JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1187–1196

Received February 05, 2009
Accepted June 08, 2009
DOI 10.1211/jpp/61.09.0008
ISSN 0022-3573

Research Paper

Retrospective population pharmacokinetic/pharmacodynamic analysis of pyridostigmine, a cholinesterase inhibitor, in Chinese males

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Abstract

Objectives We have characterised the population pharmacokinetics–pharmacodynamics of pyridostigmine given as pyridostigmine bromide.

Methods Over three days 50 healthy Chinese male subjects each received seven doses of 30 mg pyridostigmine bromide orally (3×10 mg every 8 h). Plasma concentrations of pyridostigmine and red blood cell acetylcholinesterase (AChE) activity were determined at various times within the eight hours after the first and the seventh doses. The resulting pharmacokinetic data were fitted to a single compartment open model with first-order absorption and elimination. The pharmacodynamics were modelled using an inhibitory E_{max} model. The potential influence of demographic and biological covariates on the model parameters was investigated. Nonlinear mixed effects modelling was performed using NONMEM.

Key findings The apparent clearance and volume of distribution as well as absorption rate constant of plasma pyridostigmine were estimated to be 136 l/h, 130 l and 0.68 1/h, respectively. The maximum red blood cell AChE activity decrease (E_{max}) and plasma pyridostigmine concentration producing 50% of this reduction (*EC50*) were estimated to be 9.32 AChE units per gram haemoglobin and 51.9 ng/ml, respectively. None of the tested covariates were found to be correlated with any of the model parameters. Dosing simulations suggested that 30 mg repeated every six hours might be needed to achieve steady-state trough percentage inhibition above the recommended 10% in healthy Chinese males.

Conclusions The pharmacokinetics and the effects of pyridostigmine on red blood cell AChE activity were described using a mixed effects model. For Chinese males, the dosing interval may have been shorter than that recommended for the Caucasian population. Additional studies are needed to confirm these findings.

Keywords mixed effects model; pharmacodynamics; pharmacokinetics; pyridostigmine; stochastic simulation

Introduction

The potential for exposure to nerve agents exists on the battlefield and as a terrorist threat, e.g. 1995 Tokyo subway incident. Nerve agent exposure causes a progression of toxic signs including muscle fasciculations, tremors, hypersecretions, convulsions and respiratory distress. This is directly linked to the inhibition of acetylcholinesterase (AChE) by the agent and the subsequent increase in the concentration of the neurotransmitter acetylcholine (ACh) and hyperstimulation of the cholinergic system at central and peripheral sites.[1] A combined regimen of prophylaxis and therapy is considered the most effective medical countermeasure for dealing with the threat of nerve agent poisoning to military personnel.[1–3]

With regards to the prophylactic side of medical countermeasure, one of the approaches is to sustain AChE levels by treatment with pyridostigmine bromide

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(3-dimethylaminocarbonyloxy-*N*-methyl pyridinium bromide). This carbamate cholinesterase inhibitor shields a fraction of AChE in the periphery from irreversible inhibition by the nerve agents.[4,5] It has been used for more than 40 years in the routine treatment of myasthenia gravis and may be used following surgery in the reversal of neuromuscular blockade. Following exposure to nerve agents, immediate treatment with an anticholinergic drug, such as atropine sulfate, will antagonise the effects of excess ACh at muscarinic receptor sites, and an oxime, such as pyridine-2-aldoxime methylchloride (2-PAM), is used to reactivate inhibited AChE.[6,7] Pyridostigmine bromide was approved by the US Food and Drug Administration (FDA) under its 'animal efficacy rule' for use during the 1990–1991 Gulf War to protect soldiers against possible attack by soman.[8–10]

Despite the approval of the FDA two decades ago, relatively limited pyridostigmine bromide pharmacokinetic and pharmacodynamic data have been published.[11–13] These formal trials either contributed a small number of subjects or a small number of samples, which did not allow identification of potential covariate relationships, i.e. important demographic and/or biological determinants of pyridostigmine pharmacokinetics and pharmacodynamics. One population pharmacokinetic/pharmacodynamic study was previously reported for pyridostigmine bromide.[14] The data were obtained from Caucasian subjects and only demographic covariate information was considered during model development. Unfortunately, the baseline effect attributable to pyridostigmine bromide was not directly estimated. The relevancy of dosing regimens identified for the Caucasian population to Chinese subjects and identification of other potential covariate relationships also remain to be determined.

