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

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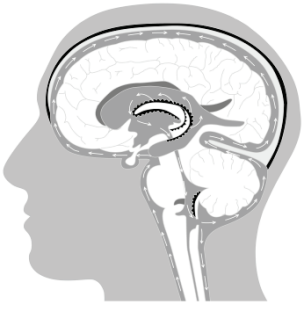
Chapter 6

Prediction of brain target site concentrations on the basis of CSF PK: General discussion and perspectives



FACTORS THAT GOVERN THE PHARMACOKINETICS IN THE BRAIN

In the development of drugs for the treatment of central nervous system (CNS) disorders, the prediction of human CNS drug action is a big challenge. In part this has been due to the sole focus on the blood-brain barrier (BBB) permeability of drugs, which, as classical paradigm, is governed by the lipophilicity and molecular weight of drugs (Levin, 1980). However, not all processes that determine drug concentrations at the relevant target site within the CNS are taken into account. Besides plasma pharmacokinetics (PK), plasma protein binding, and passive and active transport across the blood-brain barriers (BBB and the blood-cerebrospinal fluid barrier (BCSFB)), processes within the brain can also influence brain target site PK, including bulk flow, diffusion, and extra-intracellular exchange (**Chapter 1**). Moreover, it is important to distinguish between the rate and the extent of all processes. For example, passing of the BBB occurs with a certain rate and to a certain extent (Hammarlund-Udenaes *et al.,* 2008). The rate of transport across the BBB is reflecting the time needed for a drug molecule to traverse this barrier, while the extent of BBB transport expresses the ratio of unbound drug concentrations in the brain compared to those in plasma at steady state ($K_{p,uu}$). It can also be calculated as the ratio of the area under the unbound concentration-time curve ($AUC_{0-\infty}$) in brain relative to that in plasma. The rate as well as the extent of BBB transport on one hand is dependent on both the (condition-dependent) characteristics of this barrier and on the physicochemical properties of the drugs (De Lange and Danhof, 2002; Levin, 1980). Likewise, for other processes the rate and extent can be defined. Each of the different processes that determine drug concentrations at the relevant CNS target has its particular influence on the overall rate and extent, and thereby plays a more or less important role in having the drug in the *right place*, at the *right time*, and at the *right concentration*.

For many CNS active compounds, brain target site concentrations are best reflected by, or may even be equal to unbound drug concentrations in the brain extracellular fluid ($brain_{ECF}$) (De Lange *et al.,* 2000; Hammarlund-Udenaes*,* 2009; Watson *et al*., 2009). However, the possibility of direct measurement of $brain_{ECF}$ concentrations is highly limited in the clinical phase of drug

development. Therefore, unbound drug concentrations in human CSF are used as a surrogate for human $brain_{ECF}$ concentrations. It is often assumed that CSF concentrations readily equilibrate with $brain_{ECF}$ concentrations due to the lack of a physical barrier between the two (Lee *et al.,* 2001). However, the brain is a dynamic, multi-compartmental system in which all processes of entry, diffusion, metabolism, binding and elimination determine local CNS concentrations. Due to qualitative and quantitative differences in processes that govern the PK of drugs in the brain, a generally applicable relationship between CSF concentrations and $brain_{ECF}$ concentrations does not exist (**Chapter 1**, De Lange, 2013a; De Lange and Danhof, 2002; Lin, 2008; Shen *et al.,* 2004). This all implies the need for mechanistic investigations on the contribution of the different processes that govern the brain target site exposure.

