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Continuous infusion of physostigmine in patients with perioperative septic shock: A pharmacokinetic/pharmacodynamic study with population pharmacokinetic modeling

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ABSTRACT

Background: In the context of the cholinergic anti-inflammatory pathway, the clinical trial Anticholium® per Se (EudraCT Number: 2012-001650-26, ClinicalTrials.gov NCT03013322) addressed the possibility of taking adjunctive physostigmine salicylate treatment in septic shock from bench to bedside. Pharmacokinetics (PK) are likely altered in critically ill patients; data on physostigmine PK and target concentrations are sparse, particularly for continuous infusion. Our objective was to build a population PK (popPK) model for physostigmine, and further evaluate pharmacodynamics (PD) and concentration-response relationship in this setting.

Methods: In the randomized, double-blind, placebo-controlled trial, 20 patients with perioperative septic shock either received an initial dose of 0.04 mg/kg physostigmine salicylate, followed by continuous infusion of 1 mg/h for up to 120 h, or equivalent volumes of 0.9% sodium chloride (placebo group). Physostigmine plasma concentrations and acetylcholinesterase (AChE) activity were measured; concentration-response associations were evaluated, and popPK and PD modeling was performed with NONMEM.

Results: Steady state physostigmine plasma concentrations reached 7.60 ± 2.81 ng/mL (mean ± standard deviation [SD]). PK was best described by a two-compartment model with linear clearance. Significant covariate effects were detected for body weight and age on clearance, as well as a high inter-individual variability of the central volume of distribution. AChE activity was significantly reduced to 30.5%–50.6% of baseline activity during physostigmine salicylate infusion. A sigmoidal direct effect PD model best described enzyme inhibition by physostigmine, with an estimated half maximal effective concentration (EC_{50}) of 5.99 ng/mL.

Conclusions: PK of physostigmine in patients with septic shock displayed substantial inter-individual variability with body weight and age influencing the clearance. Physostigmine inhibited AChE activity with a sigmoidal concentration-response effect.

Abbreviations: AChE, acetylcholinesterase; AIC, *Akaike* information criterion; APACHE, Acute Physiology and Chronic Health Evaluation; BChE, butyrylcholinesterase; BfArM, Federal Institute for Drugs and Medical Devices; CAP, cholinergic anti-inflammatory pathway; DIVI, German Interdisciplinary Association for Intensive Care and Emergency Medicine; DSG, German Sepsis Society; EC_{50}, half maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; EudraCT, European Union Drug Regulating Authorities Clinical Trials database; FOCE, first-order conditional estimation; GCS, glasgow coma scalet; GOF, goodness of fit; Hb, hemoglobin; HPLC, high performance liquid chromatography; IIV, inter-individual variability; IL, interleukin; IMP, investigational medicinal product; IQR, interquartile range; OFV, objective function value; PD, pharmacodynamics, PK, pharmacokinetics; popPK, population pharmacokinetics; PsN, Pearl-speaks-NONMEM; ρ, Spearman's rho; RSE, relative standard error; SCM, stepwise covariate model building; SD, standard deviation; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; TNF, tumor necrosis factor; U, unit; VPC, visual predictive check

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1. Introduction

The cholinesterase inhibitor physostigmine is the major alkaloid extracted from the Calabar bean, *Physostigma venenosum* Balf. [1,2]. Its current clinical role is predominantly limited to the reversal of anticholinergic poisoning and postoperative central anticholinergic syndromes [3–5].

Despite a long history of clinical practice, knowledge of the pharmacokinetic (PK) properties and metabolism of physostigmine in humans is limited [1,6]. The cholinesterase inhibitor is usually administered as an intravenous bolus injection or a short infusion [3]. Taken together with its short half-life due to fast metabolism and elimination, treatment with physostigmine is characterized by rapid on- and offset of pharmacological effects. However, very few studies describe continuous infusion of physostigmine, and from toxicological reports, concerns have been raised about the risk of cardiotoxicity and seizures associated with the drug [7,8].

Recently, physostigmine has aroused interest in the context of the cholinergic anti-inflammatory pathway (CAP). This neuro-immune circuit has been introduced as an experimental option to reduce systemic inflammatory response and protect against cytokine-mediated diseases [9–12]. In murine models of endotoxemia and polymicrobial sepsis, treatment with physostigmine salicylate significantly suppressed systemic inflammatory response [10,13] and improved survival [13]. It is assumed that via inhibition of acetylcholinesterase (AChE), physostigmine increases the level of acetylcholine, which activates α-bungarotoxin-sensitive nicotinic alpha7 receptors on macrophages and immune cells [10,14–18]. This may result in attenuated release of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 [10,13].