The overall aim of this population analysis was to develop a model characterising the pharmacokinetic/pharmacodynamic of pyridostigmine bromide when given as 3×10 mg tablets every eight hours. An accurate description of the typical pharmacokinetic/pharmacodynamic profile and of different variability types was to be provided. In addition, an attempt was made to identify subject-specific characteristics to explain the variability of the pharmacokinetic/pharmacodynamic parameters and to further guide dosage regimen decisions for following trials. The suppression of the red blood cell (RBC) AChE activity was selected as a pharmacodynamic endpoint because it has been shown to correlate with survival following nerve agent intoxication in primate models.[15]

Materials and Methods

Study design, test volunteers and pyridostigmine bromide regimen

The study was approved by the institutional review board, and written informed consent was obtained from all participants. A total of 50 adult Chinese volunteers were included in this single-centre, non-randomized, open-label investigation. The subjects were judged to be healthy by a complete medical history, physical examination, and normal haematological and biochemical values. Various demographic as well as clinical and laboratory parameters of liver and renal functions were documented for all of the subjects (Table 1). Creatinine clearance (CL_{CR}) was calculated according to Schwartz's formula.[16] Body surface area was calculated according to DuBois and DuBois.[17]

Table 1 Variables for the study population in the pyridostigmine pharmacokinetic and pharmacodynamic analysis

Characteristic	Mean (± SD)	Range
Age (yr)	27.48 ± 5.98	19–40
Body mass index (kg/m^2)	22.22 ± 2.73	17.4–29.8
Height (m)	1.73 ± 0.06	1.58–1.91
Weight (kg)	66.92 ± 3.67	50.8–96.6
Creatinine clearance (ml/min)	106.37 ± 19.01	72.66–158.87
Total bilirubin (μmol/l)	16.02 ± 4.49	7.9–25
Alkaline phosphatase (U/l)	65.5 ± 14.79	42–104
Alanine transaminase (U/l)	21.9 ± 11.03	10–56
Aspartate transaminase (U/l)	21.12 ± 4.71	13–38
Gamma glutamyl transpeptidase (IU/l)	18.18 ± 9.27	7–58
Red blood cell count ($\times 10^6/\mu$l)	5.14 ± 0.3	4.3–5.8
Haemoglobin (g/dl)	15.36 ± 0.8	13.5–16.7
Haematocrit (%)	45.07 ± 2.27	39.1–49.1

Demographic, clinical and laboratory variables were measured. The pyridostigmine data were from a pooled population.

Subjects were admitted to the study site the day before the administration of the study drug. Subjects were orally dosed with 3×10 mg pyridostigmine bromide tablets (Mestinon, ICN Pharmaceuticals Switzerland Ltd, Birfelden, Switzerland) every eight hours for three days. A total of seven doses were administered to each volunteer under this multiple dose regimen. Subjects were allowed only water for eight hours before the initial and the final doses and no liquids two hours before and after all doses. Subjects were allowed to eat four hours after dosing. During the study, an identical diet was provided to all subjects.

Profiling of plasma pyridostigmine and red blood cell acetylcholinesterase activity levels

For each clinical trial, blood samples (10 ml each) were withdrawn just before administration of the first oral dose of pyridostigmine bromide and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 6 h post-dose. From each subject, blood samples were also obtained at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 6 h after the seventh dose.

Pharmacokinetic assay

Blood samples were vortexed for 10 s and centrifuged for 3 min at 1000*g* (Wortex). The plasma samples were stored at –22°C until analysis by a validated high-performance liquid chromatography (HPLC) system. The HPLC system was composed of a Waters Alliance 2690 separations module with a Waters 996 diode-array detection system set at 280 nm (Waters Corporation, Milford, MA, USA). After addition of the internal standard (paracetamol), 0.5 ml plasma was extracted with isopropyl alcohol. The organic layer was evaporated and reconstituted in solution of acetonitrile/Milli-Q water in pH 3.0 (20 : 80, v/v) before the injection of a sample into the HPLC system. Samples (0.1 ml) were

separated on a Waters SymmetryShield Cartridge Column RP18 (250 × 4.6 mm i.d.; particle size, 5 μm), using a gradient of 2–20% acetonitrile in Milli-Q water (pH 3.0) at a flow-rate ranging from 0.8 to 1.2 ml/min over 15 min.

Samples were quantified using peak area ratio of pyridostigmine over the internal standard. Standard calibration curves were constructed using drug-free pooled plasma mixed with a known amount of pyridostigmine. These plasma standards were used also to determine the extraction recovery, within-day and between-day precision and accuracy (n = 5) of the method. The recovery of the extraction procedure was calculated by comparing the peak area obtained after extraction with that of aqueous drug solution of corresponding concentration without extraction. Limits of detection and quantitation were determined by considering the ratio of baseline noise to calibration point (i.e. 1 : 3 and 1 : 10, respectively). The lower limit of quantitation was repeated five times for confirmation. In the assay method, calibration standards demonstrated acceptable linearity (r^2 = 0.999). The within-day and between-day coefficients of variation (CV%) for the control standards were ≤ 10.5 and 8.0%, respectively. The limit of detection of the assay was 1 ng/ml. The lower limit of quantitation of the assay was 6 ng/ml. The clean chromatogram showed no interference from endogenous substances in the plasma sample.