The rate and extent of drug penetration into the brain can be studied in the preclinical setting with several *in vitro*, *ex vivo* and *in vivo* techniques (**Chapter 1**), such as the brain perfusion technique or the brain slice technique. So far, most of these preclinical techniques determine total brain concentrations, or calculate unbound $brain_{ECF}$ concentrations using the fraction unbound in brain homogenate. When using brain homogenate, cell structures are destroyed and binding sites that are normally not accessible to a drug *in vivo* may be unmasked (Liu *et al.,* 2009). This could result in an erroneous estimation of the unbound fraction in brain tissue. Moreover, in the drug discovery phase these techniques are often used such that information on solely equilibrium distribution is obtained. However, this may limit the extrapolative power of the results to the human situation. Furthermore, most of these techniques cannot be applied to humans, which makes a direct comparison of preclinical and clinical findings impossible. In contrast, CSF sampling can be used in both animals and humans, and as it provides information on unbound concentrations (with some time-dependency) it is of special interest. Most useful would be to use the intracerebral microdialysis technique for monitoring unbound brain concentrations at one (or more) selected site(s) in the brain, but its use in humans is highly restricted. However, if applied in animals, intracerebral microdialysis may still reveal mechanistic information on the inter-relationships of different processes that govern the $brain_{ECF}$-CSF PK in different conditions *in vivo*, and may investigate such in conjunction with pharmacodynamic (PD) read-outs. Such information may provide useful links to the human situation.

PARALLEL INTRACEREBRAL MICRODIALYSIS

With intracerebral microdialysis it is possible to monitor local unbound concentrations of compounds at one or more specific sites in the brain. Thus, with the use of multiple intracerebral microdialysis probes in individual animals one can directly compare unbound concentrations in $brain_{ECF}$, CSF from lateral ventricle (CSF_{LV}) and CSF from cisterna magna (CSF_{CM}), thereby gaining insight into the relationship between $brain_{ECF}$ and CSF concentrations. However, special care should be taken in determining the concentration recovery. Because of the continuous flow of the perfusion solution through the microdialysis probe, the concentration in the dialysate will be lower than in the surrounding $brain_{ECF}$ or CSF. (De Lange *et al.,* 2000). This indicates the need for determination of the *in vivo* recovery for proper correction of the dialysate to $brain_{ECF}$ and CSF concentrations, preferably for each brain location and for each experimental condition.

The aim of the research presented in this thesis was to develop a preclinical brain distribution model, allowing the prediction of human brain target site concentrations on the basis of preclinical data. In order to be able to build a brain distribution model understanding of time-dependent (also non-steady state) kinetics of the unbound drug in $brain_{ECF}$ and CSF is essential. To that end, systematic studies on the inter-relationship of plasma PK, BBB transport, BCSFB transport and intra-brain distribution were performed in the rat by using probes at multiple brain sites in individual animals.

As a general approach, three compounds with different physicochemical properties were selected as paradigm compounds (table 1). Acetaminophen was chosen as paradigm compound for passive transport into, within and out of the brain, with a medium logP and no ionization at physiological pH (**Chapter 3**). Quinidine was selected as a paradigm compound with a high logP, indicative of high passive BBB transport, and a positive charge at physiological pH. Furthermore, quinidine is a known substrate for P-glycoprotein (P-gp)-mediated transport out of the brain. To investigate the specific contribution of P-gp-mediated transport P-gp was inhibited by co-administration of tariquidar, a selective P-gp inhibitor (**Chapter 4**). Methotrexate was selected as a paradigm compound with a low logP, indicative of low passive BBB transport, and a negative charge at physiological pH. Furthermore, methotrexate is known to be

transported by a wide variety of transporters, including the reduced folate carrier 1 (RFC1), breast cancer resistance protein (BCRP), the multidrug resistance-associated protein (MRP) family, organic anion transporters (OATs) and organic anion-transporting polypeptides (OATPs). To investigate the specific contribution of the various transporters, probenecid was co-administered as inhibitor of MRPs, OATs and OATPs (**Chapter 5**).

