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Combining Brain Microdialysis and Translational Pharmacokinetic Modeling to Predict Drug Concentrations in Pediatric Severe Traumatic Brain Injury: The Next Step Toward Evidence-Based Pharmacotherapy?

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Abstract

Evidence-based analgosedation in severe pediatric traumatic brain injury (pTBI) management is lacking, and improved pharmacological understanding is needed. This starts with increased knowledge of factors controlling the pharmacokinetics (PK) of unbound drug at the target site (brain) and related drug effect(s). This prospective, descriptive study tested a pediatric physiology-based pharmacokinetic software model by comparing actual plasma and brain extracellular fluid ($brain_{ECF}$) morphine concentrations with predicted concentration-time profiles in severe pTBI patients (Glasgow Coma Scale [GCS], ≤8). Plasma and $brain_{ECF}$ samples were obtained after legal guardian written consent and were collected from 8 pTBI patients (75% male; median age, 96 months [34.0–155.5]; median weight, 24 kg [14.5–55.0]) with a need for intracranial pressure monitoring (GCS, ≤8) and receiving continuous morphine infusion (10–40 μg/kg/h). $Brain_{ECF}$ samples were obtained by microdialysis. $Brain_{ECF}$ samples were taken from ''injured'' and ''uninjured'' regions as determined by microdialysis catheter location on computed head tomography. A previously developed physiology-based software model to predict morphine concentrations in the brain was adapted to children using pediatric physiological properties. The model predicted plasma morphine concentrations well for individual patients (97% of data points within the 90% prediction interval). In addition, predicted $brain_{ECF}$ concentration-time profiles fell within a 90% prediction interval of microdialysis $brain_{ECF}$ drug concentrations when sampled from an uninjured area. Prediction was less accurate in injured areas. This approach of translational physiology-based PK modeling allows prediction of morphine concentration-time profiles in uninjured brain of individual patients and opens promising avenues towards evidence-based pharmacotherapies in pTBI.

Keywords: microdialysis; morphine; pediatric; pharmacokinetics; traumatic brain injury

Introduction

Given that trauma continues to be a leading cause of mortality and morbidity worldwide, research into effective and safe therapies is imperative to improve patient outcome.[1] Analgosedation plays a crucial role in the supportive therapy of traumatic brain injury (TBI); however, evidence-based guidelines are lacking in both adults and children.[2,3] The latter is especially disturbing given that this concerns a vulnerable population still undergoing brain development. The significant physiological and anatomical changes that take place, especially until the age of 8 years, emphasize why extrapolation of studies from adult populations does not suffice and could even be detrimental in children.[4]

In pharmacological terms, it is imperative one first understands the pharmacokinetics (PK) of a drug, that is, ''what the body does to the drug'' in terms of absorption, distribution, metabolism, and elimination. These processes are influenced by the half-life of the drug, volume of distribution, and total body clearance. The net effect results in a dose- and time-dependent drug concentration in a certain body compartment. The following step in pharmacological research is to correlate drug concentration (PK) to drug effect or pharmacodynamics (PD; i.e., ''what the drug does to the body'').[5] Given that analgosedative drug effects are related to the unbound drug concentration at the site of action (i.e., the brain as target site), knowledge of unbound brain concentrations in TBI patients could help predict drug effect(s).[6,7] To date, such information is limited. Innovative

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developments in the field of pre-clinical and clinical pharmacology research are promising and provide tools, which could be utilized to improve our pharmacological understanding of commonly used drugs in (pediatric) TBI (pTBI).

In the clinical setting, measuring drug concentrations in the human brain is challenging. However, parenchymal microdialysis catheters as part of clinical brain monitoring in severe TBI patients allows high-frequency sampling of brain extracellular fluid ($brain_{ECF}$).[3,8] This enables the acquisition of data on unbound drug concentrations in the $brain_{ECF}$,[7,9,10] whereas human plasma drug concentrations can be obtained by serial blood sampling. In the context of this article, it must be emphasized that $brain_{ECF}$ refers to interstitial brain fluid, not cerebrospinal fluid (CSF), and reflects the closest representation of *intra*cellular brain fluid, which ultimately is the target site for drug action.