Acetylcholinesterase activity inhibition assay

To separate RBCs from plasma, 4-ml blood samples were centrifuged for 10 min at 500*g* (Wortex). Collected RBC sediments were resuspended in an appropriate dilution of red blood cell lysing buffer solution to solubilise RBC AChE and achieve a homogenous analysis matrix. Tenfold and 16-fold serial dilutions were made using 0.1 M NaH_2PO_4 buffer (pH 7.4) plus 0.1% Triton X-100 and 0.1 M NaH_2PO_4 buffer (pH 7.4), respectively, after which 50 μl of the diluted RBC was transferred to the 96-well microplate plate. Assay reagents consisted of 50 μl acetylthiocholine iodide (ASChI), 25 μl 4,4′-dithiodipyridine (PDS) and 50 μl diluted RBC. The AChE activity was measured at 324 nm (kinetic) at 37°C using a spectrophotometric microplate reader. RBC activity was measured spectrophotometrically using a modified Augustinsson assay technique to minimise spontaneous enzyme decarbamoylation reactions.[18] To account for the interference reaction of PDS chromophore with blood glutathione and sulfhydryl (SH) groups, control wells were included where the ASChI was substituted with reaction buffer. Each blood sample assay included its own corresponding control to correct for blood matrix SH group reacting with PDS. For the determination of haemoglobin, 1 : 400 dilution of each RBC sample was read at 450 nm to obtain the haemoglobin content (g/dl). Haemoglobin reading was determined concurrently with AChE assay. AChE activity (U/ml) obtained was normalised with this haemoglobin content to obtain AChE units per gram haemoglobin (U/g Hb). The limit of detection of the AChE activity assay was 0.98 U/g Hb. The intraday CV was < 3.5% while the maximum CVs between plates run on consecutive days were 3.7% and 5.1% for uninhibited and 50% inhibited blood, respectively.

Population pharmacokinetic and pharmacodynamic models

Compartmental analysis

The plasma pyridostigmine concentration–time profiles were simultaneously fit using the nonlinear regression program NONMEM (v6, NONMEM Project Group, University of California, San Francisco, CA, USA), interfaced with PDx-Pop (v2.2a, GloboMax LLC, Hanover, MD, USA) in conjunction with a G77 compiler. A structural base, i.e. covariate-free, model was first developed. The pharmacokinetic model employed was a one-compartment open model with first-order absorption and elimination. This model was implemented using the ADVAN2 and TRANS2 PREDPP subroutines within NONMEM. Model-to-data fitting was performed using the first-order conditional estimation method (FOCE) with interaction. The structural pharmacokinetic parameters that were estimated in this analysis were absorption rate constant (K_a), apparent plasma oral clearance (CL/F) and apparent volume of distribution after oral administration (Vd/F). Interindividual variability and inter-occasion variability in pharmacokinetic parameters were modelled using exponential error models:

$$\theta_{ij} = \theta \times \exp(\eta_i + \eta_j) \quad (1)$$

where θ_{ij} is the ith individual value of the parameter on the jth occasion, θ the typical value in the population, η is the random effect. Both η_i and η_j with assumed mean zero were symmetrically distributed variables with standard deviation ω_y and ω_z, respectively; ω_z was assumed to be time-independent. The covariance of the parameters was studied during the modelling process. Residual variability was best described by a standard combined additive/proportional error model.

The screening and selection of covariates was performed classically.[19] Individual pharmacokinetic parameters obtained using the post hoc option were plotted against: demographic characteristics (age, bodyweight, body surface area, body mass index (BMI)) and biological factors (serum creatinine, CL_{CR}, serum albumin, RBC count density and haematocrit) (Table 1). For these covariates, only those presenting a significant correlation with pharmacokinetic parameters were tested in the model. Each selected covariate was first tested by a univariate NONMEM analysis to confirm its relevance. A decrease in the objective function value of at least 6.64 units (corresponding to $P \leq 0.01$) was first required to consider the covariate in the intermediate model. In a second step, all such significant covariates were added simultaneously into the intermediate model, and then each covariate was independently removed from the full (intermediate) model to confirm its relevance (backward deletion strategy), and the resulting variation in objective function value (compared with the full model) examined. An increase in objective function value of more than 10.9 (P < 0.001) was required to confirm the significance of the covariate and take it into account in the final model. The performance of the final model was also evaluated informally with standard diagnostic plots of observed concentration vs model-predicted concentration and plots of weighted

residuals vs population model-predicted concentration, subject identification, and screened covariates.[20]

Red blood cell acetylcholinesterase activity

A nonlinear mixed effects modelling approach using NONMEM was utilised for the analysis of the RBC AChE activity vs time data. Individual pharmacokinetic parameters were fixed in the pharmacodynamic model. In the pharmacodynamic model, individual AChE activity values were directly related to plasma concentrations of pyridostigmine using an inhibitory E_{max} model as shown below:

$$E = E_0 - \frac{E_{max} \times C}{EC50 + C} \quad (2)$$

where E is the RBC AChE activity at the time of measurement, E_0 is the basal RBC AChE activity in the absence of pyridostigmine, E_{max} is the maximum inhibition of RBC AChE activity, $EC50$ is the plasma pyridostigmine concentration required for half-maximal inhibition and C is the predicted concentration of pyridostigmine.

