The LeiCNS-PK3.0 model development and applications: healthy-to-diseased CNS pharmacokinetic translation

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Chapter 9

The LeiCNS-PK3.0 model development and applications: Healthy-to-diseased CNS pharmacokinetic translation

General discussion and future perspectives

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Accurate prediction of the drug concentration-effect relationships in humans is a key step that can optimize and accelerate CNS drugs development [1]. A prerequisite is the evaluation of the unbound pharmacokinetic (PK) profiles of the CNS target sites, mainly at the brain extracellular ($brain_{ECF}$) and intracellular ($brain_{ICF}$) fluids [2, 3]. However, the evaluation of human brain unbound PK profiles is limited by ethical restrictions of human brain sampling and inaccuracy of the spinal cerebrospinal fluid (CSF) as a surrogate of the brain drug concentrations [4–7]. Direct translation of the (relatively) rich brain PK data of animals towards humans is hampered by the different CNS physiology [8–12]. Physiologically-based pharmacokinetic (PBPK) models has been applied to predict the PK profiles of the body organs and tissues [13], including those of the brain and the CSF [6, 14, 15]. PBPK models account mechanistically for the species physiology and thus allow the translation of the PK profiles between species.

A comprehensive CNS PBPK model has been developed within our group [14, 16], which could predict the PK profiles of small molecule drugs in multiple physiological CNS compartments based on the CNS physiological parameters and the drug physicochemical properties. The model demonstrated adequate predictions of the in vivo PK profiles of the $brain_{ECF}$ and the CSF of rats and humans. However, the model's unexplicit representation of the brain tissue non-specific binding and the pH-related drug ionization indicated the need for model improvement. In addition, the model's parameters reflect the healthy CNS physiology. Predictions of the CNS PK profiles during maturation, aging, and for CNS diseases would require the adaptation of disease- and condition-dependent changes of CNS physiology.

The main goals of this thesis were, first, to improve the healthy comprehensive CNS PBPK model, second, to explore the potential alteration of the brain and CSF PK profiles under different physiological and diseased states of the CNS, and third to provide examples on how CNS PBPK models can potentially support CNS drug development. The main thesis outcomes are:

a) The development of the LeiCNS-PK3.0 model for humans, rats, and mice, that includes the improved model description of non-specific binding and compartment-specific pH impact on drug ionization
b) The human brain and CSF PK profiles are dependent on the CNS physiological state, which applies also to brain-to-CSF PK ratio
c) Prediction of brain target site PK profiles is critical for applications in CNS drug development

In this chapter, first, the main thesis outcomes are elaborated; second, the use of scarce data to optimize PBPK model development and validation is discussed, and finally, suggestions of the future research directions are proposed.

A) Development of the LeiCNS-PK3.0 model of humans, rats, and mice

We described in **section II** the improvement of the comprehensive CNS PBPK model on the aspects of, first, brain tissue non-specific binding, where the brain cell membrane compartment has been added and, second, the impact of compartment-specific pH on drug ionization and distribution in the multiple CNS compartments. The importance of accounting for the different pH values of the CNS brain compartments is depicted in figure 1, as simulated with the LeiCNS-PK3.0 model.

In **chapter 3**, we reported development of the LeiCNS-PK3.0 model of humans and rats, which could predict adequately the in vivo PK profiles at the $brain_{ECF}$ and the CSF compartments of rats and humans. In addition, a framework was described on the translation of CNS PK profiles from rats to humans. The human LeiCNS-PK3.0 model is available as a web-based application at https://cns-pbpk.shinyapps.io/AD-SHINYAPP/. In **chapter 4**, we reported the development of the first mouse LeiCNS-PK3.0 model version, which is, to the best of our knowledge, the only PBPK model to adequately predict the mouse $brain_{ECF}$ PK profiles of small molecule drugs.

B) The brain and CSF PK profiles are dependent on the CNS physiological state

The brain and CSF tissues undergo pathophysiological changes during CNS diseases, which can potentially impact the CNS PK properties. While the CNS pathophysiological changes of CNS diseases have been addressed extensively in literature, their effect on the brain and CSF PK profiles has not been proportionally assessed [17]. In **section III**, the LeiCNS-PK3.0 model was used to explore the impact of altered CNS physiology on brain and CSF PK profiles and to predict the CNS PK profiles in diseased populations.