Acquiring sufficient plasma samples for pharmacological research can be challenging in the pediatric population because of lower circulating volumes. An approach to solve this issue is population-based PK analysis that uses the often sparse samples of various individual patients and analyzes them as a single ''population'' (after taking covariates such as age, weight, renal function, and severity of illness into account). This approach provides sufficient data points to develop a drug-specific PK model that can subsequently be used as a template for that drug in the individual patient. Identification of covariates improves the model fit per patient and explains PK differences between individual patients.[11,12] This approach (top-down) does not take into account our knowledge on physiology parameters (e.g., blood flow, organ sizes, drug metabolism enzyme activity, renal function, and blood–brain barrier function), which could be used to predict drug concentration in previously unstudied populations. The approach presented in this study: Physiology-based modeling (bottom-up) has increasingly been used and has been approved by the European Medicines Agency and the U.S. Food and Drug Administration to extrapolate adult and/or animal PK to children after adapting them to pediatric physiological parameters.

A physiology-based, multi-compartmental PK model that was recently developed on the basis of pre-clinical data in the rat allowed prediction of drug concentrations in multiple brain compartments for nine highly diverse compounds (including morphine, acetaminophen, phenytoin, and methotrexate).[13] Replacement of rat with human brain physiological parameter values in the model allowed prediction of the brain PK (i.e., unbound drug concentrations) of each of the nine compounds in multiple brain compartments for adult humans.[13] Using the same approach, we hypothesized that it would be possible to adapt this physiology-based PK model to the *pediatric* human brain and predict unbound drug concentrations in $brain_{ECF}$. This is the first step in pharmacological research: finding a method to adequately predict specific drug concentrations at the target site (in this case, the brain), which can then be correlated to PD parameters in the clinical setting.

The aim of our study was to determine whether the physiology-based pediatric brain PK model could adequately predict actual plasma and $brain_{ECF}$ unbound morphine concentrations obtained from pTBI patients.

If successful, this approach could ultimately lead to predicting brain PK profiles in individual pTBI patients.[10,13,14] This is a novel and promising approach in pharmacological TBI research, especially for children, given that it could ultimately lead to more evidence-based and tailored TBI treatment.

Methods

Study design and population

This was a single-center, prospective, descriptive pilot study performed in the pediatric intensive care unit (PICU) of Red Cross War Memorial Children's Hospital in Cape Town, South Africa, from March 2014 until April 2015.

Patients admitted with severe TBI (ages 0–13 years, Glasgow Coma Sclae [GCS] ≤8, and requiring invasive neuromonitoring) were eligible for inclusion. Standard care for pediatric TBI patients at this institution consisted of intracerebral monitoring of intracranial pressure (ICP) and brain oxygenation. In general, patient care was directed by a local algorithm based on the current recommendations for the management of severe TBI in children, including ICP, cerebral perfusion pressure, and brain oxygenation targets.[15] Microdialysis (MD) was being used for clinical monitoring of brain metabolism by the bedside. Specifically, metabolites such as lactate, pyruvate, glucose, glycerol, and glutamate were analyzed hourly by the bedside and were used to adapt clinical care based on temporal changes. Exclusion criteria were no parental/legal guardian written informed consent, severe hemorrhagic disease as contraindication for intracerebral monitoring, and unavailability of microdialysis consumables or expertise. This study was initiated after approval from the University of Cape Town's Human Research Ethics Committee (HREC reference 060/2011). The study protocol complied with the Declaration of Helsinki (2013).

Clinical data collection

Data collection included age, estimated weight, sex, and mechanism of injury. Pharmacological data collected included morphine infusion concentration and rate as well as timing and dose of morphine boluses. Intracerebral catheters are routinely placed (by the neurosurgical team) in the frontal white matter away from known areas of contusion and damage. Radiological findings were recorded, and the position of the microdialysis catheter was noted on follow-up head computed tomography (CT) scans and its location relative to any contusions (i.e., injured brain). Typically, all patients have a globally diffuse brain injury; however, areas close to contusions may demonstrate differing blood-brain characteristics. For these reasons, we defined the position of the catheter, based on the CT scans, as follows: ''Uninjured'' brain referred to positioning in diffusely injured brain with no visible contusion close to the catheter; ''injured'' brain referred to positioning near a contusion.[9,16] Note that these terms are relative. Finally, the duration of PICU and hospital stay were collected.