Table 1. Physicochemical properties of the selected paradigm compounds

Compound	MW	PSA	logP	Ionization	pKa1	pKa2	Ionized at physio-logical pH	Substrate for	Reference
Acetamino-phen	151.2	49.3	0.46	monoprotic acid	9.38	-	0% (neutral)	-	DrugBank DB00316
Quinidine	324.4	45.6	3.44	diprotic base	4.0	9.1	98% (positive)	P-gp	DrugBank DB00908
Methotrex-ate	454.4	210.5	-1.85	diprotic acid	3.4	4.1	99.9% (negative)	BCRP, MRPs, OATPs, OATs	DrugBank DB00563

Abbreviations: MW, molecular weight; PSA, polar surface area; logP, log octanol:water partition coefficient; pKa, acid dissociation constant

Since the rate of equilibration between CSF and $brain_{ECF}$ concentrations by passive diffusion is dependent on the lipophilicity and size of the compound (De Lange *et al.,* 2000; Levin, 1980), we expected the CSF and $brain_{ECF}$ concentrations to be similar for acetaminophen, because acetaminophen is a small and moderately lipophilic compound with anticipated fast transport between blood and brain. However, we have observed that $brain_{ECF}$ concentrations of acetaminophen are ~4-fold higher than its CSF concentrations. This can probably be explained by the relatively slow distribution from $brain_{ECF}$ to CSF compared to the turnover rate of CSF. This makes the CSF act as a sink, causing the observed lower concentrations in CSF compared to $brain_{ECF}$.

For the P-gp substrate quinidine we expected significant differences between $brain_{ECF}$ and CSF concentrations, since it has been well established that P-gp functions as an efflux transporter at the BBB (Schinkel, 1999), whereas there has been some evidence that P-gp could also function as an influx transporter at the BCSFB (Kassem *et al.,* 2007; Rao *et al.,* 1999). Interestingly, we found only small differences between $brain_{ECF}$ and CSF concentrations of quinidine (0.72 ±

0.20 without inhibition of P-gp and 2.22 ± 0.57 with inhibition of P-gp). On the basis of the "smaller than threefold $brain_{ECF}$-to-CSF concentration ratio paradigm" (Maurer *et al.,* 2005), this result would not be of much importance. However, in our perspective, even a small difference in PK could potentially lead to quite distinct PD, in case of a steep concentration-effect relationship, and therefore still needs to be considered. These results indicate that P-gp functionality and variations thereof may have an important effect on the $brain_{ECF}$-to-CSF concentration ratio and the extrapolation from rats to humans. For quinidine, furthermore, we also expected the unbound brain concentrations to be lower than the unbound plasma concentrations. However, to our surprise, the unbound brain concentrations in all brain compartments were significantly higher than those in plasma. Since quinidine is actively transported out of the brain, this suggests that quinidine is also transported by other transporters at the BBB and BCSFB, in the direction of the brain, possibly by organic cation transporters (Van Montfoort *et al.,* 2001). This illustrates the importance of interplay of the different transporters at the BBB and BCSFB. The influence of a particular transporter can only be dissected if specific blockers are available. Actually, only for P-gp specific blockers are available (e.g. tariquidar).

Another example of a drug that is transported by multiple active transport systems located at the BBB and BCSFB, including BCRP, MRPs, OATs and OATPs, is methotrexate. Based on differences in the direction of flux and subcellular localization of the different transporter systems at the BBB and BCSFB (**Chapter 1**), for methotrexate we were expecting significant differences between $brain_{ECF}$ and CSF concentrations. As methotrexate is a very hydrophilic compound, the extent of distribution to the brain is much lower than for acetaminophen and quinidine. Interestingly, for methotrexate we found that $brain_{ECF}$ concentrations were significantly higher than CSF concentrations (> 3-fold), and this difference seemed to be independent of probenecid co-administration. This indicates that the active transport by Mrps, Oats and Oatps does not influence the $brain_{ECF}$-CSF relationship. However, inhibition of Mrps, Oats and Oatps did result in a significant increase in both $brain_{ECF}$ and CSF concentrations. Also, for methotrexate, as transported by multiple active transport systems that cannot be inhibited in a specific manner, it becomes difficult to identify the specific contribution of each transporter. It is therefore more efficient to investigate the transport processes by systematically

influencing a subset of variables, either by varying the conditions of the system or by varying the drug properties. Using different drugs, with different drug properties, such as affinities for the different transporters, one can decipher the impact of changes at the level of these variables on the blood-brain transport and the distribution beyond.