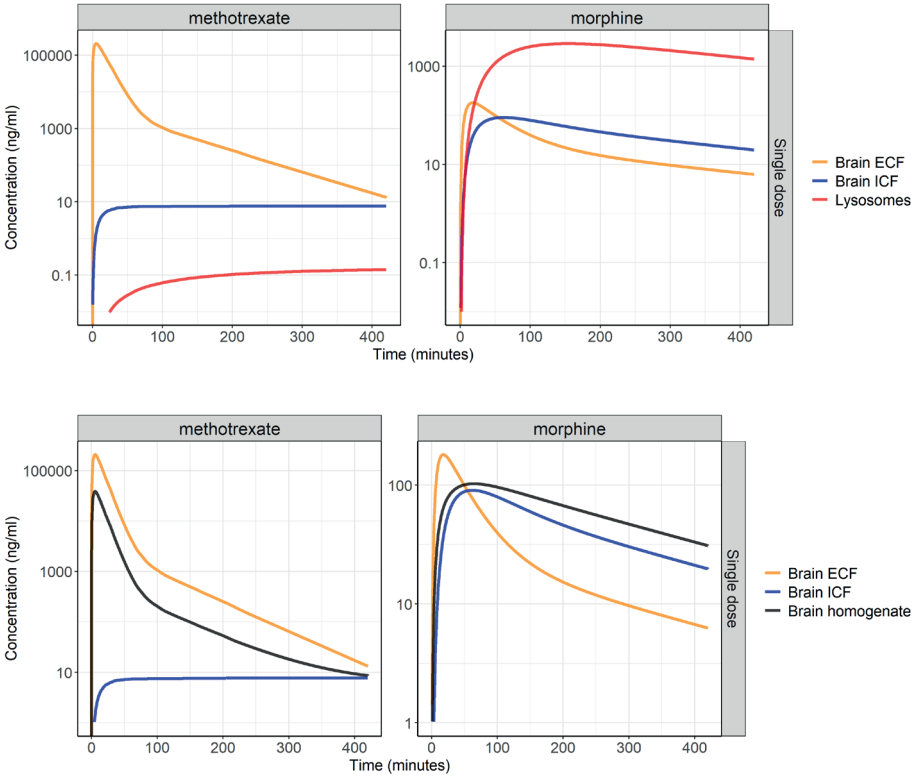

***Figure 1**. **LeiCNS-PK3.0 simulations of the unbound brain PK profiles of morphine (basic drug, pKb = 9.1) and of methotrexate (acidic drug, pKa = 3.4) across the mouse brain compartments.** a) Basic drugs will accumulate in the lysosomal acidic environment (pH = 5.0) more than in the relatively basic environment of the $brain_{ECF}$ and $brain_{ICF}$ (pH = 7.3 and 7.0, respectively) and vice versa for acidic drugs. b) The concentrations of these charged drugs when measured in brain homogenates (i.e. compartments distinction is lost) do not accurately reflect the $brain_{ECF}$ or $brain_{ICF}$ PK profiles. It is thus essential to assess and account for the physiological differences (such as pH) of the CNS compartments to distinguish their PK profiles.*

In **chapter 5**, we explored the impact of altered pH_{ICF}, pH_{ECF}, $brain_{ECF}$ volume, cerebral blood flow, and effective pore radius of the paracellular transport route ($para_{radius}$) on $brain_{ECF}$ and $brain_{ICF}$ PK profiles in a what-if simulation study using the LeiCNS-PK3.0 model. The role of these BBB passive paracellular transport and intra-brain distribution parameters on the CNS PK profiles is rarely addressed, in comparison to the role of BBB active transporters. In our what-if simulations study, the LeiCNS-PK3.0 model showed that altered cerebral blood flow and $brain_{ECF}$ volume have no effect on the $brain_{ECF}$ and $brain_{ICF}$ PK profiles. Changes of pH_{ICF} and pH_{ECF} alter the $brain_{ECF}$ and $brain_{ICF}$ PK profiles of charged drugs, i.e. those with acid and base ionization constants <7 and >7, respectively.