In view of sepsis being a global health concern [19–21] and the CAP as a promising preclinical approach, the randomized, double-blind, placebo-controlled, monocentric trial (Anticholium® per Se) addressed the possibility of taking physostigmine from bench to bedside. The study, registered at the European Union Drug Regulating Authorities Clinical Trials database (EudraCT Number: 2012-001650-26) and ClinicalTrials.gov (NCT03013322), investigated treatment of physostigmine salicylate as an adjunctive therapy in patients with perioperative septic shock [22]. Primary results have been detailed elsewhere [23].

PK data on physostigmine are sparse, particularly for continuous infusion and in critically ill patients; both target plasma concentrations and the optimal dosage have not been defined. Thus, we here report results of the embedded pharmacokinetic/pharmacodynamic (PK/PD) study, including for the first time a population PK (popPK) and PD analysis of physostigmine in patients assigned to the intervention group of this clinical trial.

2. Material and methods

2.1. Trial design, participants and outcomes

Anticholium® per Se was a randomized (1:1), double-blind, placebo-controlled, monocentric pilot trial, investigating the effect of treatment with physostigmine salicylate as adjunctive therapy in perioperative septic shock. Detailed methods are described in the protocol [22]. The clinical trial was conducted in accordance with the Declaration of Helsinki and had been approved by the Federal Institute for Drugs and Medical Devices (BfArM) and the Ethics Committee, Medical Faculty of Heidelberg University (AFmu-447/2012). For all participants, informed consent was obtained from a legal guardian, a near family member to be designated legal guardian, or a guardianship judge prior to enrolment in the study [22,23].

Eligible patients were adults (aged 18–85 years) with perioperative septic shock due to intra-abdominal infection. Septic shock was defined as infection plus systemic inflammatory response syndrome (SIRS) criteria and requirement of vasopressor support [22,23]. Primary and secondary outcomes; i.e. the mean Sequential Organ Failure Assessment (SOFA) score during treatment and subsequent intensive care, 30- and 90-day mortality, and the occurrence of side effects; were recently published [23]. PK and PD endpoints including popPK modeling, exploratory analyses of PD and PK/PD correlations are reported here.

2.2. Interventions

According to a blocked randomization list, patients allocated to the physostigmine group received an initial dose of 0.04 mg/kg physostigmine salicylate as a short infusion (with a maximum dose of 4 mg), followed by continuous infusion of 1 mg/h (= 2.5 mL/h) for up to 120 h. The placebo group was treated with 0.9% sodium chloride, accordingly. No dose adjustments were made based on renal or hepatic function. Time, amount and rate of investigational medicinal product (IMP) administration, including time and duration of interruptions of trial medication, were recorded. All patients of the physostigmine group who had received continuous infusion for at least 48 h were included in the popPK and PD analysis.

Concomitant therapies were not restricted during the clinical trial; patients were treated according to the S-2 k guideline [24] of the German Sepsis Society (DSG) and German Interdisciplinary Association for Intensive Care and Emergency Medicine (DIVI), as considered local standard. Apart from surgical infectious source control, patients received empiric antibiotic therapy. Supportive measures included hemodynamic stabilization by volume restitution with crystalloid solutions, and use of vasopressors, mechanical ventilation and renal replacement therapy where necessary.

2.3. Data collection and sampling

Patients' demographics and clinical characteristics were recorded at baseline (visit 0, prior to randomization/treatment with IMP); further potential covariates for modeling were assessed as baseline data, secondary endpoints, or derived from clinical routine measurements over the course of the study. Data on intravenous fluid therapy were retrieved from patients' files and included total volume (infusions and transfusions) hourly (± 3 h of bolus infusion) and per 24 h (day prior to and until end of IMP administration).

