Advancing the LeiCNS-PK3.0 model for prediction of CNS pharmacokinetics: nonlinear BBB transport, inter-species scaling, and machine learning

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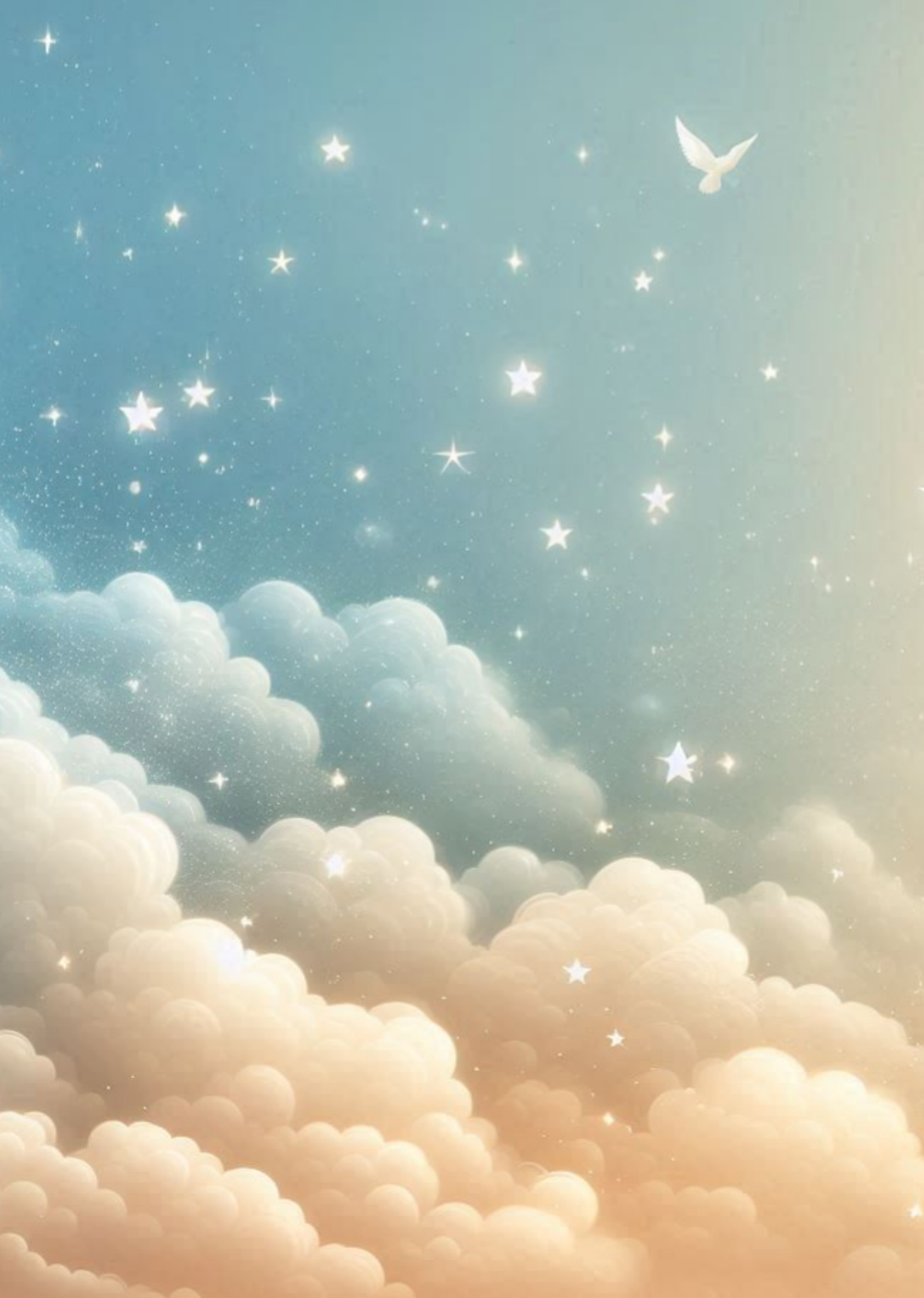