Changes of $para_{radius}$ can also alter the $brain_{ECF}$ PK profiles depending on the drug's charge, lipophilicity, and active transporters affinity. Changes of the latter three parameters, while often overlooked, can result in altered CNS pharmacokinetics. For example, traumatic brain injury (TBI) is accompanied with $brain_{ECF}$ acidosis and $para_{radius}$ widening and might explain, besides the reduction of active transporters expression, the increase of morphine concentrations in the $brain_{ECF}$ of TBI patients [14, 18, 19].

The high attrition rate of Alzheimer's disease drugs in clinical development has been attributed in part to the poor drug exposure and to PK fluctuations at the brain target sites [20–22]. Surprisingly, the $brain_{ECF}$ PK profiles of Alzheimer's disease patients has seldomly (if ever) been studied [17], although the $brain_{ECF}$ is the target site of the marketed Alzheimer's disease drugs such as memantine, donepezil, galantamine, and rivastigmine. In fact, no data could be found on the brain drug concentrations of Alzheimer's disease patients in literature and in clinical databases. The brain physiology of Alzheimer's disease patients is quite distinct compared to that of cognitively healthy adults and elderly, which implies a potentially altered brain PK profiles of Alzheimer's disease patients. In **chapter 6**, we studied the impact of Alzheimer's disease and of healthy aging on the CNS pharmacokinetics. The human LeiCNS-PK3.0 model was translated to represent Alzheimer's disease and healthy aging by replacing the model's healthy parameters with those of mild Alzheimer's disease patients and of cognitively healthy elderly; both parameter sets were identified using an extensive literature study. The LeiCNS-PK3.0 model simulations demonstrated a minimal to no impact of Alzheimer's disease and of healthy aging on $brain_{ECF}$, $brain_{ICF}$, and lumbar CSF PK profiles of the four drugs. An additional finding is that the PK fluctuations, observed during multiple dosing, can differ between the CNS compartments. PK fluctuations has been hypothesized previously to be the reason of failure of the development of semagacestat against Alzheimer's disease [22].

The brain tumor PK profiles have been shown to differ from that of the healthy brain depending on the drug, tumor type, and the extent of tumor pathophysiological changes, for example the extent of BBB breakdown [23–26]. It is thus important to account for the tumor pathophysiology to accurately assess the brain tumor PK profiles. The pathophysiological phenotypes of the brain tumors are, however, not adequately characterized, which prevents the direct prediction of the impact of the tumor-specific pathophysiology on its PK profiles. In **chapter 7**, the tumor LeiCNS-PK3.0 model was developed to predict the malignant CNS pharmacokinetics. To that end, a combined data- and knowledge-driven

approach was applied to predict the PK profiles of several anti-tumor drugs in healthy and malignant brain tissues. The values of cerebral blood flow, brain microvascular volume, $brain_{ECF}$ volume, and $pH_{ECF/ICF}$ in different tumor models were collected from literature. Information on pathophysiological changes of $para_{radius}$ and active transporters in the different brain tumors were, however, unavailable. The tumor model specific values of these two parameters were estimated from the available in vivo PK data of the healthy and malignant brain tissue and showed tumor and drug dependency. With our approach, we could adequately predict the $brain_{ECF}$ PK profiles of methotrexate, temozolomide, ganciclovir, gemcitabine, and letrozole in rat brain glioma, of methotrexate in rat rhabdomyosarcoma, and of methotrexate in human brain glioma. Thus, combining (patho)physiological knowledge and PK data allowed the estimation of unknown LeiCNS-PK3.0 parameters and in turn the prediction of tumor PK profiles. This approach is useful to explore the target site PK profiles of brain tumors, particularly in the absence of enough information on tumor pathophysiological changes [27].

Altogether, findings of **section III** highlight the importance of accounting comprehensively for the CNS physiological differences for adequate translation of the brain and CSF PK profiles across the human populations.