Sample collection

Plasma samples were retrieved from remnant blood taken during routine laboratory rounds. A minimum volume of 0.5 mL was required, which was immediately centrifuged at 4000 rpm for 5 min to obtain plasma, which was separated from the precipitate. Brain MD catheters were placed in the brain parenchyma by a separate burr hole at the time of ICP monitoring. We used M Dialysis 71 High cut-off brain MD catheters (shaft diameter, 0.9 mm; pore size, 100 kDa) with a bedside CMA 106 pump infusing sterile and artificial central nervous system (CNS) perfusion fluid at a set flow rate of 0.3 μL/min (M Dialysis AB, Stockholm, Sweden). Microdialysate was collected hourly in capped microvials. When hourly dialysate was <10 μL, the time interval between vial change was increased to 2 h to guarantee this minimum volume for drug concentration analysis. $Brain_{ECF}$ and plasma samples were frozen at −80°C until drug analysis was performed.

Analysis of concentrations in plasma and dialysate

Drug concentrations in plasma and microdialysate were determined at the clinical pharmacology laboratory of the Academic Medical Center in Amsterdam, The Netherlands. Morphine, morphine-3-glucuronide, and morphine-6-glucuronide concentrations were analyzed using liquid chromatography/tandem mass spectrometry in the positive ionization mode on a Shimadzu LC-30

(Shimadzu Corporation, Nishinokyo-Kuwabaracho, Japan) system coupled to an AB Sciex 5500 QTRAP mass spectrometer (AB Sciex, Framingham, MA). To 10 μL of sample, 75 μL of acetonitril/methanol 84:16 (v/v%) containing the internal standard, morphine-d3, morphine-3-glucuronide-d3, and morphine-6-glucuronide-d3, was added to precipitate proteins. Samples were vortexed, stored at –20°C for 30 min, vortexed again, and centrifuged. For determination of morphine, morphine-3-glucuronide, and morphine-6-glucuronide, 3 μL was injected onto a Thermo Scientific Hypersil Gold HILIC (50×2.1mm, 1.9 μm; Thermo Fisher Scientific, Waltham, MA) column. A step-wise chromatographic gradient was applied using acetonitril and water with a constant 5% addition of 1% ammonium formate/2% formic acid in water. Flow was 600 μL/min for the hydrophilic interaction liquid chromatography method; column-oven temperature was 40°C. Morphine, morphine-3-glucuronide, and morphine-6-glucuronide were measured as [M+H+], using the mass transition of 286.1/165.1, 462.2/286.2, and 462.2/286.2, respectively. The method was validated over a range of 2–500 ng/mL. Accuracies ranged from 93.5% to 105.5%, intraday precisions were below 9.6%, and interday precisions were below 12.9%.

Translational modeling methods

A physiology-based PK model consists of three parts that each contain variables which can be measured and entered into the model. These are variables from the ''system'' (i.e., the biological system, such as the human body, and relate to weight and age), plasma variables (i.e., the measured concentration of drug in the blood, which is influenced by plasma volume and rate of clearance), and finally variables concerning the drug itself (i.e., drug dosing, drug characteristics such as lipophilicity, which influence diffusion characteristics). Subsequently, modeling assumptions are made to predict how the drug will spread to other compartments in the body (or system) such as the brain. These assumptions are based on the expected volume of these (brain) compartments and factors that govern drug distribution and clearance (i.e., brain transport mechanisms). The validity of the physiology-based PK model can be tested by comparing prediction to observed concentrations. If these correlate, then the PK model can be used as a template for that specific drug.

The previously published physiology-based multi-compartmental brain PK model used in this pilot study was based on healthy rat CNS physiological parameters and PK information of multiple CNS compartments.[13] The CNS compartments in this model encompassed brain extra- and intracellular fluid and the CSF compartments of the lateral ventricle, third- and fourth ventricle, cisterna magna, and subarachnoid space. This model was developed and subsequently translated to humans (adults) for nine compounds, including morphine. The details of the model structure and translational methods are described in Yamamoto and colleagues.[13]