The influence of the covariates on the pharmacodynamics of pyridostigmine was assessed using the methodology described previously for the selection of the final pharmacokinetic model. As per the statistical model for the pharmacokinetics, interindividual error in all pharmacodynamic model parameters and residual variability of percentage AChE activity were best described by an exponential variance model and a combined additive/proportional error model, respectively.

To check the robustness of the final model and its ability to predict the data, the visual predictive check method was used. This approach is similar to the previously described posterior predictive check, but assumes that parameter uncertainty is negligible relative to interindividual and residual variance.[21] The basic premise is that a model and parameters derived from an observed data set should produce simulated data that are similar to the original observed data. The visual predictive check is a useful adjunct to typical diagnostic plots, in that it provides information about the performance of random-effects parameter estimates, whereas typical diagnostic plots are primarily informative about the fixed-effects parameter estimates. The visual predictive check model evaluation step was performed by using the final pharmacokinetic/pharmacodynamic model and its parameter estimates to simulate data under the same experimental design of the original data.

Five hundred Monte Carlo simulation replicates of the original dataset were generated using the final model. Distributions of the simulated plasma pyridostigmine concentrations and RBC AChE activity values were compared with those in the observed dataset. The simulated data from each of the 500 virtual trials were assembled, and the similarity between the actual observed data and simulated data was examined by comparing the 95% prediction intervals of the simulated data with the original observed data.

Results

Population pharmacokinetic analysis

In total, 879 plasma pyridostigmine concentration–time points and 1100 RBC AChE activity–time points (Figure 1) were available from the subjects for analysis. The data

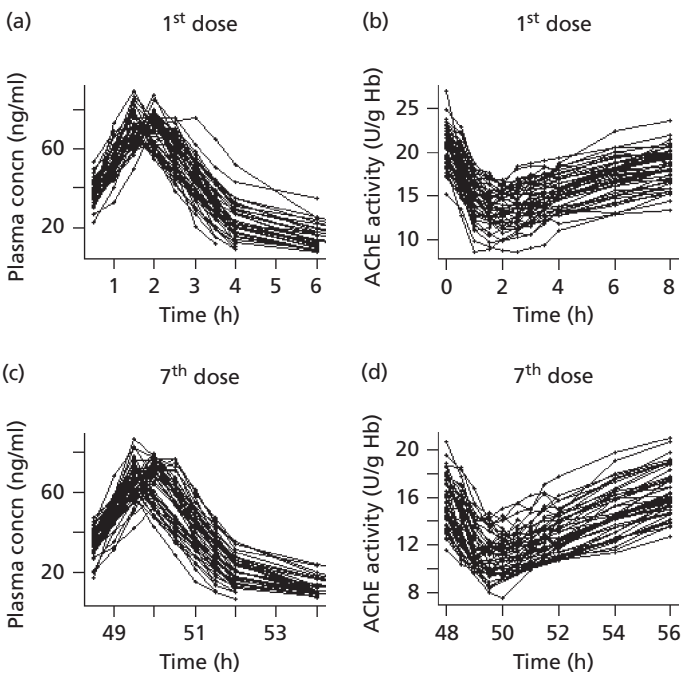

Figure 1 Plasma pyridostigmine concentration vs time (a,c) and red blood cell acetylcholinesterase activity vs time (b,d) plots. The plots shown are following the 1st (a,b) and the 7th (c,d) doses (3 × 10 mg pyridostigmine bromide) included in the population pharmacokinetic and pharmacodynamic analyses. AChE, acetylcholinesterase.