For PK analyses, blood was collected on visit 1.1 (3 ± 2 min after the start, and at the end of the initial dose ± 2 min), during continuous infusion [visit 1.2 (10 ± 2 min, 20 ± 2 min, and 30 ± 2 min after the start of continuous infusion), visit 1.3 (after 1 h ± 10 min), visit 2 (2 h ± 30 min), visit 3 (24 ± 2 h), visit 4 (48 ± 2 h), visit 5 (72 ± 2 h), visit 6 (96 ± 2 h), visit 7.1 (at the end of the infusion period ± 2 min)] and after the end of IMP administration [visit 7.2 (10 ± 2 min, 20 ± 2 min, and 30 ± 2 min after the end of the infusion period), visit 7.3 (after 1 h ± 10 min and 2 h ± 10 min) and visit 8 (6 d ± 4 h)]; a flow chart of visits and blood sampling may be found in the Supplements (A1). Blood was drawn into heparinized tubes, previously spiked with neostigmine bromide to prevent degradation of physostigmine, and immediately centrifuged on the ward (10 min, 3000 rpm). The obtained plasma was then put on dry ice and stored at −70 °C until analysis. Plasma concentration of physostigmine and its metabolite eseroline were measured by high performance liquid chromatography (HPLC) as previously described [25], with an extended calibration range (0.05–100 ng/mL) for physostigmine. For both analytes, precision and accuracy were < 9% and < 11%, respectively.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity were measured with the point-of-care device ChE check mobile (Securetec, Neubiberg, Germany) [26,27]. Measurements were performed immediately after blood sampling (visits 0, 1.3 and 2 to 8) using 10 µL of whole blood from residual arterial blood gas tubes. For ChE check mobile, correlation with the reference method [28] is specified with $r^2 > 0.9$, precision < 5% and inter-operator variability < 7% [27].

IL-2, IL-6 and TNF-α were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits.

2.4. PopPK model

Dataset preparation was performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Data pre-analysis and graphical output was created in R, Version 3.4.0 (R foundation for statistical computing, Vienna, Austria) with additional use of the Xpose package, Version 4.5.3 [29–31]. Model building was performed using NONMEM, Version 7.3 (ICON Development Solutions, Ellicott City, MD) with the ADVAN 6 subroutine and the first-order conditional estimation (FOCE) +I estimation method. Visual predictive checks (VPC) and stepwise covariate model building (SCM) were performed with the Pearl-speaks-NONMEM (PsN) module, Version 4.6.0 [32,33].

The basic structural model was tested with linear one- and two-compartments models.

For nested models, the difference in the objective function value (OFV) was considered the best parameter to quantify model improvement. A drop in OFV of 3.84 corresponding to a 5% level of significance with a model change by 1 ° of freedom was defined to indicate an adequate model improvement.

Inter-individual variability (IIV) was tested for clearance terms, central and peripheral volume of distribution using exponential random effects. Additive-, proportional- and combined-error models were explored to best describe the residual unexplained variability.

The effect of potential covariates was consecutively tested on clearance terms, central and peripheral volume of distribution. Continuous covariates were documented time dependently; a linear interpolation was performed if no covariate was available at the time of physostigmine measurements. Continuous covariates included

- age, body weight, body surface area
- Acute Physiology and Chronic Health Evaluation (APACHE) II score at baseline
- creatinine, bilirubin, albumin
- scoring of organ dysfunction (daily SOFA score), renal replacement therapy
- parameters of infection (C-reactive protein, procalcitonin, IL-2, IL-6, TNF-α, body temperature)

To describe the changing fluid balances, the overall hydration at different time points throughout the study was assessed, taking hydration in the last 1, 2, 3, 4, or 5 h before the actual sampling time-point as potential covariates.

Gender was tested as categorical covariate.

Covariate testing was performed using SCM in PsN with a 5% forward inclusion and 1% backward elimination criterion. Model performance was evaluated by creating goodness of fit (GOF) plots. VPCs were created to test the predictive performance of the model during crucial steps of model building.

2.5. Cholinesterase activity and PK/PD investigations

Statistical analyses were performed with SAS, Version 9.4. PD outcomes were analyzed with descriptive and exploratory statistics.

Additionally, the Wilcoxon-Mann-Whitney test was used for group comparisons (physostigmine versus placebo); the Wilcoxon signed-rank test was applied to paired data comparisons (between visits). Associations between physostigmine plasma concentrations and PD parameters were investigated using Spearman's rank correlation coefficient (ρ), and included absolute values (e.g., cholinesterase activity) and ratios (e.g., enzyme activity corrected for and divided by baseline activity). The influence of hepatic function on cholinesterase activity was also assessed. Correlation coefficients > 0.5 were considered associations.

2.6. PD modeling and target evaluation

Based on the PK model, a consecutive PD model, using data from the physostigmine group only, was evaluated by testing a linear and a sigmoidal effect function, as well as a turn-over effect compartment model to account for lag-times in the hydrolytic regeneration of the enzyme. The individual PK parameters were derived from the final PK model. The best PD structural model was then further developed using data from the placebo group. For nested models, the difference in OFV was considered to quantify model improvement; non-hierarchical models were compared using the *Akaike* information criterion (AIC).