C) Brain target site PK predictions are critical for CNS drug development applications

The unbound drug concentrations at CNS target sites in the $brain_{ECF}$ and $brain_{ICF}$ drive the drug effect and thus are most relevant to model the concentration-effect relationship of CNS drugs [3]. Nevertheless, the assessment of the brain unbound PK profile is often based on a single lumbar CSF sample, under the assumption that it is an accurate surrogate of the $brain_{ECF}$ and $brain_{ICF}$ PK profiles [12, 28, 29]. The LeiCNS-PK3.0 model simulations in **chapters 3** and **6** provided evidence against this argument, in line with previous research [4–6]. In **chapter 3**, simulations of the human LeiCNS-PK3.0 model showed that altered CSF volume and flow, as often reported in CNS diseases, result in altered lumbar CSF PK profile, with no impact on that of the $brain_{ECF}$. This implies that lumbar CSF-to-$brain_{ECF}$ drug concentration ratio is dependent on the physiological context and is sensitive to alterations of the CSF volume and/or flow. Thus, the $brain_{ECF}$ to lumbar CSF PK ratio should not be used for translation between populations, particularly in the context of diseases involving a change of CSF dynamics.

In **chapter 6**, the LeiCNS-PK3.0 model simulations showed that memantine concentrations are lower in the human lumbar CSF than in the human $brain_{ECF}$. These simulations explained the unexpected findings of a clinical trial that assessed the concentration-effect relationship of the NMDA receptor antagonist memantine in Alzheimer's patients [28]. In the clinical trial, memantine concentrations measured in the lumbar CSF were unexpectedly below the in vitro IC_{50} value of NMDA receptors, which did not rationalize the pharmacological effect observed in the study participants [28]. However, the human $brain_{ECF}$ PK profile of memantine, when predicted with the LeiCNS-PK3.0 model, was indeed above the in vitro IC_{50}, which explains the memantine pharmacological effect observed in the patients. Memantine is a substrate of an unidentified proton coupled organic cationic transporter [30, 31]. Organic cationic transporters such as OCTs and OCTNs function as uptake transporters at the BBB [32, 33] but as an efflux transporter at the BCSFB [33]. Such transporter activity might explain the difference of memantine concentrations between the $brain_{ECF}$ and the lumbar CSF.

Overall, our findings stress the importance of the assessment of $brain_{ECF}$ and $brain_{ICF}$ PK profiles, particularly in relation to measures of drug effect. In addition, they do not support the use of the drug concentration of the lumbar CSF to predict that of the brain.

Should, however, drug developers stop sampling the lumbar CSF of patients and use LeiCNS-PK3.0 predictions instead? The answer is *"not yet"*, as these clinical PK measurements provide important information on the drug penetration into the CNS and are essential to establish the confidence in the models' predictions [34]. The LeiCNS-PK3.0 model and other PBPK models predictions cannot yet replace PK outcomes of the preclinical and clinical studies. They rather provide a framework to integrate multilevel data from physiology, drug physicochemical, in vitro, in vivo, and (when available) clinical information to predict as accurately as possible the target tissue PK profile.

Importantly, PK prediction of the relevant CNS target site(s) using the LeiCNS-PK3.0 model could be used to prioritize the drug candidates with favorable CNS pharmacokinetics, which can potentially optimize CNS drug development [35]. This proposal was explored in **chapter 8** using COVID-19 as an example. SARS-CoV-2, while primarily a respiratory virus, has been shown to also infect and persist in the human brain cells, implying the need to treat the viral load in the $brain_{ICF}$. To that end, the LeiCNS-PK3.0 model was used to select the COVID-19

drug(s) with adequate $brain_{ECF}$ and $brain_{ICF}$ exposure. The predicted $brain_{ECF}$ and $brain_{ICF}$ PK profiles of nirmatrelvir, remdesivir, and molnupiravir were compared against the in vitro EC_{90} of the delta and omicron variants. Our simulations showed that nirmatrelvir is more likely to achieve effective concentrations in the human brain. The $brain_{ECF}$ concentrations of remdesivir and EIDD1931 and the $brain_{ICF}$ of their active metabolites, GS443902 and EIDD2061 respectively, were below EC_{90}. Thus, based on the LeiCNS-PK3.0 model simulations, nirmatrelvir is a promising candidate for future (pre)clinical studies investigating the CNS efficacy of the COVID-19 drugs.

