{brain,1}$ are the unbound volumes of distribution in the central blood and brain compartments, respectively, k_{in} is the rate constant from blood to brain and k_{out} represents the rate constant out of the brain.

The residual error was described by slope-intercept models in both tissues, and the unbound arterial concentration and the unbound brain concentrations, corrected for the recovery, were predicted from the model according to:

$$y_{ij} = \frac{A_1}{V_1} \cdot \left(1 + \varepsilon_{1,ij}\right) + \varepsilon_{2,ij} \quad (14)$$

$$y_{ij} = \frac{A_3}{V_{brain,1}} \cdot \left(1 + \varepsilon_{3,ij}\right) + \varepsilon_{4,ij} \quad (15)$$

where y_{ij} corresponds to the *j*:th prediction in the *i*:th individual and A_1 and A_3 the amount of drug in the blood and the brain compartment, respectively. The proportional errors are represented by ε_1 and ε_3. ε_2 and ε_4 represent the additive error.

The inclusion of probenecid as a categorical covariate affecting the systemic clearance improved the model fit (Paper I). The typical value [RSE (%)] of the decrease in clearance upon probenecid co-administration was estimated as 21(30) %.

The CL_{in}/CL_{out} ratio was estimated as 0.27 after morphine administration and 0.39 upon morphine and probenecid co-administration (Paper I). These values were similar to the ratio between the steady state concentrations in the brain to those in the blood ($C_{u,ss,brain}/C_{u,ss,blood}$) obtained from the NCA (Table 3). By applying modelling to the data it was possible to distinguish between the effect of probenecid on CL_{in} and on CL_{out}. For morphine, the model fit was substantially improved by the inclusion of probenecid as a covariate that decreases $CL_{out.}$ In contrast, there was only a small improvement in the correlation between the observed and the predicted concentrations of morphine in the brain if probenecid was assumed to increase the CL_{in}. It was, therefore, concluded that probenecid mainly decreases the brain efflux of morphine and that the influence on the influx is minor.

The integrated model

In Paper V the integrated model was developed using the morphine data from the first experimental day presented in Paper I. Using this model, all data including the total arterial concentrations, the brain and blood dialysate concentrations and the recovery measurements were analysed simultaneously.

In contrast to the restricted model, the types of concentrations predicted with the integrated model were the same as the types of observed concentrations, i.e., the total arterial concentrations (Equation 16) and the integrated venous (Equation 17) and brain (Equation 18) dialysate concentrations.

$$y_{ij} = \left(\frac{A_1}{V_1/2} \right) \Big/ fu \cdot \left(1 + \varepsilon_{1,ij}\right) + \varepsilon_{2,ij} \tag{16}$$

$$y_{ij} = \frac{C_{dialysate,blood}}{TIN} \cdot \left(1 + \varepsilon_{3,ij}\right) + \varepsilon_{4,ij} \tag{17}$$

$$y_{ij} = \frac{C_{dialysate,brain}}{TIN} \cdot \left(1 + \varepsilon_{5,ij}\right) + \varepsilon_{6,ij} \tag{18}$$

TIN is the time of the collection interval for the dialysates, and f_u is the unbound fraction of the drug in the blood. The central volume of distribution (V_1) was equally divided between the two blood compartments.

The typical values [RSE (%)] of the recovery were estimated as 45.9 (4.2) % with an IIV of 14 (55) % and 10.4 (5.5) % with an IIV of 12 (66) % in the blood and brain probes, respectively.

The systemic pharmacokinetics of morphine was best described by a two-compartment model with no delay in the distribution between the arterial and the venous blood compartments (Figure 16, Equations 19-21). In the brain a one-compartment model with bi-directional transport of morphine across the BBB, sufficiently described the data (Figure 16, Equation 22).