Ultimately, the $brain_{ECF}$ can be seen as the best representation of the target site for drug therapy, as sampling of human *intra*cellular fluid ($brain_{ICF}$) is not feasible. Therefore, the Yamamoto generic multi-compartmental CNS distribution model was translated to predict morphine plasma and $brain_{ECF}$ concentrations for each *pediatric* patient in four steps: 1) development of a plasma PK model using individual plasma PK data; 2) replacing system-specific parameters by individual pediatric patient values (e.g., age and weight); 3) applying allometric scaling to the drug-specific parameters; and 4) predicting the $brain_{ECF}$ concentrations using estimated human plasma PK parameters, replacing system-specific parameters and scaling drug-specific parameters. The specific parametric scaling is detailed in Supplementary Table 1. (see online supplementary material at http://www.liebertpub.com) The scaling method of each parameter (plasma, drug, or system specific) is indicated with color coding, and the entire model is illustrated in Figure 1. Simulation was performed using NONMEM software (version 7.3; ICON Development Solutions, Hanover, MD). The plots were conducted using R software (R Foundation for Statistical Computing, Vienna, Austria). This approach enabled prediction of $brain_{ECF}$ morphine concentrations for each individual pTBI patient, based on a few individual plasma data points. The predicted morphine $brain_{ECF}$ concentrations could then be visually compared to the observed morphine $brain_{ECF}$ concentrations.

Statistical analysis

This study was descriptive with exploratory aims (no interventions yielding comparison of patient groups), and, as such, no formal power analysis was performed. Given the small study

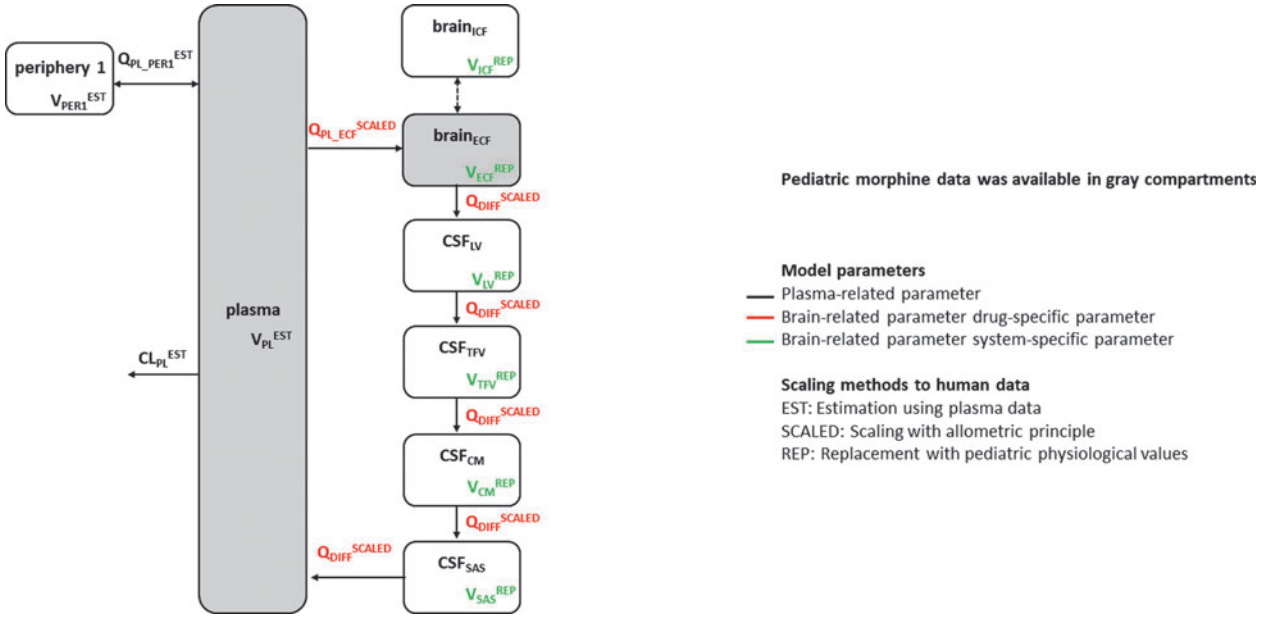

FIG. 1. Multi-compartmental brain pharmacokinetic model structure. V, volume; CL, clearance; Q, flow; CSF, cerebrospinal fluid; DIFF, diffusion; PL, plasma; PER, periphery; ICF, intracellular fluid; ECF, extracellular fluid; LV, lateral ventricle; TFV, third and fourth ventricle; CM, cisterna magna; SAS, subarachnoidal space.