Data scarcity as a challenge in PBPK modelling

The development and validation of PBPK models require extensive physiological information and in vivo PK data, respectively. The unavailability of both data types is one of the major challenges of PBPK modeling that reduces the confidence in PBPK models outcomes and thus limits their applications in drug development [36–38]. The available scarce physiological and PK data should, however, be used to optimize the development and validation of PBPK models. The first point of this section focuses on the value of combining PBPK models and in vivo PK data to improve the predictive quality of the models. The second point provides a brief perspective on PBPK models validation and on the use of in vitro and in vivo PK data to increase the credibility of PBPK models.

The value of integrating PBPK models and in vivo PK data

PBPK models account for the physiological processes in a mechanism-based manner and thus can predict beyond and independent of the in vivo PK datasets. The mechanistic structure of PBPK models require extensive information on the system's physiological processes and parameter values, on the drug physicochemical properties, on the drug-systems (i.e. biological) properties, and on complex in vitro-in vivo extrapolates; the values of which are often unavailable. The unavailable information might limit the model's PK predictions that are based merely on the physiological knowledge. For example, the limited knowledge on the active transport processes at the human BBB does not support their mechanistic inclusion in PBPK models and consequently, the $brain_{ECF}$ PK profile of active transporters substrates cannot be accurately predicted.

It is often debated whether PBPK models predictions should be based only on physiological and drug parameters or account in addition for the available in

vivo PK data. Models are, however, developed as fit for purpose tools to address drug development questions and thus, PBPK modelers should not refrain from the use of in vivo PK data, where available, for more accurate PK predictions. PK data, in this case, can be used to estimate uncertain physiological parameters (**chapter 7**, handshake approach) or to act as a pivot for accurate human PK predictions **(chapters 3)**. Notwithstanding, in vivo PK data should not be an essential input to the PBPK model's predictions, as in this case, the PBPK model might lose its main value, i.e. predicting the unmeasurable PK data.

An example on the value of integrating in vivo PK data and PBPK models is presented in **chapter 3**. Morphine is a P-gp transporter substrate; the information on P-gp's capacity and rate at the human BBB are unavailable. To predict the human brain$_{ECF}$ PK profiles of morphine, the LeiCNS-PK3.0 model was combined with morphine PK data measured at the rat brain$_{ECF}$ and with physiological and in vitro information on rat-to-human differences of P-gp expression and capacity at the BBB, respectively. By using the preclinical PK data as a pivot, the LeiCNS-PK3.0 model could predict the morphine PK profiles of the human brain$_{ECF}$ with less than two-fold error.

Integrating PBPK models and PK data is useful to address the (patho) physiological information gap. Physiological parameters estimated with this approach can be used to predict the PK profiles of other drugs, where the PK data are unavailable [27] or can be translated, with the necessary corrections, to other species or populations **(chapters 3 and 6)**. Two challenges against accurate estimation of physiological parameters from the scarce PK data are the data's (potential) heterogeneity and parameter identifiability issues. PK data heterogeneity (e.g. from different physiological states, animal species, drugs, etc.) might not allow the estimation of the "true" physiological parameter value. Parameter identifiability issue arises when the uncertain parameters cannot be uniquely estimated from the available experimental data, i.e. an infinite set of parameter values result in the same prediction [39]. Both can however be minimized by assessing the biological plausibility of the estimated parameter value and reducing the number of estimated parameters based on a sensitivity analysis [40, 41].

Validation of predictive PBPK models

A major stumbling block that prevents the broader application of PBPK modeling in drug development is that the model predictions cannot be validated against (the often unmeasurable) human PK data [37]. This understanding of PBPK

model validation stems from confusing the traditional, empirical population pharmacokinetic-pharmacodynamic models, which describe a certain PK dataset but with limited capacity to extrapolate beyond it, and the mechanistic PBPK models, which are developed to predict unmeasurable scenarios that are beyond the dataset [40]. Thus, PBPK models predictions will be rejected because of the challenge they were developed to tackle in the first place, i.e. the unmeasurable PK data (figure 2) [42]. Wrong and unvalidated PBPK models are still useful (at the least) to identify wrong PK hypotheses and physiological knowledge gaps. Notwithstanding, the validation of a PBPK model is a prerequisite to its application in drug development [43]. Box 1 and the next paragraph provide a perspective on how to approach the validation of PBPK models.