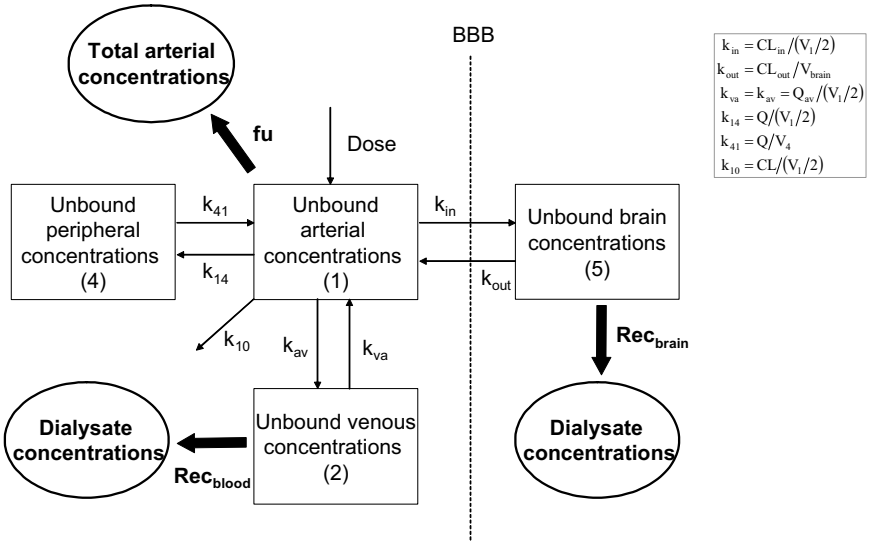


Figure 16. *The integrated model describing the systemic pharmacokinetics and the blood-brain barrier transport of morphine. The elliptical boxes represent the observed data and the thick arrows show the corrections that are made within the model to obtain the concentrations describing the distribution of unbound drug between the blood and the brain. The thin arrows represent mass transport. The compartment numbers used in Equations 19-22, 25 and 26 are given within parentheses. The conversion from the rate constants to the estimated parameters is given in the legend. All abbreviations are explained in the list of Abbreviations.*

$$\frac{dA_1}{dt} = R_{exp} + k_{va} \cdot A_2 + k_{41} \cdot A_4 + k_{out} \cdot A_5 - (k_{14} + k_{av} + k_{in} + k_{10}) \cdot A_1 \quad (19)$$

$$\frac{dA_2}{dt} = k_{av} \cdot A_1 - k_{va} \cdot A_2 \quad (20)$$

$$\frac{dA_4}{dt} = k_{14} \cdot A_1 - k_{41} \cdot A_4 \quad (21)$$

$$\frac{dA_5}{dt} = k_{in} \cdot A_1 - k_{out} \cdot A_5 \quad (22)$$

R_{exp} represents the rate of the exponential infusion of morphine. A_1, A_2, A_4 and A_5 correspond to the amount of morphine in the arterial, the venous, the peripheral and the brain compartments, respectively. The rate constants k_{14} and k_{41} represent the flow between the arterial and the peripheral compartments, k_{av} and k_{va} the rate constants between the arterial and venous compartments, k_{10}, the transport out of blood, k_{in} the transport from blood to brain and k_{out} the transport from brain to blood. The unbound parameter estimates that were derived from these equations were similar to the values obtained from the restricted model and the NCA.

In a manner similar to that adopted for the restricted model, the BBB transport was parameterised in terms of CL_{in} and CL_{out} and, therefore, an estimate of V_{brain} was required. In theory it is possible to estimate V_{brain} using the integrated model. However, since the amount of drug in the brain is small it will have a low impact on the systemic pharmacokinetics, thereby making V_{brain} difficult to estimate. Although it was possible to estimate the V_{brain} for morphine in this particular case, the parameter estimate was highly sensitive to the initial estimate of the parameter, effectively requiring the value of V_{brain} to be known. CL_{in} and CL_{out} were calculated according to:

$$CL_{in} = k_{in} \cdot (V_1/2) \tag{23}$$

$$CL_{out} = k_{out} \cdot V_{brain} \tag{24}$$

The dialysate concentrations in the venous blood and in the brain were expressed as the integral over each collection interval, represented by t_1 and t_2 (Equations 25 and 26). Mass transfer from the tissues into the dialysates was neglected.

$$C_{dialysate,blood} = \int_{t_1}^{t_2} \frac{A_2}{(V_1/2)} \cdot Recovery_{blood}\ dt \tag{25}$$

$$C_{dialysate,brain} = \int_{t_1}^{t_2} \frac{A_5}{V_{brain}} \cdot Recovery_{brain}\ dt \tag{26}$$