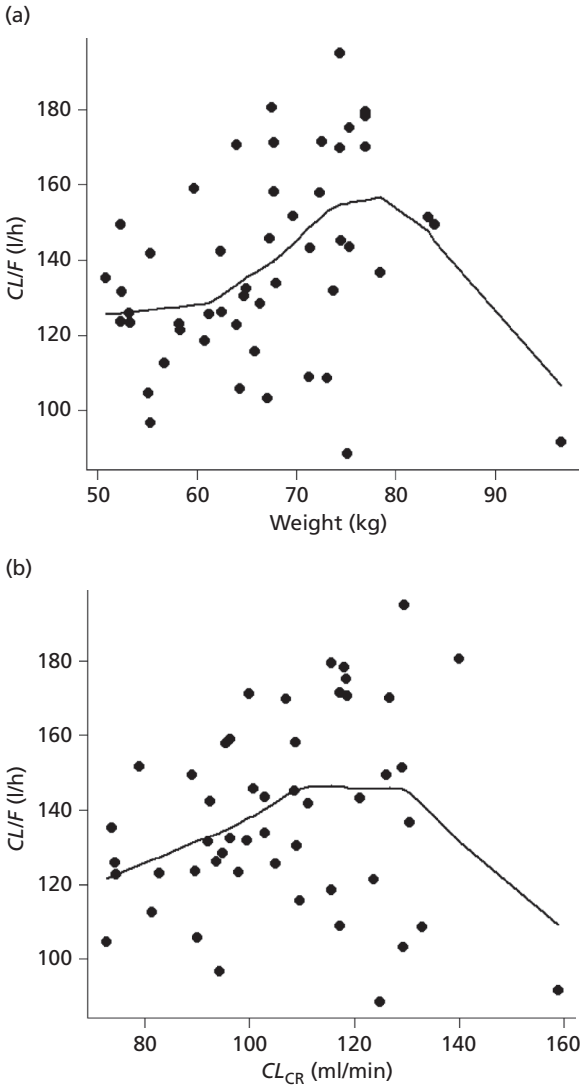

Figure 2 Plots of pyridostigmine apparent oral clearance vs bodyweight and creatinine clearance. Subjects were administered seven doses of 30 mg pyridostigmine bromide orally (3 × 10 mg every 8 h). The plots show apparent oral clearance (*CL/F*) vs (a) bodyweight and (b) creatinine clearance (CL_{CR}). The continuous lines are local regression smooths.

Table 2 Parameter values for the final population pharmacokinetic and pharmacodynamic model for pyridostigmine

Parameter	Estimate (%SE)
Pharmacokinetics	
CL/F (l/h)	136 (2.85)
Vd/F (l)	130 (5.08)
K_a (1/h)	0.68 (6.04)
Lag time (h)	0.223 (4.75)
Pharmacodynamics	
E_0 (U/g Hb)	17.8 (1.65)
E_{max} (U/g Hb)	9.32 (5.33)
EC50 (ng/ml)	51.9 (10.1)
Interindividual variability	
Pharmacokinetics	
CL/F (%)	19.2 (18.7)
Vd/F (%)	37.7 (28.8)
K_a (%)	40.2 (17.9)
Lag time (%)	ne
Pharmacodynamics	
E_0 (%)	9.75 (27.5)
E_{max} (%)	27.3 (32.1)
EC50 (%)	39.2 (31.2)
Residual variability	
PK proportional error (%)	15.8 (9.84)
PK additive error (ng/ml)	3.79 (18.2)
PD proportional error (%)	11.5 (29.9)
PD additive error (U/g Hb)	1.28 (33.2)

Mean pharmacokinetic and pharmacodynamic parameters and estimates of the interindividual variability for the final model are shown. Pharmacokinetics (PK) were analysed by a one-compartmental model parameterised by apparent clearance from the central compartment (*CL/F*), apparent volume of distribution (*Vd/F*) and absorption rate constant (K_a). Pharmacodynamics (PD) were analysed by an inhibitory E_{max} model with E_0 (baseline RBC AChE activity), E_{max} (maximum effect attributable to pyridostigmine) and *EC50* (plasma pyridostigmine concentration producing 50% of the maximum effect). SE is the standard error of the estimate. Each variability was expressed as CV (%). ne, not estimated.

supported the inclusion of an absorption lag time in the population pharmacokinetic model. Consideration of interoccasion variability in K_a, *CL/F* and *Vd/F* neither significantly reduced the objective function value nor improved the model goodness-of-fit. Plots of the individual pharmacokinetic parameters vs potential covariates suggested relationships between bodyweight or CL_{CR} and *CL/F* (Figure 2). Univariate analysis with NONMEM, however, showed that the inclusion of bodyweight and/or CL_{CR} did not improve the fit or decrease the interindividual variability of *CL/F*. Accordingly, no covariate was retained in the final pharmacokinetic model.

Parameter values for the final population pharmacokinetic and pharmacodynamic model (to be discussed below) are shown in Table 2. The estimated mean (±SE) times (t_{max}) for peak concentration to occur after a dose was 1.43 ± 0.12 h, calculated from each subject's conditional estimates of K_a and K_e by the standard formula $t_{max} = \ln(K_a/K_e)/(K_a - K_e)$ for a one-compartment extravascular model. Typical values for *CL/F* calculated from conditional estimates for the studied population was 136.1 ± 3.9 l/h. The typical values of *Vd/F* and K_a over all subjects were 130.4 ± 6.6 l and 0.68 ± 0.04/h, respectively. The interindividual variability about *CL/F*, *Vd/F* and K_a was 19.2%, 37.7%, and 40.2%, respectively. Random effect correlation between *CL/F* and *Vd/F* was 0.655 (percent standard error, 21.9%). The terminal half-life ($t½$), derived from the expression $t½ = (0.693 \times Vd/F)/(CL/F)$ with individual estimates of *CL/F* and *Vd/F*, for plasma pyridostigmine concentrations was 1.41 ± 0.36 h.