The subsequent model was tested for significant covariate effects with a 5% forward inclusion and 1% backward elimination criterion.

GOF plots were created. With the final PD model, VPCs were used to test the predictive performance of the model.

3. Results

3.1. Patients

Twenty patients were enrolled in Anticholium® per Se between January 2015 and February 2017. All patients assigned to the physostigmine group (n = 10) were included in the popPK and PK/PD analysis; analysis of cholinesterase activity and PD modeling additionally involved the placebo group (n = 10).

Demographic data and baseline characteristics of the physostigmine group are shown in Table 1.

3.2. Treatment and plasma concentrations

The median initial dose of physostigmine salicylate was 3.7 mg (range 2.6–4.0 mg), and median duration of IMP administration in the physostigmine group was 119.3 h (range 54.3–120.2 h). There were no deviations from infusion rates, no dose adjustments and no preliminary terminations of trial medication. For two subjects, minor interruptions of IMP infusions of less than 0.4 h were recorded [23].

Physostigmine plasma concentrations during visit 3–7 (steady state) were 7.43 ng/mL median (range 2.79–15.22 ng/mL); all measurements, including those immediately after the initial bolus, ranged from 0.33 ng/mL to 77.56 ng/mL for physostigmine.

During visit 3–7, eseroline plasma concentrations were 1.13 ng/mL median (range 0.29–3.32 ng/mL; for all time points, measured values did not exceed 3.45 ng/mL for eseroline. Physostigmine and eseroline plasma concentrations showed no association except for visit 4 (ρ = 0.886, p = 0.019).

Table 1
Patients' characteristics for the popPK analysis.

Parameter	Physostigmine group	Placebo group
Age, median (range), years	63 (50–79)	59.5 (39–79)
Gender (males/females)	9/1	8/2
Body weight, median (range), kg	91.5 (65–170)	78.5 (54–113)
SOFA score at baseline, median (range)[a]	12 (7–15)	13.5 (9–21)
APACHE II score at baseline, median (range)[b]	30.5 (21–40)	33.5 (16–38)
PK samples, No.	146 (physostigmine), 117 (eseroline)	

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; PK, pharmacokinetic; SOFA, Sequential Organ Failure Assessment.

[a] Scale for SOFA score ranges from 0 to 24, with higher scores indicating greater severity of organ failure; calculated with suspected GCS (Glasgow Coma Scale) score if patients were sedated.

[b] Scale for APACHE II score ranges from 0 to 71, with higher scores indicating greater severity of illness.

3.3. PopPK model

The basic structural PK model consisted of two compartments with a combined proportional and additive error model. Introducing IIV on clearance and central volume of distribution was found to significantly improve the model.

For these variabilities, a full covariance matrix was estimated, but did not result in a significant model improvement. Covariate testing was performed using SCM in PsN. Additionally, due to the limited number of patients and potential bias in SCM, covariate diagnostic plots of variability parameters against all documented covariates were reviewed.

A linear covariate effect of body weight on clearance, as well as age on clearance was found to improve the model significantly, according to Eq. (1):

$$CL = \theta_{CL} * e^{\eta_{BASE}} * (1.02 * (Weight - 70)) * (0.98 * (Age - 60)) \quad (1)$$

No other covariates had a significant impact on clearance or volume of distribution in our model. Of note, the marked difference in the patients' intravenous hydration profile could not explain the high IIV detected for the central volume of distribution.

The final PK model parameters are given in Table 2.

Table 2
Population-based pharmacokinetic parameters of the final model. Clearance estimated for a typical individual represented by a body weight of 70 kg and aged 60 years.

Model parameter	Estimate (% RSE)	IIV (% RSE)
Clearance [L/h]	107 (10)	14.6 (31)
Central volume of distribution [L]	34.9 (44)	134 (20)
Peripheral volume of distribution [L]	105 (9)	–
Intercompartmental clearance [L/h]	403 (8)	–
Additive residual error [ng/mL]	0.34 (27)	–
Proportional residual error [%]	23 (13)	–
Weight on clearance	0.02 (17)	
Age on clearance	–0.02 (8)	

Abbreviations: IIV, inter-individual variability; RSE, relative standard error.

A VPC illustrates the predictivity in the continuous infusion setting of the clinical trial (Fig. 1); GOF plots for observed data versus individual and population predictions may be found in the Supplements (A2).

3.4. Cholinesterase activity and PK/PD investigations

During treatment with IMP (visit 1–7), AChE activity was significantly lowered in the physostigmine group, whereas in the placebo group, enzyme activity was virtually constant over time (Fig. 2).