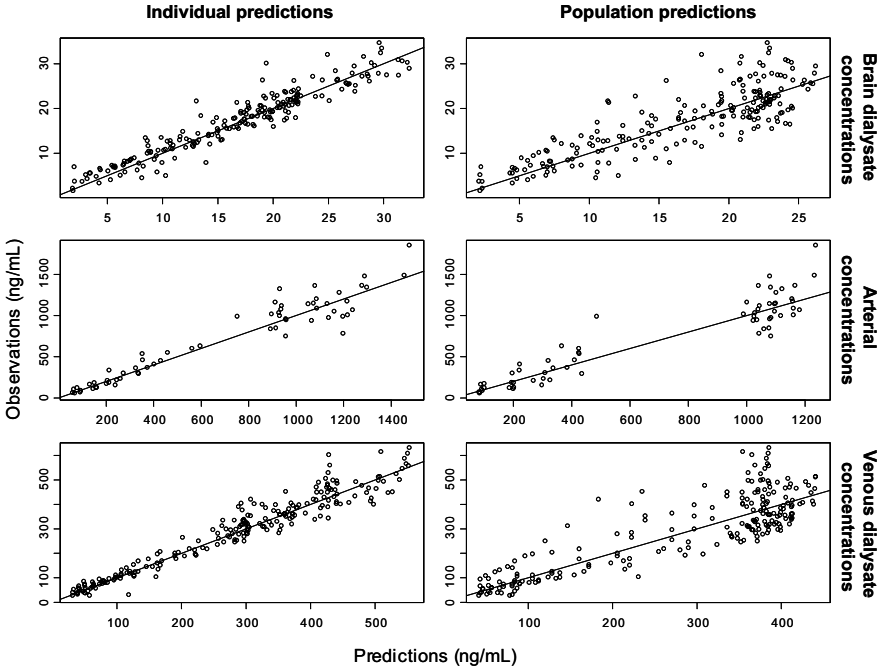


Figure 17. *The observed data and population predictions and individual predictions of the brain dialysate concentrations, the venous dialysate concentrations and the total arterial concentrations obtained from the integrated model (Paper V).*

Figure 17 shows the observed data and the population predictions and the individual predictions of the arterial concentrations and the venous and the brain dialysate concentrations obtained from the integrated model.

The integrated model, developed in Paper V, was applied to the M6G data obtained in Paper II. The typical value [RSE (%)] of the blood probe recovery was estimated as 54.1 (6.4) % with an IIV of 17 (45) %. The brain probe recovery was best described by a typical value which varied at random between one experimental day and another (inter occasion variability). The typical value was estimated as 6.4 (12) % with an inter occasion variability of 43 (30) %.

A model comprising two compartments in both the blood and the brain adequately described the systemic and the brain pharmacokinetics of M6G. Similarly to morphine, there was no delay in the distribution of M6G between the two blood compartments. Considering that M6G is a low extraction drug in the liver, no arterio-venous differences were expected. The inclusion of probenecid as a categorical covariate affecting the systemic

clearance of M6G improved the model fit and, in the typical individual, the clearance decreased by 22 (15) % upon probenecid co-administration. The unbound fraction, which was estimated as 85 (5) % with an IIV of 6.8 (55) %, was similar to a previously reported value of 83% (Bickel *et al.*, 1996).

The BBB transport of M6G was unaffected by probenecid, as previously discussed (see "Blood-brain barrier transport of M6G (Paper II)").

Comparing the different strategies for data analysis

Population pharmacokinetic modelling was originally developed for the analysis of sparse data (Sheiner *et al.*, 1977), which is often the reality in clinical studies. However, it has been shown that the population approach is also applicable to rich data (Jonsson *et al.*, 2000), as is obtained with microdialysis. The advantages with population modelling are that, in addition to the residual error, the variability can be assessed between individuals, and between different occasions. In addition, it allows more complex models to be characterised, compared to individual analysis.