The observed vs population-predicted and individual-predicted concentrations are presented in Figure 3a and b, respectively. Overall the fit was judged to be good. The distributions of weighted residuals as a function of population-predicted values, sampling time or subject number were

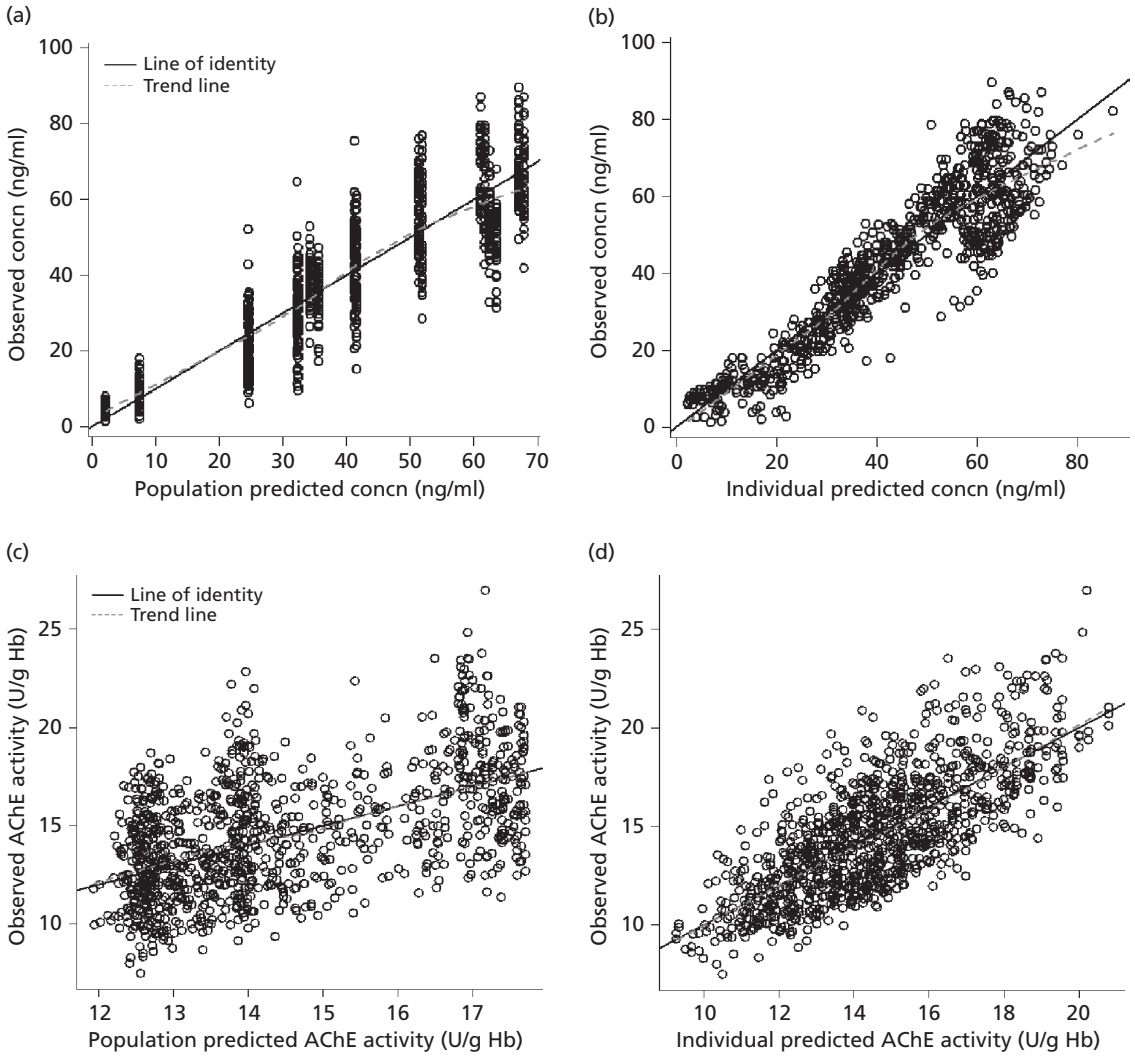

Figure 3 Goodness-of-fit plots for the final pharmacokinetic and pharmacodynamic model. (a) Observed vs population predicted plasma concentrations of pyridostigmine; (b) observed vs individual predicted plasma concentrations of pyridostigmine; (c) observed vs population predicted red blood cell (RBC) acetylcholinesterase (AChE); and (d) observed vs individual predicted RBC AChE activity. The gray broken lines are local regression smooths.

homogenous indicating a good fit of the model to the data (data not shown). The visual predictive check showed the observed data from the subjects to be symmetrically distributed about their respective 50th percentile profiles, with approximately 6% of the data distributed outside the 5th- to 95th-percentile boundaries (Figure 4).