Chapter 8

General discussion and future perspectives

Predicting central nervous system (CNS) drug exposure is essential to further optimize analgesic drug therapies. After administration, drugs distribute into the blood circulation, after which distribution across the blood-brain barrier (BBB) and target sites within the CNS can occur, followed by subsequent drug-receptor binding associated with therapeutic effects. The rate and extent [1] at which these processes occur is both dependent on drug- and biological system (i.e., organism) specific properties. Physiological-based pharmacokinetic (PBPK) modeling offers a powerful strategy to address this challenge and predict CNS drug exposure. In this context, the LeiCNS-PK3.0 model [2] was developed to predict PK profiles of small drugs in the human and rat brain and cerebrospinal fluid compartments. In this thesis we build on the LeiCNS-PK3.0 model in order to enhance the prediction of CNS drug exposure, with a primary focus on applications towards analgesic therapies. We studied the impact of saturable nonlinear blood-brain barrier (BBB) transport and its effects on receptor occupancy, as well as the potential role of P-gp efflux transporter mediated drug-drug interactions. Furthermore, we expanded the translational capabilities of the model beyond rats and humans towards mice. Finally, we evaluated the utility in applying machine learning in combination with the LeiCNS-PK3.0 model to improve prediction of CNS drug exposure.

Nonlinear transport morphine transport – brain$_{ECF}$ exposure

In **Chapter 2**, we studied how nonlinear transport of morphine across the BBB could affect relative differences in brain$_{ECF}$ exposure of morphine and its metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). For BBB transport a linear relationship between plasma and brain PK is often assumed. For morphine, however, BBB transport is influenced by active transport mechanisms that include efflux transport by P-glycoprotein (P-gp) [3–5] and probenecid sensitive transporters [6], as well as a yet unidentified saturable influx transporter [4, 7]. Together these transporters can lead to nonlinear transport of morphine across the BBB, dependent on the plasma concentration present. The impact of this nonlinear BBB transport of morphine on human brain$_{ECF}$ exposure and its relation to metabolites has not been studied before. To this end we implemented the nonlinear BBB transport process in the LeiCNS-PK3.0 model, relating the plasma concentration dependent unbound partition coefficient of the BBB ($K_{p,uu,BBB}$) to the unbound steady state concentration in plasma ($C_{u,SS,plasma}$). We found that on a relative scale, increased morphine brain$_{ECF}$ exposures occur at lower, clinically relevant doses [8], whilst this effect was reduced at higher morphine doses. The impact of nonlinear BBB transport of morphine at lower dosing regimens is of relevance, given the interest in novel combination drug therapies for chronic pain based on the combination of opioids with other CNS-active drugs [9]. Moreover, as the route of administration can lead to differences in relative concentrations of morphine, M3G and M6G, nonlinear BBB transport of morphine further influences the relative concentrations of these compounds at the CNS targets sites, and thereby the CNS effects. This highlights a potential opportunity for optimization of dosing strategies that can optimize treatment effects. More generally, the approach described in this chapter is of relevance to support and demonstrate how consideration of nonlinear BBB transport processes for CNS drug distribution may be inform optimization of dosing schedules of opioids as well as other CNS-targeting agents with enhanced efficacy or improved safety profiles. Here, the use of PBPK approaches are of particular relevance due to their utility in making predictions towards special patient populations such as pediatric patients.