Table 1. Patient Characteristics

Patient	*Age (years)*	*Sex*	*Weight (estimated kg)*	*Injury mechanism*	*Morphine infusion range (μg/kg/h)*	*PICU LS (days)*	*HLS (days)*
1	4.2	M	16	Pedestrian–MVA	20–40	23	35
2	9.5	F	28	Pedestrian–MVA	20–40	30	48
3	8.3	M	26	Gunshot	20–40	11	18
4	3.5	F	15	Gate crush	0–40	6	8
5	13	M	30	Passenger–MVA	20–40	7	15
6	7.7	M	22	Pedestrian–MVA	20–40	6	8
7	2.8	M	14.5	Pedestrian–MVA	10–40	6	22
8	11.7	M	55	Pedestrian–MVA	0-40	2	5

PICU LS, pediatric intensive care unit length of stay; HLS, hospital length of stay; MVA, motor vehicle accident.

population, clinical data are presented as median with range and categorical variables presented as proportions (%).

Results

Eight patients were included in this pilot study during the 1-year study period. Median age was 8 years (range, 2.8–13.0), median weight 24 kg (range, 14.5–55.0), and 75% were male. All patients survived to hospital discharge. Morphine infusion was commenced on admission to the PICU as per local protocol. Table 1 provides an overview of patient characteristics. Table 2 describes the various intracerebral injuries of the head CT scan at admission to the hospital and positioning of the MD catheter.

Microdialysis brain$_{ECF}$ sampling: feasibility

The first step of this study was to determine whether brain$_{ECF}$ sampling with MD for analysis of brain$_{ECF}$ morphine concentrations was feasible in pTBI patients. We were able to determine morphine concentrations in low-volume MD samples (volume, ≥10 μL) as detailed in the Methods section. It was necessary to adjust the brain$_{ECF}$ sampling time from 1 to 2 h in 5 patients to obtain the minimum volume of 10 μL (patients 1, 2, 4, 5, and 6).

Of the 8 patients included in this study, we were able to use 6 patients' data to investigate the translated multi-compartmental brain PK model: In 1 patient, the MD catheter failed to yield dialysate, in another no blood samples were collected, and no further modeling was possible. Total duration of brain MD sampling for morphine PK varied per patient with a median collection time of 90 h ($n=6$; range, 57–128). Table 3 illustrates the number of samples collected per patient and the median unbound morphine concentration in blood and brain$_{ECF}$.

Physiology-based pharmacokinetics modeling: comparison of predicted and observed pediatric morphine concentrations

Plasma PK parameters were estimated with good precision and the developed plasma PK model described pediatric plasma PK of morphine well in all patients, with 97% of the morphine plasma sample concentrations falling within the 90% prediction interval (Fig. 2). As for brain$_{ECF}$ morphine concentrations, the model captured the observed values more accurately (i.e., within the 90% prediction interval) when sampling was from relatively uninjured brain (patients 1, 2, and 4). This was not the case for brain$_{ECF}$ morphine concentrations sampled from relatively more injured brain regions (patients 5, 6, and 7). Plotting of the measured concentrations in the 90% prediction interval for these patients demonstrated diverse patterns ranging from diffuse scattering to a trend on the upper or lower limit, respectively.

Discussion

This study demonstrates that collecting brain$_{ECF}$ samples to determine drug concentrations is possible in pTBI patients. Using these data, we also show that a physiology-based PK model can be

Table 2. Overview of Intracerebral Injuries on Admission Head CT Scan and MD Catheter Position

Patient	*CT scan on admission*	*MD catheter location*	*MD sample location*
1	Left frontal lobe hemorrhage, intraventricular bleeding, and subarachnoid hemorrhage	Right frontal lobe	Uninjured
2	Subarachnoidal bleeding, contusions, intraventricular bleeding, and generalized swelling	Right frontal lobe	Uninjured
3	Right parietal subdural hematoma (max. 7 mm) with right hemispheric swelling and midline shift to left (max. 10 mm)	Right frontal lobe	Injured
4	Right fronto-parietal subdural hematoma (max. 7 mm), left frontal hemorrhagic contusion, and midline shift to left (4 mm) with cerbral edema (R > L)	Left frontal lobe	Uninjured
5	Right frontal lobe hemorrhagic contusion, generalized swelling	Right frontal lobe	Injured
6	Small punctate hemorrhagic contusions at right gray/white matter interface, suggestive of DAI	Right frontal lobe	Injured
7	Diffuse axonal injury, subarachnoidal bleed, and interventricular bleed	Right frontal lobe	Injured
8	Small frontal subdural hematoma	Right frontal lobe	Injured

CT, computed tomography; MD, microdialysis; DAI, diffuse axonal injury.