SYSTEMS-BASED PHARMACOKINETIC MODELING

In order to predict human CNS effects, different mathematical modeling techniques can be applied (Danhof *et al.,* 2008). The most commonly applied has been the compartmental model analysis (Fleishaker and Smith, 1987), in which the brain compartment is modeled as an effect compartment (Hammarlund-Udenaes *et al.,* 1997; Sheiner *et al.*, 1979). Here the plasma concentration is the driving force for brain concentrations, without uptake into or elimination from the brain influencing the concentration-time profile in blood. Extrapolation of animal PK parameters to the human situation can sometimes be performed reasonably well by allometric scaling, using bodyweight or body surface area as the main determinant of PK parameters.

The physiologically-based pharmacokinetic (PBPK) modeling approach has provided the basis for interspecies extrapolation. It has focused on quantitative modeling of mass transport into and out of physiological compartments and made highly significant contributions to knowledge of the body (system) and the fates of drugs (Rowland *et al*., 2011). It has not, however, taken into account the distinction between the bound and unbound drug. Inclusion of unbound concentrations, however, will provide more accurate information on specifically membrane transport processes and can be named systems-based pharmacokinetic (SBPK) modeling.

Information on species- and/or condition-dependent differences in abundance levels and activities of the different active transport proteins and drug-metabolizing enzymes at the BBB and BCSFB, as well as at the liver and kidney, under healthy or diseased conditions, is essential for extrapolation purposes. With the use of advanced SBPK modeling the contributions of individual mechanisms in animals can be revealed to serve as links to the human situation. Thus, SBPK models integrate drug-specific and system-

specific physiological parameters that vary between species, subjects, or within subjects with different age and/or disease state (Colburn, 1988; Espié *et al.,* 2009; Ings, 1990). However, even though the whole body SBPK approach would provide the best information for prediction, it requires an extensive amount of information to be able to identify the impact on specific parameters, making the whole body SBPK modeling approach highly time-consuming and costly. We therefore chose to limit the SBPK approach to the brain only, with the plasma kinetics to be defined by a simple compartmental modeling approach to determine the input function; the PK exposure of the brain. In the SBPK brain model the data that were produced on (unbound) concentrations in plasma, $brain_{ECF}$, CSF_{LV} and CSF_{CM} from single animals were used to define the time-dependent parameters on exchange between plasma, $brain_{ECF}$ and CSF concentrations between several real brain compartments with their volumes and surfaces, by diffusion, fluid flows, and active transport processes. This was all performed using non-linear mixed-effects modeling using the NONMEM software package. Thereby, also the relationship between $brain_{ECF}$ and CSF concentrations could be determined.

Using the same structural model for all three paradigm compounds, the impact of drug characteristics on brain kinetics and the $brain_{ECF}$-CSF concentration relationship is investigated in a mechanistic manner. This will contribute to the predictability of human brain target site concentrations on the basis of preclinical data.

EXTRAPOLATION TO THE HUMAN SETTING

Given that CSF concentrations are considered to be the best available surrogate for $brain_{ECF}$ concentrations in humans (Fridén *et al.,* 2009; Kalvass and Maurer, 2002; Liu *et al.,* 2006; Liu *et al.,* 2009; Maurer *et al.,* 2005), we focused on predicting human $brain_{ECF}$ concentrations. Thereby human acetaminophen CSF concentrations as presented by Bannwarth *et al.* (1992) were used as a reference in **Chapter 3**. By changing the different values of the physiological parameters of the rat to their corresponding human values, and by fitting the human plasma data to our model while extrapolating the plasma-brain exchange in a systems-based manner, we were able to adequately predict human lumbar CSF

concentrations as observed by Bannwarth *et al.* (1992). For acetaminophen in humans, it was predicted that $brain_{ECF}$ concentrations are on average ~2-fold higher than unbound plasma concentrations, whereas the $brain_{ECF}$-to-CSF (from the subarachnoid space) concentration relationship is highly dependent on the time after dose. Though we do not have data on human acetaminophen $brain_{ECF}$ data, the data as predicted for human CSF lumbar concentrations that are in line with observed lumbar concentrations (Bannwarth *et al.,* 1992) gives confidence in the usefulness of our model.