Nonlinear transport morphine transport – $brain_{ECF}$ binding kinetics

In **Chapter 3**, we used the LeiCNS-PK3.0 model to evaluate the impact of nonlinear BBB transport differences of morphine on μ-opioid receptor occupancy for morphine and its metabolites M3G and M6G. Both morphine and its metabolites bind to μ-opioid receptors, which are predominantly present on cells present in the $brain_{ECF}$ and CSF_{SAS} and responsible for the analgesic effects of morphine [10–12]. While morphine and M6G have a high binding affinity for the μ-opioid receptor, M3G has lower binding affinity and is even debated to contribute to hyperalgesia [13–17]. It has been previously proposed that receptor binding by morphine and its metabolites may influence the balance between pro- and antinociceptive effects. To further quantitatively study this question, the extended LeiCNS-PK3.0 model implemented in **Chapter 2**, was further extended by incorporating a binding kinetics (BK) model for assessing μ-opioid receptor occupancy, while accounting for μ-opioid receptor expression differences across the CNS pain matrix regions. We found that nonlinear BBB transport of morphine results in different relative μ-opioid receptor occupancies of morphine and its metabolites, in a dose and CNS region dependent manner. In most of the CNS pain matrix regions such as cerebral cortex, thalamus, midbrain, pons and medulla oblongata except the subarachnoid space (CSF_{SAS}), morphine μ-opioid receptor occupancy was increased at lower doses, while at higher doses, μ-opioid occupancy was increased for M6G. M3G, however, exhibited relatively low receptor occupancy across all doses. Finally, we found also the route of administration to be of importance. Although predictions in this chapter require further validation, the results offer possible insights into potential sources for interindividual variability in responses to morphine treatment. When comparing plasma PK, CNS PK and μ-opioid receptor occupancy of morphine and its metabolites, our findings indicate that plasma PK profiles may not be optimal predictors of CNS μ-opioid receptor occupancy. Our integration of a PBPK and BK modeling approach represents a first key step towards further prediction of μ-opioid receptor mediated drug effects. Ultimately, the developed PBPK-BK model framework can be integrated with incorporation of cellular signaling pathways modulated through receptor activation using quantitative systems pharmacology (QSP) approaches [18], which will enable opportunities for optimizing translational drug development and dosing strategies.

Drug-drug interaction at the BBB

In **Chapter 4**, we investigate the potential impact of P-gp-mediated drug-drug interactions (DDIs) at the BBB on morphine $brain_{ECF}$ exposure using the LeiCNS-PK3.0 model. P-gp is one of the key transporter proteins involved in the transport of morphine across the BBB [19, 20]. As such, we hypothesized co-administration of other drugs that may inhibit P-gp functionality might lead to DDIs with regard to P-gp mediated BBB transport of morphine, leading to an increase in morphine exposure in $brain_{ECF}$, and therefore improving drug penetration into the CNS. This could in turn mitigate the need for higher systemic doses of morphine which are associated with peripheral side effects [21, 22], thereby improving the overall safety and efficacy of the treatment, particularly in DDI scenarios where P-gp inhibitors are co-administered. In humans, positron emission tomography (PET) studies have shown that brain distribution of P-gp substrate drugs like verapamil and loperamide are increased when P-gp is inhibited by agents like tariquidar, cyclosporin-A or quinidine [23–26]. This indicates a potential impact of DDIs on P-gp-mediated transport at the BBB in humans, however, the clinical effect of such DDIs on morphine $brain_{ECF}$ exposure remains unclear. In this study, we demonstrated how the

LeiCNS-PK3.0 model in **Chapter 2**, extended with nonlinear BBB transport of morphine, can be used as platform model to systematically evaluate P-gp mediated DDIs at the BBB, through integration with inhibitory constants. A comprehensive analysis evaluating a larger number of clinically relevant CNS drugs was evaluated for their potential role in CNS-associated DDIs. Our findings indicated that, at clinically relevant intravenous doses of morphine, the impact of P-gp-mediated DDI on $brain_{ECF}$ exposure in human is minimal. However, in this study only P-gp inhibition was included, while morphine is transported by other BBB transporters as well. As a next step, inclusion of additional transporters in the analysis could affect our current conclusions. More generally, this study provided a relevant modelling framework to evaluate the effects of P-gp and other efflux transporters at the BBB, including their influence on drug exposure in the brain. The model framework can be readily expanded by including additional active BBB transporters. As such, it provides the flexibility to integrate DDIs at the level of the BBB, with intestinal, hepatic and renal transporter mediated DDIs that have shown to influence plasma PK, PD and toxicity [27], thereby allowing for a more generalized assessment of transporter mediated DDI effects on morphine and other CNS active drugs. Transporter mediated DDIs PBPK modeling is increasingly included in regulatory submissions [28–30]. Given this increased regulatory focus on transporter mediated DDIs, the developed modeling framework can be considered to study BBB transporter mediated DDI effect on $brain_{ECF}$ predictions to optimize further drug treatments.