Table 3. Overview of Patient Samples and Unbound Morphine Concentrations

Patient	*Blood samples* (n)	*$Brain_{ECF}$ samples* (n)	*Unbound morphine concentration blood* (*μg/L*)	*Unbound morphine concentration $brain_{ECF}$* (*μg/L*)
1	11	31	43.6 [18.8–60.2]	13.1 [1.0–17.5]
2	5	15	36.5 [31.1–40.1]	6.7 [2.6–8.8]
3	1	0	3,5	NA
4	5	19	47.6 [22.3–209.0]	6.9 [2.1–13.1]
5	4	11	7.6 [7.5–7.7]	6.8 [2.1–19.7]
6	5	23	26.5 [16.6–53.9]	15.0 [10.0–37.5]
7	4	29	30.7 [21.1–32.4]	3.6 [2.4–6.9]
8	0	4	NA	33.7 [28.7–42.3]

Unbound morphine concentrations are demonstrated as median concentration and [range].
NA, not applicable; *n*, number of samples.

used to adequately predict morphine concentrations in plasma and $brain_{ECF}$ in the uninjured brain regions of pediatric TBI. The importance of this finding is that it enables prediction of morphine PK in the target site (i.e., the brain) and holds the potential of developing a model-based approach from which further research into evidence-based pharmacotherapy in (pediatric) TBI is possible.

Analgosedation is one of the pillars of supportive therapies in protocols for TBI and other acute brain injury conditions worldwide.[2,3,17] Frequently used drugs include midazolam, pentobarbital, fentanyl, morphine, and propofol with the aim of providing adequate sedation to reduce secondary brain injury and relieve pain and anxiety. However, evidence-based dosing regimens are lacking, which raises concerns about efficacy and safety of drugs currently used, attributed to under- and overdosing. Current guidelines provide level 2 (adults) and 3 (children) evidence at best, and, although disturbing, it is no surprise the recommendation still reads: ''*…the choice of sedative, analgesics and neuromuscular blocking agents …should be left to the treating physician.*''[3] Inevitably, this leads to diverse analgosedation regimens in TBI patients dependent on clinician experience and preference. This is often guided by the effect on hemodynamic stability, further hampering constructive comparison of such treatment regimens. Further, there is a general paucity of PK and PD understanding of the CNS drugs at target sites.[10] These factors, together with ongoing poor outcome post-TBI and the high failure rate in CNS drug development, have sparked renewed interest in unraveling the mechanisms of drug passage across the blood–brain barrier (BBB) and blood–CSF barrier (BCSFB) to improve our pharmacological understanding and ultimately develop evidence-based, adequate therapies.[7,9,10,14,18,19] Combining innovative techniques from both pre-clinical and clinical research enables us to take the next step in determining PK properties of commonly used drugs and holds the promise of developing individualized analgosedation.

As a future implication of our data, it will be feasible to use a translated (''humanized'') pre-clinical multi-CNS compartment PK model, combined with population-based PK statistical analysis, to develop a pediatric template for prediction of morphine PK in $brain_{ECF}$. In addition, our findings underline the necessity of knowledge about $brain_{ECF}$ morphine concentrations to understand morphine PK in total. This pilot study shows that a given drug dose with similar plasma morphine concentration can lead to *different* brain morphine levels dependent on whether the $brain_{ECF}$ sampling was from injured or uninjured brain. This confirms the important role of the BBB and BCSFB drug passage mechanisms in drug distribution at the target site of the brain as other covariates (such as weight and age) are accounted for in the model.

The successful development and validation of a physiology-based PK model could enable further pharmacological research without the need for large patient numbers requiring invasive procedures and numerous samples per patient. This is important because microdialysis studies, particularly in children, are rare. These are currently some of the obstacles that are especially relevant in pediatric pharmacological research and account for the paucity of evidence-based drug dosing data in this patient group. If using the physiology-based PK model as a template for predicting pediatric morphine PK profiles is successful in linking brain PK to PD, it may open new avenues for determining evidence-based dosing regimens for other analgosedative drugs in (pediatric) TBI.