Population pharmacodynamic analysis

Consideration of interoccasion variability in E_0 and E_{max} significantly reduced the objective function value. However, the inclusion of interoccasion variability in *EC50* did not allow further model improvement and was estimated to be < 5% if included. When taking into account interoccasion variability on E_0 and E_{max} concomitantly, interoccasion variability was 42.3% (range 27–96.5%) on the basal RBC AChE activity E_0, while interindividual variability seemed significantly lower (5.87%). Interoccasion variability was lower on E_{max} (17%) when compared with interindividual variability (23.1%). Taking into account interoccasion variability only on E_{max} and not on E_0 induced a significant decrease of the objective function value, but interoccasion variability was then much higher than the interindividual variability estimate (28.1 vs 1.3%), the latter being poorly estimated (very large %SE) for all parameters. Therefore, interoccasion variability was not retained for any parameters in the final model (Table 2).

As with the pharmacokinetic models, estimates of the individual pharmacodynamic model parameters were plotted as a function of the subjects' covariates. None of the pharmacodynamic parameters demonstrated any statistically significant correlation with these characteristics and therefore no covariates were included in the final pharmacodynamic model.

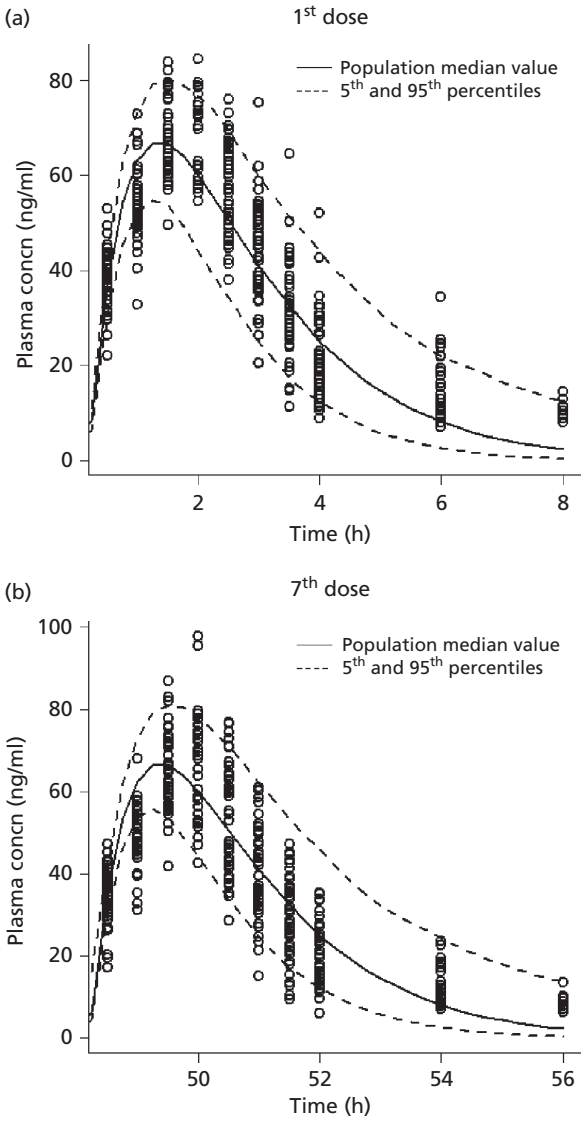

Figure 4 Visual predictive check of the final population pharmacokinetic model for pyridostigmine zero to eight hours post 1st (a) and 7th (b) doses (3 × 10 mg pyridostigmine bromide). The population predicted profile (50th percentile) of plasma pyridostigmine concentration is shown by the solid centre line, and the 95th percentile prediction intervals estimated from 500 simulated concentrations are encompassed by the broken lines in each plot.