Next, for quinidine (**Chapter 4**), the inclusion of the influence of P-gp-mediated transport at the blood-brain barriers was taken into account. It was clear that P-gp functionality is an important factor in the relationship between CSF and $brain_{ECF}$ exposure, given the fact that the relative distribution of quinidine over the brain compartments changes with blocking P-gp-mediated transport by co-administration of tariquidar. No data were available on quinidine CSF distribution in human, so at this moment in time this observation cannot be validated for the human situation.

For methotrexate there is quite some clinical data available, including $brain_{ECF}$ concentrations in humans (Blakeley *et al.,* 2009). However, all published human data (children and adults) has been obtained from patients with different disease states. It is therefore not logical to expect proper prediction of diseased human concentrations in different brain compartments on the basis of a preclinical model developed on data obtained in healthy rats. This is because diseases may influence the rate and extent of several processes that govern brain target site concentrations of (also non-) CNS active compounds. Actually, it is of high value to identify disease-specific induced changes in particular PK processes (and therewith PD impact). Assuming proper predictions of human brain concentrations under healthy conditions by the preclinical derived model, deviations of particular brain concentrations in disease conditions may as well be used to identify parameter "suspects" responsible for or contributing to changes in brain compartment concentrations.

In **Chapter 5** we therefore applied the SBPK model on literature data on methotrexate brain distribution, first, to predict data obtained in other healthy rats (plasma and $brain_{ECF}$ data), then, to investigate the impact of disease-status on the PK of methotrexate. By using the same PK parameter values that were estimated based on our data, we were able to predict the methotrexate plasma

and $brain_{ECF}$ concentrations in other healthy rats reasonably well. For earlier reported $brain_{ECF}$ concentrations of methotrexate in brain tumor-bearing rats (De Lange et al., 1995) the predictions by the preclinical brain distribution model were found to be significantly lower, indicating increased distribution of methotrexate at the brain tumor site. The next step was to use our SBPK model to predict plasma and CSF concentrations in healthy dogs. When taking into account that the hepatic elimination of methotrexate in dogs is only a fraction of the renal clearance (Henderson *et al.,* 1965), whereas in rats the hepatic elimination of methotrexate is estimated to be over 5-fold higher than the renal clearance, the predictions of plasma and CSF concentrations were reasonable.

In the case where a disease condition is the variable in a cross-compare designed study, the SBPK brain distribution model can be used in helping to identify which parameters (e.g. the elimination from plasma or the blood-brain transport) are possibly influenced. Furthermore, provided that the SBPK brain distribution model is able to describe the different processes well in healthy conditions, simulations will help in our understanding of the impact of parameter changes in disease conditions. With the assumption that our SBPK brain distribution model can appropriately predict methotrexate brain distribution in healthy humans, this model could be used to identify changes in methotrexate distribution brought about by disease conditions (like for the tumor-bearing rats).

In humans, methotrexate undergoes extensive enterohepatic circulation, effectively reducing the hepatic elimination rate to the same level as the renal elimination rate (Hendel and Brodthagen, 1984). With this information incorporated into the model, the prediction of human unbound methotrexate plasma concentrations is reasonable. However, under the given disease conditions, the $brain_{ECF}$ and CSF concentrations are significantly higher than predicted for healthy conditions. Simulations indicate a possible decreased active efflux from the $brain_{ECF}$ as well as a lower CSF flow could be the cause of these higher $brain_{ECF}$ and CSF concentrations under the given disease conditions. The reduced CSF flow as "suspect" contributor to changed methotrexate brain PK is in line with the observation that several adult patients had an obstruction to normal CSF flow (Glantz *et al.,* 1998).