Limitations

There are various limitations and learning points from this study. This is a small patient group from a single center. Larger patient numbers, included from multiple centers, would be necessary to further validate this (pediatric) morphine PK model. Further, this pilot study focuses solely on drug PK, and, as such, no assumptions can be made about clinical outcome measures (PD) at this stage.

The pooling of samples over 2-h intervals could be seen as a suboptimal measurement, yet the overall trend is important, which, in general, remained within the 90% predicted interval for samples derived from relatively uninjured brain. Median duration of sampling was 90 h in this pilot study. Given the natural history of secondary brain injury, which evolves over the course of days, it is of interest to assess whether the currently observed correlation between model prediction and measured drug concentrations remains consistent over time because of changes in blood-brain-passage mechanisms secondary to variations in brain swelling and evolving infarction. Further, biofouling of the MD catheter membrane (e.g., clogging of the membrane pores) within 5 days potentially affects catheter recovery rate, which may affect sample integrity. These are some mechanisms that might influence sampling and the $brain_{ECF}$ morphine concentrations over time and need further investigation.

The distinction between uninjured and injured brain is relative, given the global nature of TBI, but is a well-established practice in microdialysis TBI studies for brain chemistry.[9,16] The reason for this distinction was to determine how accurate the physiology-based PK model was given that this is currently based on uninjured animals. Contusions and pericontusional regions of the traumatized brain may behave in a dysregulated manner in which regulatory mechanisms affecting hemodynamic and BBB characteristics are different from uninjured brain. We hypothesized that the physiology-