In the physostigmine group, AChE measurements distinguished well between baseline activity and enzyme inhibition, with median (interquartile range [IQR]) activities of 39.3 (34.0–45.2) and 14.9 (9.4–21.2) U/gHb before (visit 0) and under treatment (visit 1.3), respectively. Median AChE activities under physostigmine salicylate infusion were below 18.6 U/gHb for all time points, indicating a strong decrease/enzyme inhibition, while median AChE activities in the placebo group did not fall below 26.6 U/gHb. In relation to baseline enzyme activity, median AChE activity was reduced to 30.5%–50.6% during physostigmine salicylate infusion.

Accordingly, BChE activity was lower in the physostigmine group compared with the placebo group (Fig. 3). In the physostigmine group, median (IQR) BChE activities were 1109.5 (811.0–1603.9) and 539.6 (413.1–641.6) U/L at baseline and after the first hour under treatment, respectively. In the placebo group, median (IQR) BChE activities at baseline were 1378.9 (1106.1–1587.6) U/L and did not exceed 1609 U/L (cut-off for strong decrease/enzyme inhibition) over the entire study.

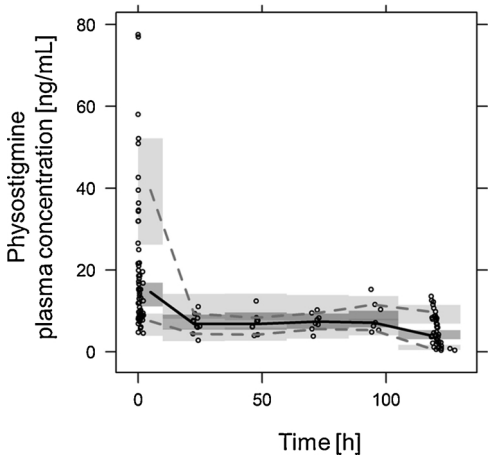

Fig. 1. Population pharmacokinetic model. Visual predictive check (final model). Solid line median of the observations; dashed lines 5% and 95% percentiles of the observations; shaded areas 95% confidence intervals for simulated data (1000 simulated data sets) for the corresponding percentiles.

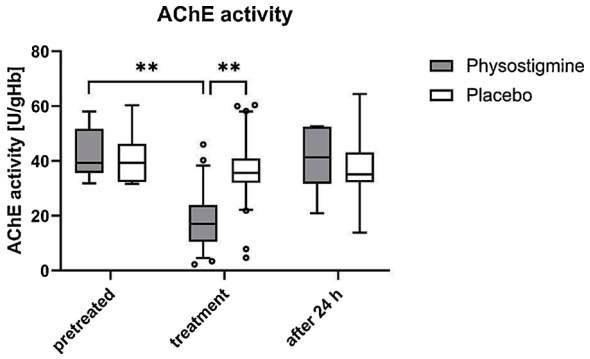

Fig. 2. Acetylcholinesterase activity in physostigmine and placebo group. Boxplots represent lower and upper quartiles, solid lines median, whiskers 5% and 95% percentile. Values outside the whiskers are plotted individually.

Median residual BChE activity during physostigmine salicylate infusion was between 48.7%–71.3% of the baseline enzyme activity.

Mean steady state physostigmine plasma concentrations (visit 3–7) were inversely correlated with mean AChE activity ($\rho = -0.750$, $p = 0.020$). For BChE activity, the association with physostigmine plasma concentrations was less clear. We found no association between BChE activity and APACHE II score or SOFA hepatic subscore at baseline; however, a high level of bilirubin was positively correlated with BChE activity at visit 0 ($\rho = 0.647$, $p = 0.003$).

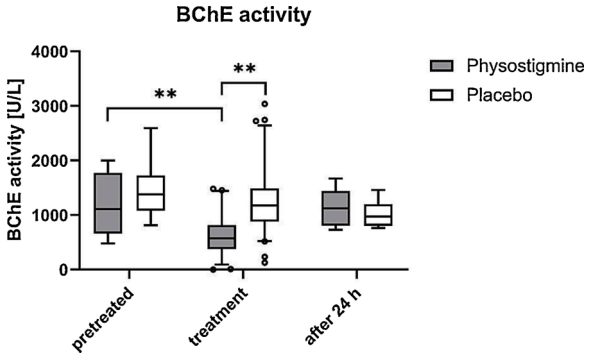

Fig. 3. Butyrylcholinesterase activity in physostigmine and placebo group. Boxplots represent lower and upper quartiles, solid lines median, whiskers 5% and 95% percentile. Values outside the whiskers are plotted individually.