So, interestingly, apart from blood-brain transport, the CSF flow seems to play an important role in the $brain_{ECF}$-CSF relationship. For acetaminophen and

methotrexate, the CSF acts as a sink, causing the observed lower concentrations in CSF compared to $brain_{ECF}$. As the relative rate of CSF turnover in rats is much higher than in humans, the sink effect in humans could be smaller as compared to that in rats. Then, certain drugs and certain diseases may influence CSF formation. This indicates that CSF turnover should also be considered in the $brain_{ECF}$-CSF relationship.

FUTURE PERSPECTIVES

To be able to predict CNS drug effects in humans on the basis of preclinical data, it is essential to study the underlying processes and mechanisms that govern the ultimate concentration-effect relationship. Therefore, it is of importance to investigate the inter-relationship between plasma PK, BBB and BCSFB transport, intra-brain distribution, target binding, target activation, transduction, homeostatic feedback, and disease processes (Danhof *et al.,* 2007; De Lange, 2013b; De Lange *et al.,* 2005). The current preclinical SBPK brain distribution model is a first step into that direction. It allows the investigation of the relationship between plasma PK, BBB and BCSFB transport and intra-brain distribution, in a systems-specific manner.

By systematically varying one (or a subset) of conditions (such as P-gp functionality), one can decipher the impact of changes on brain distribution in integrative cross-compare designed studies. To that end we also need advanced mathematical modeling procedures to dissect contributions of individual mechanisms, being key to translation from one condition to the other (De Lange, 2013b). The current preclinical SBPK brain distribution model follows that approach, and needs to be further developed/refined by using more data on other drugs with distinct physicochemical properties. By doing so we will be able to pin-point the influence of particular drug properties on the pharmacokinetic brain distribution behavior of drugs. Furthermore, the PK of a drug at different sites in the brain should be connected to (biomarkers of) the effect in order to unravel those concentrations that can be considered as target site concentrations.

Other aspects that need to be included for improving the SBPK brain distribution model are target-mediated drug disposition and target association

and dissociation kinetics. This may cause non-linearity in PK and/or PD, which may complicate the characterization of the PK-PD relationship. When drugs are bound with high affinity and to a significant extent (relative to the dose) to their target sites, the drug can be retained much longer in target rich tissue spaces than expected on the basis of the plasma elimination rate (Levy, 1996; Mager, 2006; Mager and Jusko, 2001). As an example, this may hold for the antipsychotic drugs risperidone and paliperidone with their targets being the dopamine D_2 and serotonin 5-HT_{2A} receptor. For these compounds, information on the regional brain distribution, together with information on the target density as well as the target association and dissociation kinetics provides a better understanding of processes that govern the PK-PD relationship (Johnson *et al.,* 2011; Kozielska *et al.,* 2012).

Thus, apart from blood-brain and intra-brain transport processes, target-mediated disposition adds on to the limited value of plasma PK to predict target site PK and stresses the importance of having additional information on target site PK that actually drives the PK-PD relationship. Since CNS target site concentrations cannot be obtained directly from humans, the aim should be to predict target site concentrations and effects in humans on the best indirect way, such as based on preclinical data.

The value of intracerebral microdialysis in this prediction is clearly exemplified by recent work by Stevens *et al.* (2012). They have shown that the effect of remoxipride, a dopamine D_2/D_3-receptor antagonist, on prolactin concentrations in plasma could be directly linked to remoxipride $brain_{ECF}$ concentrations as measured by microdialysis in the rat. To that end, human $brain_{ECF}$ remoxipride concentrations were predicted by allometric and physiological scaling of the rat data, which were then used to predict human plasma prolactin concentrations by applying the same structural PK-PD model as was developed on the basis of the rat data. The predicted human plasma prolactin concentrations show a great similarity to clinically observed plasma prolactin concentrations, indicating that advanced PK-PD modeling of preclinical data allows the prediction of drug effects in humans.