Both modelling approaches used in Paper V resulted in similar structural models. The parameter estimates from the two models and the NCA were also comparable. In addition, the reanalysis of the morphine data, presented in Paper I, using the integrated model resulted in comparable parameter estimates and a similar structural model to that obtained with the restricted model and the NCA. However it should be recognised that there are several advantages related to the modelling approach and, in particular, to the use of the integrated model. In Table 5 the benefits and drawbacks associated with the three data analysis strategies are summarised.

The main difference between the two models presented in this thesis is that the integrated model describes all data in a single model. This means that the recovery and the protein binding are parameters that are estimated within the model, in addition to parameters such as CL_{in} and CL_{out}. Thereby, the uncertainty, the inter probe variability and the inter occasion variability in these parameters are taken into account. In contrast, they are neglected in the restricted model and in the NCA, since the observed concentrations from the dialysis are simply corrected for the average value of the recovery prior to the data analysis. It should also be noted that constructing the data file is more practical and less open to error when using the integrated model, since none of the observed concentrations are adjusted prior to the data analysis.

Table 5. Benefits and drawbacks of using the non-compartmental methodology, the restricted model and the integrated model for the analysis of microdialysis data, with the emphasis on brain drug delivery.

Characteristics	*Non-compartmental analysis*	*Restricted model*	*Integrated model*
No data transformation required prior to the data analysis			•
No assumptions required about the time of the observation of the microdialysis data			•
Uncertainty in the measurement of the recovery is recognised			•
Uncertainty in the measurement of the protein binding is recognised	NA		•
All blood data are used to estimate the systemic pharmacokinetics			•
Any time delay in drug distribution between arterial and venous blood can be investigated			•
Low level of complexity	•		
Little computer capacity is required	•		
The extent of blood-brain barrier transport can be estimated	•	•	•
The rates of blood-brain barrier transport can be estimated		•	•
The volume of distribution in the brain can be estimated			•
Can be used for simulations			•

NA, not applicable

For studies of the BBB transport of drugs, the modelling approach is preferable since estimates of both CL_{in} and the CL_{out} can be assessed. In contrast, only the ratio between these two parameters, expressed as $C_{u,ss,brain}/C_{u,ss,blood}$, can be calculated using the NCA. Thus, with the NCA it is only possible to measure changes in the extent of BBB transport, for

example as a consequence of transport modulation. On the other hand, using the modelling approach it is possible to determine whether an inhibitor of a certain transporter increases the CL_{in} or decreases the CL_{out}. Thereby greater mechanistic insight is gained.

In summary, the integrated model is superior to the restricted model and the NCA because all the data are described with just a single model, no assumptions regarding the time of the dialysate observations need to be made, and corrections for the recovery and the protein binding are carried out within the model. This enables these sources of variability in the data to be taken into account, thereby yielding more reliable parameter estimates, and allowing the study design to be optimised through simulations.

Conclusions

Both morphine and M6G are substrates for efflux transporters at the BBB. In addition, the extent of BBB transport (i.e., the exposure of the drug to the brain compared to the blood) is similar for these compounds, although the transport across the BBB is slower for the metabolite. The exposure of morphine to the brain is increased by probenecid co-administration, while the concentrations of M6G in the brain are unaffected by probenecid. This demonstrates that of the drugs tested only morphine is a substrate for the probenecid-sensitive transporters at the BBB. However, the exposure to unbound morphine is lower in the brain than in the blood despite probenecid being administered, confirming that the probenecid-sensitive transporters are not the only transporters involved in the brain efflux of morphine.

In this thesis similarities in the BBB transport are investigated in rats, pigs and humans, using morphine as the model compound. It is demonstrated that morphine undergoes active efflux at the BBB in the species investigated, although the extent of transport differs between species. In addition, the half-life of morphine is significantly longer in the brain than in the blood in the three species, indicating that the cerebral pharmacokinetics of morphine differs from the systemic pharmacokinetics. These results show the predictive capacity of two animal models for the brain penetration of morphine in humans. However more drugs need to be investigated, if more general conclusions are to be drawn.