3.5. PD modeling and target evaluation

Starting with the data of the physostigmine group only, the sigmoidal direct effect PD model best described inhibition of AChE activity by physostigmine. As of the highest AIC, the linear direct effect model showed the worst fit. Compared to the sigmoidal direct effect model, AIC, VPC, as well as parameter precision and GOF plots did not show a significant improvement through addition of the turn-over compartment. The combined residual error model was a significant improvement to either of the structural models. Thus, the sigmoidal direct effect PD model was implemented. IIV was tested for baseline AChE activity and the half maximal effective concentration (EC_{50}); only the IIV on the baseline parameter was of significance in the observed population.

The placebo effect supplied additional information on baseline AChE activity and variability. A time dependent change in slope for baseline activity was assessed as well, but did not improve the model significantly.

For the PD model containing physostigmine and placebo effect, visual covariate exploration of IIV versus covariates showed an association of body weight and baseline AChE activity; additionally, a link between the SOFA score and baseline AChE activity was observed (Fig. 4). Although the forward inclusion step detected a model improvement on a 5% level of significance for both, highly unprecise parameter estimates indicated uncertainty of the resulting model, thus the covariates were omitted in the final PD model.

No other covariates had a significant impact on the PD effect parameters in our model. Parameters of the final PD model are shown in Table 3; a VPC stratified for physostigmine and placebo group is shown in Fig. 5, GOF plots may be found in the Supplements (A3).

Table 3
Final pharmacodynamic model.

Model parameter	Estimate (% RSE)	IIV (% RSE)
Baseline activity [U/gHb]	38.1 (5)	20 (16)
EC_{50} [ng/mL]	5.99 (18)	
Proportional residual error [%]	17.3 (32)	
Additive residual error [U/gHb]	5.02 (33)	

Abbreviations: Hb, hemoglobin; EC_{50}, half maximal effective concentration; IIV, inter-individual variability; RSE, relative standard error; U, unit.

4. Discussion

Physostigmine plasma concentrations in this study showed a wide variability during bolus infusion, but under continuous infusion and steady state conditions, comparable and reasonable levels were reached in all ten patients.

The PK data were best described by a linear two-compartment model with a combined-error model; model fit was improved by inclusion of the covariates body weight and age on clearance. However, a high IIV of the central volume of distribution was detected. This was to be expected in critically ill patients, as it is well known that pharmacokinetics may be affected by diseases such as septic shock [34–36]. Altered drug distribution due to compromised tissue perfusion, enhanced capillary permeability and fluid extravasation, as well as changes in plasma protein binding and body water are possible reasons for variations in the volumes of distribution [34]. Organ dysfunction, including sepsis-induced liver hypoperfusion and liver failure, may contribute to an altered metabolism and decreased clearance of high-extraction drugs [34]. In addition to pathophysiological changes, therapeutic interventions such as excessive fluid administration can alter the drug distribution and further PK properties of a drug [34,35].

Thus, intravenous infusion volumes administered were tested as covariates on the central volume of distribution in the PK model, but no improvement of fit was found. Although seven blood sampling time points were implemented during the first 2 h of IMP treatment, covering the anticipated time to steady state concentrations, additional PK data during the first 24 h may have further improved the model. As none of the patients in the physostigmine group had received RRT, this potential covariate could not be evaluated.

To the best of our knowledge, this is the first study performing popPK modeling for physostigmine. Even in healthy young volunteers [37] and patients with Alzheimer's disease [38], the variability found in PK parameters was high. Hartvig et al. [39] investigated pharmacokinetics of physostigmine in neurosurgical patients after extubation; after intravenous administration of 1 mg physostigmine, plasma clearance ranged from 47 to 163 L/h and volume of distribution ranged from 14 to 74 L, respectively. With an open two-compartment linear model, large inter-individual differences in PK parameters were found in their study, but no relationship between clearance, volume of distribution, elimination rate and age or body weight was identified, which is in contrast to our findings.