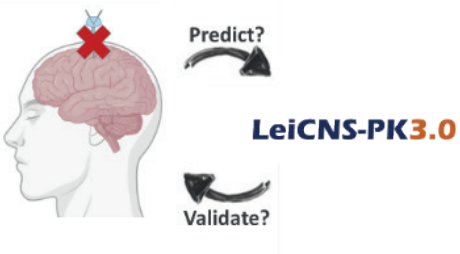

Figure 2. The PBPK modeling dilemma: should we accept or reject PBPK models that are not fully validated? *PBPK models are developed to predict PK profiles and scenarios, where no PK data can be measured for time or technical constraints. However, PBPK predictions are often not widely accepted, since the predictions are not validated with the experimental PK data that are unmeasurable and for that the model was developed in the first place. PBPK model's predictions should be interpreted in the context of the model's goal, development, and validation. The usefulness of PBPK models should not be determined only based on the aspect of the "line going through the data".*

In general, planning the validation process should precede any model development activities [34, 44] and include explicit specification of the amount and type of PK data required to validate the model. The amount and type of the PK data should be determined based on the intended modeling goal, context of model use, and the model's position in the clinical development process [43]. PBPK models validation should not be limited to comparing the model's predictions to measured concentration-time profiles but should also evaluate the aspects and assumptions of the model against the available experimental information. For example, unbound PK profiles measured in animals can be useful to validate the animal versions of a PBPK model, which would provide confidence in the structure and related assumptions of the human model version, if the physiological structure

and assumptions are preserved across species [8–10, 14, 16, 45]. In vitro PK parameters such as unbound fraction and unbound volume of distribution can also be used to validate the model's structure and assumption. It is also important to evaluate the suitability of the PK data to the validation context. For instance, PK data sampled in disease animal models might bias the validation process if the animal model does not reflect the full disease pathophysiological phenotype [46]. In summary, the validation process of PBPK models is crucial for the acceptance of their outcomes but should not limit their broader applications. The process should not be restricted to evaluating how "the line goes through the data" but also evaluate the model's structural aspects.

Box 1. Lessons learned on PBPK model validation

- Plan the validation early, before model development
- Validate the model with different data types
- Evaluate the suitability of PK data to the model validation
- Select the right evaluation tool, e.g. error measure
- Wrong/unvalidated models cannot inform drug development…
- …but are useful to identify knowledge misconceptions and gaps

Future directions of the LeiCNS-PK3.0 model

Perspective on how the LeiCNS-PK3.0 model can support drug development and treatment

We believe that the LeiCNS-PK3.0 model should be regarded as an indispensable component of the different stages of CNS drug development programs. The model could optimize the early stages of CNS drug development by distinguishing drug candidates with potentially (un)favorable brain PK profile, as described in **chapter 8**. Exploring hypothetical scenarios with the model prior to the actual experiments can result in better choice and planning of preclinical studies, eventually refining and reducing, if not replacing, animal experimentation. In the early clinical development, the model can support the determination of the first-in-human doses and the refinement of dosing regimens. The model can also be used in the later clinical development stages to assess the need for dose optimization for the different CNS patient populations and the impact of potential drug-drug interactions at the BBB on the CNS pharmacokinetics. The model can provide a mechanism-based interpretation of clinical trials findings and an evaluation of controversial hypotheses, as explained in **chapters 3 and 6** on the inadequacy of lumbar CSF to brain surrogacy and the memantine clinical trial findings, respectively. Our model can be combined with a pharmacodynamic model to link the predicted target site concentration to the observed drug effect.

Box 2. Applications of the LeiCNS-PK3.0 model

- Prediction of the CNS target site PK profiles
- Interspecies and interpopulation translation of CNS PK profiles
- Exploring hypothetical, what-if scenarios
- Mechanistic interpretation of clinical trials findings
- Assess the potential of drug-drug interactions at the BBB

Regional brain PK profiles

A potential improvement of the LeiCNS-PK3.0 model is to address the PK profiles of the various brain regions, which might differ depending on the brain region's physiology [47, 48]. For example, the regional difference of lipid composition of brain tissue [49, 50] and of active transporters expression at the BBB [51] have resulted in regional differences of brain tissue non-specific binding [52] and drug partitioning across the BBB as measured with the $Kp_{uu,BBB}$ [53], respectively. Recently, an adaptation of our group's CNS PBPK model [16] has been published [47], which accounts for the regional differences of brain non-specific binding and predicts the brain PK profiles of the brain cortex, basal ganglia, thalamus, and the rest of brain.