Interspecies translation of LeiCNS-PK3.0

In **Chapter 5**, we extended the applicability and translatability of the LeiCNS-PK3.0 model by implementing a mouse-specific version. This extension is of relevance as mouse models are frequently used to study CNS PK and PD [31]. Translating PK between species is commonly done using allometric scaling, which relies on empirical relationships related to body size [32]. For scaling of CNS PK, allometric relationships may not hold due to physiological differences between species regarding CNS drug disposition, including transport processes [33]. In contrast, PBPK modeling provide a more mechanistic framework for addressing these limitations. By incorporating species-specific anatomical, physiological, and biochemical data, PBPK models offer the ability to simulate drug distribution, including CNS penetration, with greater precision. This mechanistic approach allows for the integration of parameters like transporter kinetics, brain tissue composition, and species-specific BBB dynamics, which are often absent in traditional allometric methods. Previously, the LeiCNS-PK3.0 model was developed and validated for rats and subsequently for humans [2, 34, 35]. To expand the LeiCNS-PK3.0 model towards mice, mouse-specific CNS physiological parameters were collected from literature. Data on CNS distribution of unbound drugs in mice was found to be very scarce. For 10 drugs, unbound plasma PK and associated (microdialysis based) $brain_{ECF}$ data were available. These observed data were used to validate the LeiCNSPK3.0 mouse model predictions of $brain_{ECF}$ PK profiles. The model gave good predictions of $brain_{ECF}$ PK profiles for 7 out of 10 drugs. Further validation of the PK predictions of the mouse model for other CNS compartments such as $brain_{ICF}$, $CSF_{ventricle}$, CSF_{SAS} and either whole brain remains pending availability of PK data on the different CNS compartments. These validation steps are important to further evaluate the predictive value of the mouse CNS PK predictions. To the best of our knowledge, the mouse LeiCNS-PK3.0 model is the only PBPK model that adequately predict the mouse $brain_{ECF}$ PK profiles of small molecule drugs. As such, the mouse LeiCNS-PK3.0 holds promise for further development to be

useful as a translational tool to predict the healthy, ultimately the CNS PK profiles in diseased humans, also from using PK data obtained from healthy and diseased mice.

QSPR model for $K_{p,uu,BBB}$ predictions

In **Chapter 6**, we developed a quantitative structure-property relationship (QSPR) model to predict $K_{p,uu,BBB}$ values, which is a key drug-specific input parameter for the LeiCNS-PK3.0 model required to estimate unbound drug concentrations in $brain_{ECF}$. The parameter $K_{p,uu,BBB}$ describes the extent of BBB transport, which is critical as it provides a quantitative measure of whether the BBB transport of a drug is dominated by passive diffusion, active efflux, or active influx. Additionally, $K_{p,uu,BBB}$ is a key parameter for making accurate CNS PK profile predictions using the LeiCNS-PK3.0 model. However, this parameter is often unavailable, and obtaining it may require animal studies. To reduce the use of animal experiments, we explored if machine learning (ML) approaches can help to predict the $K_{p,uu,BBB}$. We compared multiple algorithms to predict the $K_{p,uu,BBB}$ values based on chemical structural properties. In the literature, several QSPR models for $K_{p,uu,BBB}$ exist [36–43]. These models were based on data derived from the combinatory mapping approach which can include microdialysis data but also brain homogenate, brain slice and equilibrium dialysis [44], which do not take into account the intact physiological context of the processes that govern CNS drug distribution, whilst the data used to develop the QSPR model in the current chapters was built solely using microdialysis data, which were considered most physiologically relevant for BBB transport. We first constructed a QSPR model by collecting available rat $K_{p,uu,BBB}$ values measured via microdialysis and calculate the physicochemical descriptors for each of the collected drugs. Various ML algorithms were evaluated, and the selected QSPR model was subsequently used to predict $K_{p,uu,BBB}$ values. The in vivo microdialysis-derived $K_{p,uu,BBB}$ values, were used as input for the LeiCNS-PK3.0 model, and the in vivo observed $brain_{ECF}$ concentrations were compared with the predicted ones. We found that the developed QSPR model provided acceptable predictions for $K_{p,uu,BBB}$ considering the statistical criteria on the predictive performance of the train and test set [45]. For most compounds the predicted $brain_{ECF}$ concentrations, generated with the LeiCNS-PK3.0 model using both QSPR-predicted and microdialysis-measured $K_{p,uu,BBB}$ values, closely matched the observed data. To further improve QSPR models such as developed in this chapter, further studies could incorporate additional data on drug-BBB transporter interactions. Including the molecular interactions of drugs with BBB transporters would be particularly beneficial [43]. Previous research has demonstrated that incorporating such data can enhance QSPR model prediction performance [46]. Our study is in line with the emerging paradigm, whereby ML or artificial intelligence can be helpful, in this case in combined with (CNS) PBPK modeling to predict (PK) parameters as input data for PBPK models [47]. The integration of our QSPR model in the CNS PBPK model has the potential to enhance the performance of PBPK models, possible support to other PBPK models and reduce their reliance on in vitro or in vivo animal studies, thereby contributing to a reduction in animal usage.