Steady state plasma concentrations in our study (7.60 ± 2.81 ng/mL [mean ± SD]) slightly exceed the range of 3–5 ng/mL, recommended for antagonism of postoperative somnolence by Hartvig et al. [39]. However, in view of limited PK studies and the fact that physostigmine plasma concentrations are not assessed in clinical routine, it is largely unknown whether this target level is achieved and by which dose. In case of a female patient (age 45, weight 65 kg) [40], who received an initial bolus of approximately 0.57 mg, followed by 0.92 mg/h of physostigmine, infusion rates had been calculated based on the authors' previous data [39], aiming plasma concentrations of 10 ng/mL at steady state. Observed steady state concentrations in their

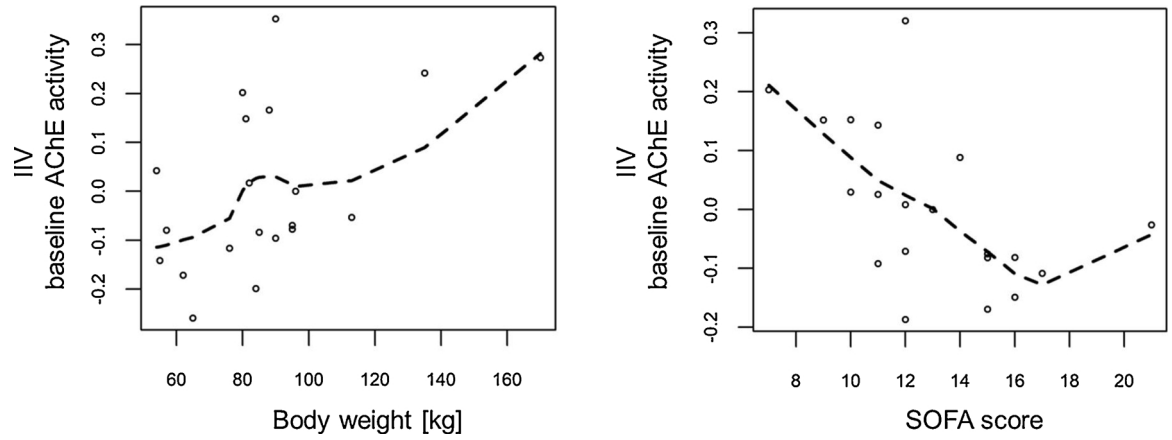

Fig. 4. Pharmacodynamic model. Covariate effect of body weight and the Sequential Organ Failure Assessment (SOFA) score on unexplained variability in baseline acetylcholinesterase activity, expressed as eta.

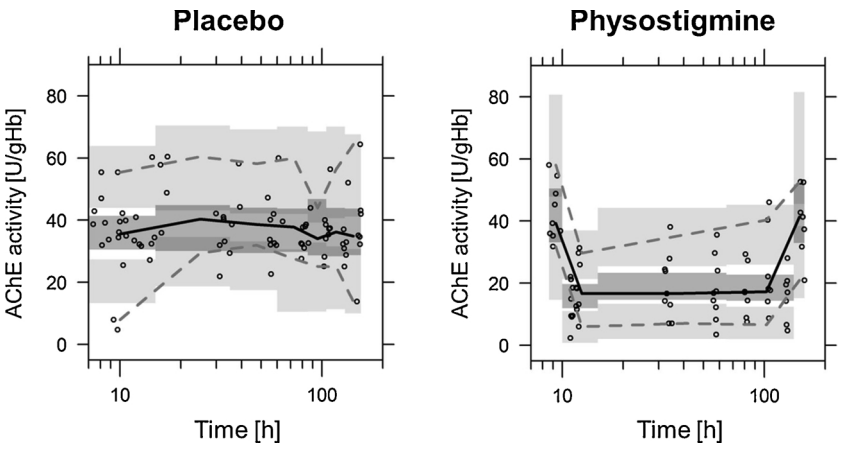

Fig. 5. Pharmacodynamic model. Visual predictive check (final model) for acetylcholinesterase activity versus time, stratified for placebo and physostigmine group. Solid line median of the observations; dashed lines 5% and 95% percentiles of the observations; shaded areas 95% confidence intervals for simulated data (1000 simulated data sets) for the corresponding percentiles.

study were 5.2 ng/mL and calculated plasma clearance approximately 225 L/h. While the bolus infusion accounted for about 1/6 of the initial dose in our study, the continuous infusion rates and steady state plasma concentrations were comparable. Thus, though the bolus was well tolerated in the present study [23], a high initial dose might not be necessary to obtain these concentrations.

Unlike the initial dose, continuous infusion in our study was not adjusted to body weight; an impact of body weight on physostigmine clearance was observed in the popPK model. However, as obvious by the factor 0.02 (Table 2), the impact of body weight is not very pronounced, indicating that dose adjustment based on body weight may not be necessary.