Further development of the preclinical SBPK brain distribution-effect model lies in improvement of the quality of the CNS effect data. Often, the focus has been on a single biomarker to reflect the CNS drug effect. However, given the complexity of brain diseases, it can be seen that the search for a single

biomarker to explain the disease relative to the healthy condition, and/or changes in the disease condition by (drug) treatment will never lead to a success. Actually we do not deal with "the" effect, but a composite of effects. The search should therefore be on "fingerprints" of multiple biomarkers, in a time-dependent manner, for investigations on the "effect spectrum". With metabolomics as an emerging scientific tool, many more compounds in brain fluids and in plasma can be measured in parallel, in a quantitative and time-dependent manner. Furthermore, the emphasis should lie on measures that can be obtained both preclinically and clinically, to enhance translational insights and therewith predictive power of preclinically obtained information (De Lange, 2014).

In conclusion, the future perspective is that by combining drug-specific and system-specific information on brain target site distribution with mechanistic information on the concentration-effect spectrum relationship (as they vary in between species, between subjects, or within subjects with age and/or disease state) will ultimately result in a systems-based PK-PD model that is anticipated to be able to predict human CNS drug effect on the basis of preclinical PK data (figure 1).

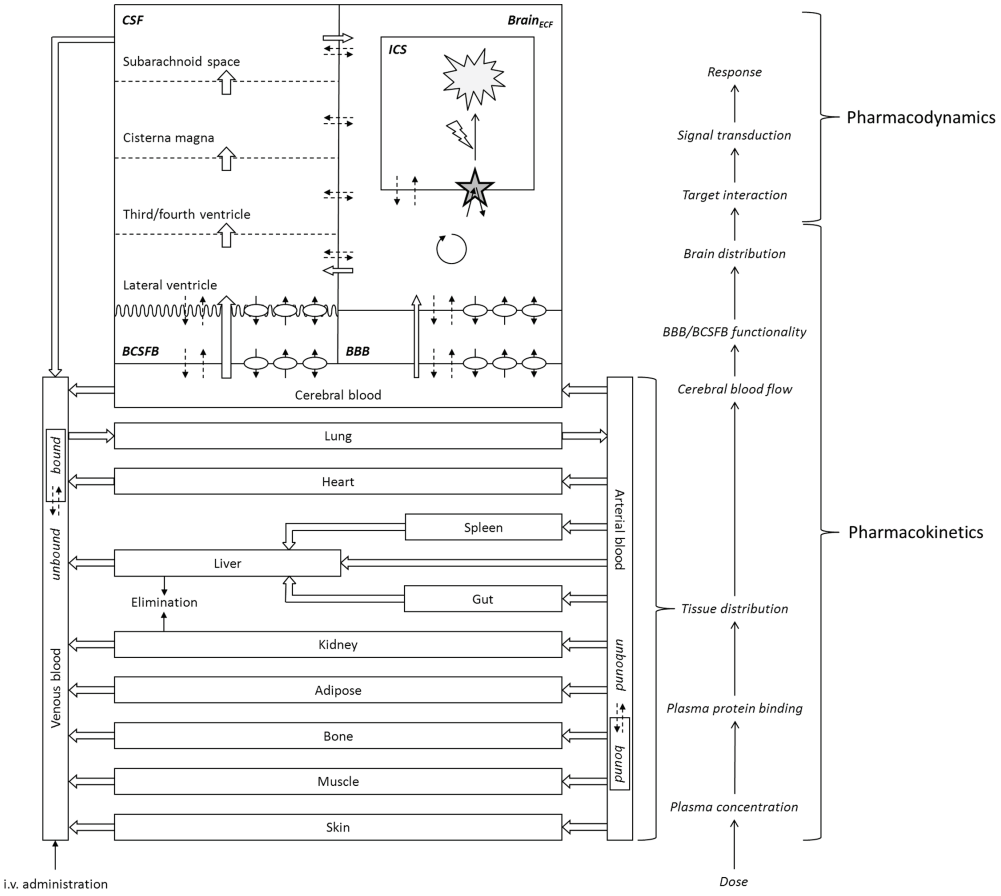


Figure 1. Schematic representation of a systems-based PK-PD model. On the right several underlying processes or mechanisms that are important for the pharmacokinetics and pharmacodynamics of a (unbound) drug are highlighted

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