Mechanistic inclusion of active transport and metabolism

The LeiCNS-PK3.0 model can also be improved to account for other physiological processes such as active transport and metabolism. Description of the BBB active transporters in the model is one of our top priorities, the implementation of which is however challenged by, first, the incomplete understanding of the active transport process, for example the interplay of different transporters and, second, the large variability of the in vitro measures and the transporter expression levels.

Extension to large drugs: antibodies and cells

An aspiring future direction is to develop a LeiCNS model to predict the kinetics of biologics, such as antibodies and cell therapy. A CNS PBPK model has been developed that could predict the antibody kinetics in rats and translated to mice, monkey, and humans [54]. This model used the structure of our group's comprehensive CNS PBPK model [16] and included the antibody transport mechanisms across the BBB and BCSFB. In addition, a whole-body PBPK has been developed that predict the CAR-T cell kinetics in the whole body, including in the brain, but did not however account for the CNS compartments, which would be necessary to support the assessment of CAR-T cells exposure in the brain following intravascular versus locoregional delivery [55].

LeiCNS-PK3.0 Shinyapp

We have developed and published a Shinyapp of the LeiCNS-PK3.0 model **(chapter 6)**, with the goal of promoting the use of our model. Shinyapps are web-based applications with a user-friendly interface that allow the performance of model simulations without prior modeling or coding knowledge. The current Shinyapp (https://cns-pbpk.shinyapps.io/AD-SHINYAPP/) is the beta version of the application and allows limited functionalities of the model. An updated version is currently under development with full access to all model functionalities, which will allow interested scientists from academia and industry to use the model as a component of their drug development plan.

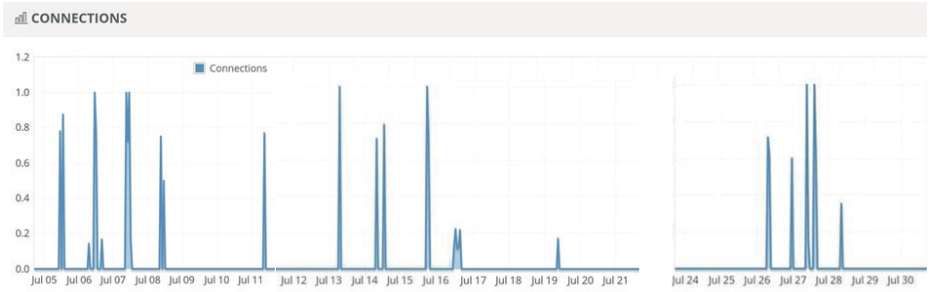

Figure 3. LeiCNS-PK3.0 Shinyapp log records in July, demonstrating a lot of interest in the Beta version of the application. *Usage data on July 22nd and 23rd were unavailable. For hands-on experience with LeiCNS-PK3.0, please follow the link to the app (https://cns-pbpk.shinyapps.io/AD-SHINYAPP/).*

Conclusion

We have developed the LeiCNS-PK3.0 CNS PBPK model, which includes a brain cell membrane compartment to address the brain tissue non-specific binding. In addition, the model accounted for the compartment-specific pH effect on drug ionization and distribution. The LeiCNS-PK3.0 model demonstrated adequate prediction, within two-fold errors of the $brain_{ECF}$ of rats, mice, and humans and of multiple CSF compartments of healthy rats and humans. We have shown, based on our model simulations, the importance of PK assessment of the CNS target site(s) in the $brain_{ECF}$ and $brain_{ICF}$ and that lumbar CSF PK profiles are not accurate surrogates of those of the brain. Furthermore, we highlighted the importance of accounting comprehensively for the changes of the CNS physiology during healthy aging and in CNS diseases for accurate predictions of the brain and CSF PK profiles. Altogether, our work highlighted key lessons on CNS pharmacokinetics that are potentially relevant to streamline CNS drug research and development.

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