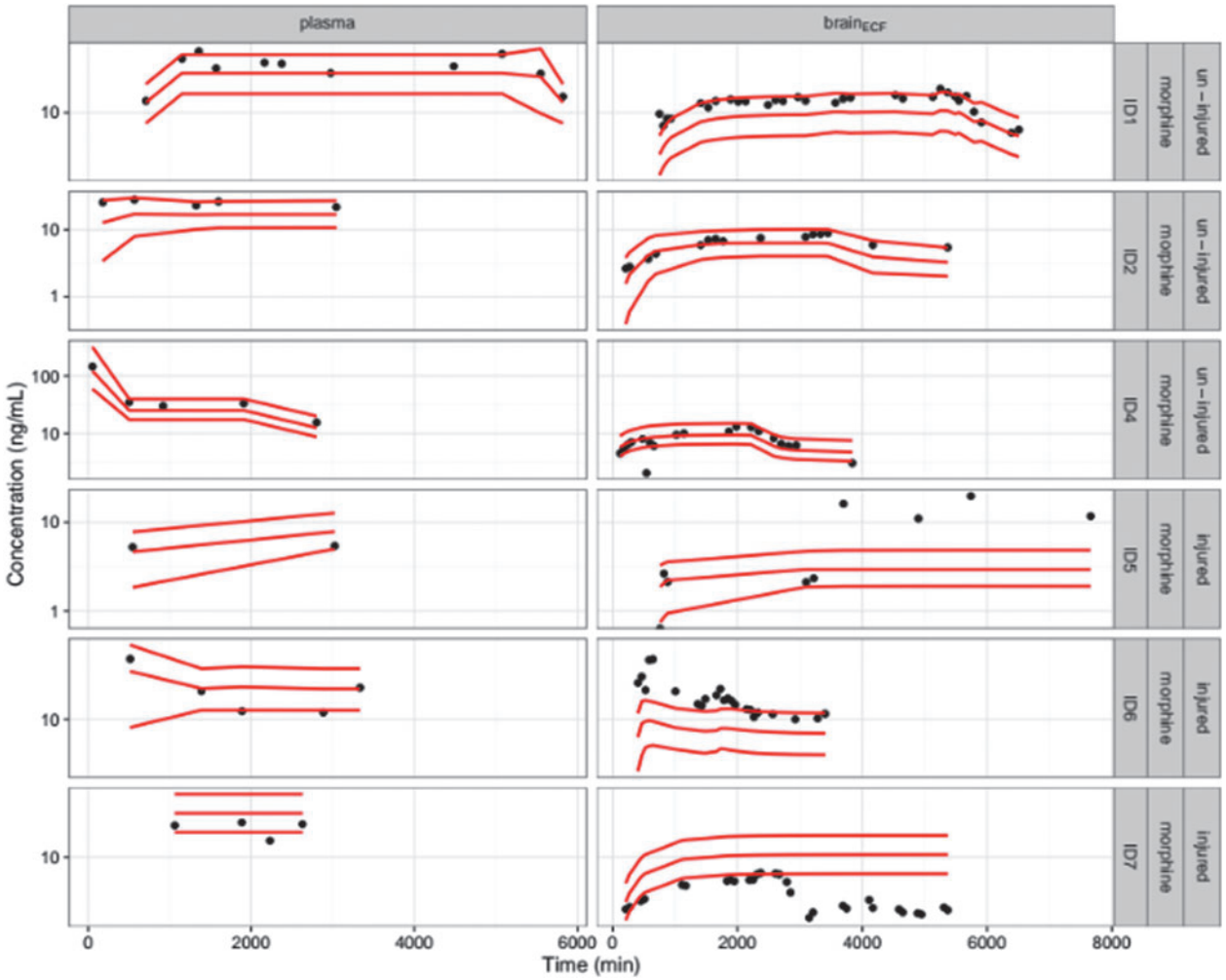

FIG. 2. Observed versus predicted morphine concentrations in plasma and brain$_{ECF}$. Red lines represent the 5th, 50th, and 95th percentile, respectively, of predicted morphine concentrations for that specific patient. Black dots represent the observed morphine concentrations over time. ECF, extracellular fluid.

based PK model would therefore provide better prediction of those samples collected from relatively uninjured brain, which seemed to be the case as illustrated in Figure 2. This finding is in accord with the brain$_{ECF}$ morphine concentrations found in adult TBI patients by Ederoth and colleagues, which demonstrated both lower and higher brain$_{ECF}$ morphine concentrations in injured brain compared to relatively uninjured brain.[20] Their suggestion was that the injured brain shows altered efflux mechanisms and BBB permeability for morphine. In our study, patients 5, 6, and 7 had values above and below the outer percentiles for observed versus predicted cerebral morphine concentrations. This may be the result of a new balance between BBB influx and efflux mechanisms and permeability as a result of localized brain injury. This finding is important given that it demonstrates that only measuring drug plasma levels can over- or underestimate drug levels in the brain. Subsequent PKPD studies could lead to misinterpretations of drug efficacy and safety, if only *plasma* PK is taken into account in pharmacological studies that focus on clinical outcome measures such as depth of sedation and pain control. In addition, it also demonstrates that the current physiology-based PK model dose not fully capture the local PK changes that take place in injured brain. Therefore, it is important to translate this physiology-based PK model for injured brain by changing system-specific parameter values according to alterations in injured brain. Hypothetically, this could enable better prediction of morphine PK in injured regions. However, potential regional differences in cerebral drug target site concentrations within patients raise the question of what the focus for adequate drug dosing should be (i.e., injured vs. uninjured brain). This is similar to questions about targeting physiological parameters in clinical care.

Finally, we have only focused on predicted and observed morphine concentrations in this pilot study. It is imperative to also determine whether (active) metabolites of morphine, such as morphine-6-glucunoride (M6G), can be predicted accurately. This is of importance for future PK/PD modeling. Preliminary data, not presented in this article, show that it is possible to determine morphine metabolites in both blood and brain$_{ECF}$ in pediatric TBI.

Future steps toward compiling an evidence-based dosing regimen for morphine in pediatric TBI patients include adding other centers in data acquisition to validate the physiology-based PK model. In addition, a physiology-based PK model for injured brain will be developed to assess whether this predicts drug concentrations from injured brain more precisely. Once the physiology-based PK model has been validated, the next step will be to compile a PD profile for morphine with the aid of multi-modal neuromonitoring.[8] This

approach will enable defining more PD markers, such as brain-oxygenation levels, lactate/pyruvate ratio, and the presence of seizures, which, together with ICP, may better reflect local dynamics of cerebral blood flow and metabolic demand as well as establish both efficacy and safety of CNS drugs used in (pediatric) TBI.

Conclusion

Level 1 evidence-based dosing regimens for commonly used drugs in analgosedation in pediatric TBI currently do not exist. Modalities such as brain microdialysis combined with physiology-based PK modeling and population-based PK statistical analysis are promising tools in designing a PK template for a variety of drugs that could assist in developing evidence-based dosing strategies for effective and safe therapy in vulnerable patient groups. Our data demonstrate the feasibility of this concept and warrant further studies, which are currently in progress.

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