AChE activity was confirmed as a suitable parameter to monitor enzyme inhibition as a direct and prompt PD effect of physostigmine. While normal values for AChE activity are in the range of 26.7–50.9 U/gHb, slightly lowered AChE activity is indicated by ≤ 26.6 U/gHb, and a strong decrease, i.e. enzyme inhibition, by ≤ 18.6 U/gHb [27]. Median values during the treatment phase were below the cut-off for enzyme inhibition in the physostigmine group, but not in placebo group.

In contrast, regardless of treatment group, median BChE activity was below the cut-off for enzyme inhibition (1609 U/L), and the majority of patients remained below normal enzyme activities (2300–7000 U/L) throughout the study. Thus, although BChE activity under treatment was lower in the physostigmine group compared to the placebo group, BChE measurements failed to clearly differentiate between inhibited and non-inhibited enzymes in the population studied here. The underlying disease, sepsis, critical illness, and attenuated liver function may be explanations for the reduced enzyme activities observed [41–44]. However, in our study, no association of BChE activity and surrogates for disease severity (APACHE II score) or liver failure (SOFA hepatic subscore) was obvious, while patients with elevated BChE activity even had higher levels of bilirubin.

Regarding PK/PD correlations, the strongest association of physostigmine plasma concentrations and AChE activity was found when steady state concentrations had been reached. In addition, excessive fluid resuscitation and vasopressor use, characterizing the early phase of septic shock, were reduced at later time points.

Compared to literature [38,45], correlations between physostigmine concentrations and AChE or BChE inhibition found in our study were lower than anticipated. Of note, comparison to other data is impeded by differences in patients' characteristics and statistical methods. In a study with healthy male volunteers receiving oral physostigmine [45], approximately 30% inhibition of AChE activity was already reached at plasma concentrations of about 1 ng/mL, suggesting that concentration-response linearity may be limited to plasma concentrations lower than those observed in our study. Similarly, patients with Alzheimer's disease treated with physostigmine salicylate intravenously [33] showed nearly 45% inhibition of BChE at plasma concentrations of 3 ng/mL physostigmine. Further PK/PD data have been reported from animal models for AChE [46] and BChE [47–50]; however, remarkable interspecies differences in basal enzyme activity and inhibitory properties were found studying human and rat cholinesterases *in vitro* [51], thus precluding extrapolation of animal data to the clinical setting.

The AChE baseline effect estimate of our PD model is comparable to reference data based on AChE activity assessments in 242 healthy volunteers [52]. The authors found a marked IIV, but AChE activity only appeared to be dependent of age and gender [52]. Additionally, PD models developed for AChE inhibition by another cholinesterase inhibitor, pyridostigmine, were unable to identify a significant impact of covariates on PD parameters as well [53,54].

Estimate for EC_{50} derived from our PD model was 5.99 ng/mL for a typical individual, which is slightly below median physostigmine steady state plasma concentrations observed during continuous infusion in our study, and in good agreement with the target concentration suggested by Hartvig et al. [31]. The aforementioned study assessing AChE activity [45] has not reported EC_{50} estimates; plasma concentrations were lower than in our study and maximum enzyme inhibition did not exceed 35%. However, data from *ex vivo* inhibition of human erythrocytes, incubated with physostigmine, suggested an EC_{50} in the range of 6.7–7.4 ng/mL [55,56], which is in line with our findings.

5. Conclusions

Plasma concentrations resulting from physostigmine salicylate infusion (initial bolus and continuous infusion) in patients with septic shock were found to be sufficient for about 50%–70% inhibition of erythrocyte AChE activity. With regard to the rapid onset of cholinesterase inhibition and steady state concentrations, a high initial bolus might be dispensable. AChE inhibition resulting from the observed physostigmine plasma concentrations showed a sigmoidal concentration-response effect.

Although unexplained variability of the central volume of distribution was high, a linear two-compartment popPK model with inclusion of the covariates age and body weight on clearance was predictive for physostigmine plasma concentrations during continuous infusion.

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Authors' contributions

NP, JBZ and SS conceptualized the study and made substantial contributions to the interpretation of data for the work. NP conducted the study, including acquisition of data for the work, and drafted the manuscript. JBZ acted as principle investigator. SG, GW and GH wrote the NONMEM models and revised the manuscript; TB was involved in statistical analysis. THT and MAW made substantial contributions to the design of the study. All authors have read and approved the final manuscript.

Conflict of interest

NP, JBZ, SG, GW, GH, TB, THT and SS declare no competing interests. MAW has served as speaker for Dr. Franz Köhler Chemie GmbH. The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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Appendix A. Supplementary data

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