In the pig, the exposure of the brain to morphine is increased in the presence of experimentally induced meningitis in comparison to the healthy condition. However, the time-course of the morphine concentrations in the brain is unaffected by meningitis. These results can be explained by an increase in the passive diffusion or a decrease in the active efflux over a BBB that has been damaged by meningitis. Consequently, if these results are consistent with those in patients suffering from meningitis, and if a drug has similar properties to morphine at the BBB, the dose of that drug should be decreased while retaining the dosing interval in these patients. In contrast, there is no significant difference in the exposure of the “better” brain tissue and the “worse” brain tissue to morphine in brain trauma patients.

The data obtained with microdialysis differ from the data collected by regular blood sampling because microdialysis only measures a fraction of the true unbound tissue concentration and the data is collected in fractions. To handle this type of data an integrated model was developed. In that model all of the collected data could be analysed simultaneously, and thereby no assumptions needed to be made of error free recovery and protein binding. In contrast to the restricted model that was previously used, no assumptions need to be made regarding the time of the observation of the microdialysis data with the integrated model. Moreover, as all the data collected in the blood are included in the model, any delay in the distribution of the drug between the arterial and the venous blood compartments can be investigated.

Perspectives

The goal in drug treatment is to attain the desired pharmacological effect with as few adverse events as possible in each patient. In order to get the right dose and dosing interval for the individual patient it is crucial to understand the mechanisms underlying the operation of the drug that will affect the drug concentrations at the site of action. Consequently, for drugs that are targeted to the brain, the brain concentrations should be measured under a variety of conditions. At present, the pharmacokinetics at the target site is rarely studied during drug development. As a result of this it is difficulty to predict the consequences of disease or local drug interactions at for example the BBB on the concentrations of drugs in the brain.

In this thesis the importance of investigating the local pharmacokinetics of drugs was demonstrated using morphine and M6G as a model compounds. Furthermore, the value of using microdialysis in these types of studies was emphasised. For example, the effect of meningitis and probenecid co-administration on the brain exposure of morphine would not have been recognised if blood samples only had been collected. Until now microdialysis has been used to measure local drug concentrations in many tissues. Yet it is not a technique commonly used in drug development in the pharmaceutical industry. Nevertheless, because of the unique data that is generated by microdialysis, I believe that it will be used more frequently in the pharmaceutical industry in the future. In particular, I think the method will be used in confirmatory studies, for example to verify that a drug reaches its target site. In addition, for CNS-acting drugs with small therapeutic ranges I think that microdialysis will be used to investigate drug interactions at the BBB.

For the identification of the specific transporters that are involved in the brain efflux of drugs, *in vitro* methods are superior to *in vivo* models. However, to get the overall picture, *in vivo* experiments using either specific transport inhibitors or knock-out animals are preferable. At present, only a few drugs have had their transport across the BBB characterised *in vivo*. If more drugs were investigated, the properties of the molecules that are important for their transport across the BBB could be identified. Thereby it would be possible to predict the penetration of a drug into the brain on the

basis of the physico-chemical properties of the drug and the affinity it has to specific transporters present at the BBB. In addition, local drug interactions at the BBB could be foreseen and handled to avoid toxicity in patients that are treated with more than one drug. Thus, this knowledge would increase the probability of getting the right dose to the patient.

Populärvetenskaplig sammanfattning

Målsättningen med läkemedelsbehandling är att i varje patient uppnå en önskad farmakologisk effekt, utan att patienten upplever svåra biverkningar. Den farmakologiska effekten styrs av läkemedelskoncentrationen i målorganet. För läkemedel som har sin effekt i hjärnan är det viktigt att ha kunskap om de mekanismer vid blod-hjärnbarriären som påverkar koncentrationen av läkemedlet i hjärnan.

Blod-hjärnbarriären separerar hjärnan från övriga kroppen, och fungerar som ett skydd för hjärnan från giftiga ämnen. Den skyddande effekten är kopplad till barriärens struktur med tätliggande celler och transportproteiner som ofta pumpar främmande ämnen ut ur hjärnan. Dessa egenskaper gör att många läkemedel har svårt att nå tillräckligt höga koncentrationer i hjärnan.