CNS PBPK model applications

CNS PBPK modeling, as highlighted in this thesis, holds large potential for predicting CNS drug distribution under diverse physiological conditions. One of the aims of this research was to investigate, integrate, and better understand the impact of physiological changes caused by brain disorders on drug concentration-time profiles in the CNS. By leveraging existing data on CNS physiology, CNS PK in healthy and diseased conditions from databases and literature, we can explore ”what-if” scenarios to predict how these changes might affect drug behavior. This knowledge can contribute to improving and accelerating the development of drugs for brain disorders, as it allows us to predict CNS, specifically $brain_{ECF}$, concentration-time profiles based on the properties of (candidate) drugs, without relying solely on clinical trial data. Furthermore, this approach can enhance the design of clinical trial studies by providing a better understanding of how drugs will behave in patients with specific disease conditions. Another significant benefit of CNS PBPK modeling approaches is the potential to drastically reduce, and perhaps ultimately even replace, the use of animal testing in studies related to CNS drug disposition. By applying non-invasive imaging techniques, optimal drug dosages for individual patients may be ultimately determined based on their specific brain (patho)physiology. Finally, this research opens the door to exploring the repurposing of existing drugs for the treatment of CNS disorders, providing an efficient pathway to address unmet medical needs.

CNS PBPK model data integration, validation and future perspectives

Data integration methodologies

PBPK modeling is a mechanistic approach that offers valuable predictions on drug behavior in the body. These predictions can be further developed and improved by integrating various (clinical) methodologies with microdialysis, thereby providing the most valuable insights into unbound drug concentration time profiles in $brain_{ECF}$, a compartment often directly associated with a drug its target site, and CSF. Ethical constraints however limit the use of microdialysis in humans, necessitating reliance on alternative methods such as animal studies or non-invasive imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), or positron emission tomography (PET) [48–52]. Among these, PET is particularly suited for studying CNS drug distribution, as it provides spatial and temporal information on a PET tracer incorporated into a drug. However, PET data reflects total drug concentrations and does not distinguish between extracellular, membrane-bound, or intracellular drug locations. Thus, to estimate unbound concentrations, PET data must be corrected for brain tissue binding and intracellular distribution [53] for which experimental data is required.

Experimental data

CNS microdialysis approaches represents a key experimental method to gain insight into CNS drug disposition [54–56]. This method provides real-time data on the dynamic changes in the chemical composition of the brain, offering insights into PK, drug distribution, and the biochemical processes underlying neural activity. Moreover, microdialysis can provide insights into both the extent and rate of BBB penetration, and the role of active transport mechanisms. The brain slice method when combined with steady-state brain homogenate and plasma concentrations is an alternative approach to gain information on drug BBB transport and extra-intracellular distribution [57, 58]. This experimental technique preserves the structural and functional integrity of brain tissue to study drug

transport and distribution. However, direct determination of BBB transporters involved remains a challenge, as often the information relies on overall BBB partitioning. This underscores the importance of integrating diverse experimental methodologies to improve the reliability of CNS PBPK models. Obtaining knowledge on BBB transport mechanisms can be approached through advanced experimental strategies, imaging data integration, and using mechanistic modeling [59]. Beside microdialysis and the brain slice methodology, other techniques such as MRI, SPECT, PET and CSF-sampling can give information on the influx and efflux of compounds across the BBB. Combining the information on efflux and influx obtained by in vivo measurements with in vitro measurements on receptor expression, receptor binding, uptake and luminal to abluminal transport can give in depth information on BBB transport mechanism. The resulting information can help better to understand and accurately predict for example the $K_{p,uu,BBB}$ values and subsequently CNS PK. Understanding, improving and validation is key to advancing CNS drug distribution predictions and enabling their broader application in drug development and regulatory science.

Model validation and qualification

Validation is an essential step in the development of PBPK models, including CNS PBPK models, providing the foundation for their credibility and applicability. Once a model demonstrated sufficient predictive capability and aligns with experimental and/or clinical data, it represents an essential tool to inform and guide further drug development decision making. However, while validation of PBPK model predictions is essential, it currently remains challenging to achieve, because only after clinical data is collected such validation can be performed. The fact that validation remains often difficult does not mean CNS PBPK models cannot be of significant value, as these models allow to address gaps in our understanding of CNS drug disposition. Whilst validation of CNS PBPK models may often be challenging, a robust PBPK model must nonetheless align with its intended purpose, acknowledge its assumptions and limitations, and justify them appropriately [60, 61]. We envision that a stepwise strategy, wherein PBPK models are continuously improved and evaluated is the only way forward to develop models which are able to predict with increasing certainty CNS drug disposition across biological and drug-specific properties. Here, also regulatory agencies have an important role in providing guidelines to ensure consistency and transparency in CNS PBPK model reporting for regulatory use beyond those currently available for general PBPK models [62–65].

Conclusion

In this thesis the LeiCNS-PK3.0 model has been used to understand step-by-step the factors related to morphine dose-effect pathway whereby taking into account the metabolites and possible DDI effect. While performing these studies, multiple modeling approaches have been developed which can be applied and extended towards other compounds. Furthermore, we expanded the use of the LeiCNS-PK3.0 model by translating the model to the mice and made a first step of making the model independent of in vivo measured values by combining ML and PBPK modeling. Overall, the studies described in this thesis demonstrate the value of CNS PBPK modeling approaches to support and inform drug development and treatment optimization strategies.

Future perspectives

To enhance CNS PBPK predictions, particularly those using LeiCNS-PK3.0, and broaden its scope, several measures can be implemented. Collecting and sharing physiological datasets across various populations, including pediatric groups, and incorporating species-specific parameters, can improve model predictions. Moreover, combining CNS PBPK frameworks with modeling approaches that concern subsequent receptor binding and pharmacodynamic effects, represent a next step for paving the way for personalized treatments and to further support drug development.

In this thesis, the LeiCNS-PK3.0 model was utilized to simulate morphine PK by integrating drug-BK and DDI data. This methodology can be adapted to predict the behavior of other CNS-active compounds. Future iterations of LeiCNS-PK3.0 could include additional physiological elements, such as metabolism, sex, comorbidities, polymorphism, genetic variations in transporters, receptors and enzymes, either individually or in synergy. Preliminary findings indicate that parameters such as pH, cerebral blood flow, passive paracellular transport, sex differences, and the impact of chronic pain over time exert minor influences on $brain_{ECF}$ pharmacokinetics of CNS drugs. Moreover, metabolism—occurring not only in systemic circulation but also within the BBB and brain —may exhibit notable variation between males and females males [66–68], necessitating further exploration. Furthermore, the predicted $brain_{ECF}$ PK can be used besides for prediction of CNS BK, also the cellular signaling and transduction pathway which can provide information on signal transmission via neural circuits.

For CNS PBPK modeling to effectively support clinical research and optimize dosing regimens, developing a user-friendly interface for LeiCNS-PK3.0 will be critical. Such an interface would enable accessibility for healthcare professionals and other stakeholders, fostering wider adoption in drug development, enhancing collaboration, and promoting efficient data exchange.

Building on the findings of this thesis, future research can further explore the application of the LeiCNS-PK3.0 model across a broader range of CNS-acting compounds, particularly those with complex metabolic pathways or potential drug-drug interactions. Furthermore, expanding the model to include disease-specific conditions, genetic variability, or transporter alterations may improve its translational value. Ultimately, these developments can contribute to more efficient drug discovery, personalized medicine approaches, and optimized treatment strategies for CNS disorders.

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