Syftet med den här avhandlingen var att studera hur blod-hjärnbarriärtransporten av morfin och morfin-6-glukuronid (M6G), en nedbrytningsprodukt till morfin, påverkas av probenecid som blockerar vissa transportproteiner som finns i blod-hjärnbarriären. Jag har också undersökt om transporten över blod-hjärnbarriären skiljer sig åt mellan försöksdjur (råtta och gris) och människa och dessutom hur hjärnhinneinflammation och skallskada påverkar mängden läkemedel som når hjärnan. I dessa studier använde jag morfin som modellsubstans. Alla försök utfördes med mikrodialys som är en metod som mäter obundna läkemedels-koncentrationer, t.ex. i hjärnan. Mikrodialys mäter koncentrationer på ett annorlunda sätt än vanliga blodprover. Därför utvecklade vi en matematisk modell som klarar av att ta hand om informationen från mikrodialysförsök.

Resultaten visar att koncentrationen morfin är lägre i hjärna än i blod i råtta, gris och människa. Det innebär att morfin påverkas av transportproteiner i blod-hjärnbarriären hos dessa tre species. En annan likhet är att det morfin som tar sig in i hjärnan sedan har svårt att ta sig tillbaka till blod. Alltså verkar experiment både i gris och i råtta kunna förutsäga hur morfin beter sig vid blod-hjärnbarriären i människa. För att veta om dessa försöksdjur kan användas för att förutsäga hur läkemedel i allmänhet beter sig vid blod-hjärnbarriären i människa behöver man undersöka fler läkemedel.

I grisar med hjärnhinneinflammation är koncentrationen av morfin lika i hjärna och i blod. Det tyder på att de transportproteiner som brukar pumpa morfin ut ur hjärnan är utslagna, och/eller att blod-hjärnbarriärens celler ligger glesare vid hjärnhinneinflammation än vid friskt tillstånd. Under förutsättning att hjärnhinneinflammation påverkar funktionen av blod-hjärnbarriären på ett liknande vis i människa, bör därför morfin ges i lägre doser till patienter som lider av denna sjukdom. Likaså bör dosen minskas av andra läkemedel som transporteras över blod-hjärnbarriären på ett liknande sätt som morfin. Tvärtemot visar den kliniska studien att det inte är någon skillnad i morfinkoncentration i ”friskare” och ”sjukare” hjärnvävnad hos patienter som råkat ut för svår skallskada.

När både morfin och probenecid ges till råtta är koncentrationen av morfin i hjärnan högre än när enbart morfin ges. Det tyder på att de transportproteiner vid blod-hjärnbarriären som blockeras av probenecid också hindrar morfin att ta sig in i hjärnan. Resultaten från studien visar dock att även andra transportproteiner än de som blockeras av probenecid påverkar hur mycket morfin som tar sig in i hjärnan. Till skillnad från morfin så påverkar inte probenecid blod-hjärnbarriärtransporten av M6G i råtta. Det innebär att morfin och M6G påverkas av olika transportproteiner vid blod-hjärnbarriären.

I det sista arbetet i avhandlingen utvecklade vi en matematisk modell för att analysera koncentrationsmätningar i blod och i hjärna från mikrodialysförsök. En fördel med en sådan modell är att den både kan beskriva hur snabbt och hur stor del av en dos av ett läkemedel som transporteras över blod-hjärnbarriären. En annan mer generell fördel med en modell är att den kan användas för att förutsäga t.ex. vilken koncentration som uppnås i hjärnan efter att en viss dos av ett läkemedel getts. Vid utvecklingen av den nya modellen lades tonvikten på hur man ska hantera koncentrationmätningar från mikrodialysförsök på bästa sätt. I jämförelse med en tidigare använd modell görs färre antaganden i den nya modellen, vilket bör ge mer pålitliga värden på de parametrar som uppskattas från modellen.

Genom att identifiera vilka transportörer som påverkar koncentrationen av ett läkemedel i hjärnan är det möjligt att förutse interaktioner mellan olika läkemedel vid blod-hjärnbarriären. Sådan kunskap är väsentlig för patienter som behandlas med flera läkemedel eftersom dessa interaktioner kan leda till oväntat höga eller låga koncentrationer av ett läkemedel i hjärnan. Ur patientsynpunkt är det också viktigt att förstå hur olika sjukdomstillstånd kan påverka koncentrationen av läkemedel i hjärnan.

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