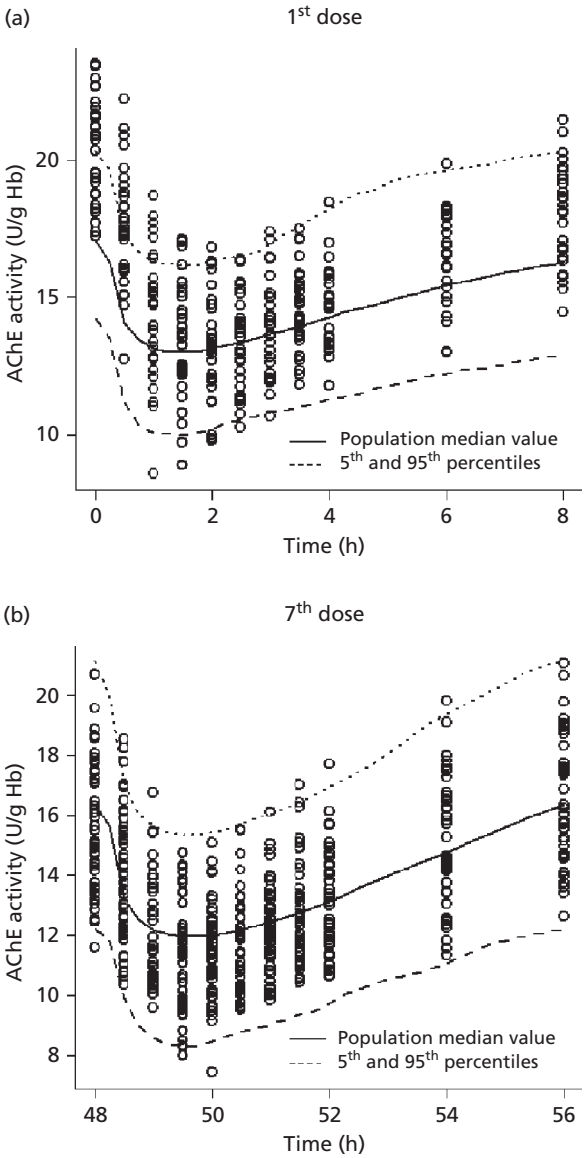

Figure 5 Visual predictive check of the final population pharmacodynamic model for red blood cell acetylcholinesterase activity zero to eight hours post 1st (a) and 7th (b) doses (3 × 10 mg pyridostigmine bromide). The population predicted profile (50th percentile) of acetylcholinesterase (AChE) activity is shown by the solid centre line, and the 95th percentile prediction intervals estimated from 500 simulated AChE activity are encompassed by the broken lines in each plot.

The goodness-of-fit plots for the final pharmacodynamic model are shown in Figure 3, where (c) and (d) show the consistency between the population- or individually-predicted and observed RBC AChE activity levels, as the values closely approximate the line of unity. The weighted residuals were evenly distributed around zero without trend across the model-predicted RBC AChE activity levels, sampling time or over subject number (data not shown). Figure 5 displays median, 97.5th, and 2.5th quantiles of the simulated data as lines with the observed data plotted as individual points. Less than 5% of the observed data were outside these 95% prediction intervals. No biased pattern or any tendency for over- or underestimation was noted.

Discussion

To our knowledge, this is the first investigation in which nonlinear mixed effects modelling has been applied to investigate oral pyridostigmine pharmacokinetics/pharmacodynamics and determine predictors of exposure and efficacy in Chinese males. In general, the parameters and related variability terms were estimated with good precision, and there were no trends in the goodness-of-fit plots or other model evaluation criteria, indicating that the model described the data well. The appropriateness of the final model was

further substantiated by results from visual predictive checks, which showed no biased pattern or any tendency for over- or underprediction with respect to the measured data.

High interoccasion variability estimates were obtained in this study, greater than interindividual variability, which rendered the inclusion of interoccasion variability in the model questionable. Indeed, at the basis of a strategy with interoccasion variability, a same individual is recognised as such on each occasion, but with some variability. However, interoccasion variability is supposed to be less than interindividual variability, which was not the case in our study. This suggested that a given individual in our sample was as different to himself on another occasion as to another individual. If interoccasion variability is much higher than interindividual variability, it is reasonable to assume the individual as being a different subject on each occasion.[22] Secondly, the covariate analysis did not find any significant relationship between any covariates and model parameters. This finding is important because Karlsson and Sheiner[23] concluded that when interoccasion variability is omitted, erroneous covariate models may sometimes be found, which was not the case here. Finally, clinical reasoning also advocated for the choice of the modelling strategy where each occasion is considered as a different individual.

In this study, an inhibitory E_{max} model was used to describe the inhibition of RBC AChE activity after drug administration. A similar model was utilised by Marino *et al*.[14] for mixed effects modelling of plasma concentrations and pharmacodynamic data from Caucasians, albeit with an effect compartment linked to the central compartment. During our pharmacodynamic model-building process, the hypothetical effect compartment model resulted in a statistically insignificant reduction in objective function value compared with the inhibitory E_{max} model without the effect compartment. In selecting the latter model as the final pharmacodynamic model, the baseline RBC AChE activity was also successfully estimated, which represented an improvement over the 'link' type approach implemented by Marino *et al*.[14], where E_0 was set to be equal to E_{max}. It follows that the estimated mean values of E_{max} and *EC50* in this study were also different from those reported in Marino *et al*.[14] (10.4 U/ml and 69 ng/ml, respectively).

The results showed that the apparent oral clearance of pyridostigmine increased with CL_{CR} and bodyweight. This was expected as the drug was eliminated through the kidneys and kidney function scales with CL_{CR} and bodyweight.[24,25] However, no significant influence by these covariates on the oral pyridostigmine pharmacokinetics and pharmacodynamics was obtained by NONMEM analysis. In our study population, the subjects' renal functions were within normal limits and the bodyweights showed a relatively narrow range compared with other covariates (Table 1). These may be reasons why inclusion of CL_{CR} and/or bodyweight as a covariate in the mixed effects model did not improve the fit or decrease interindividual variability of model parameters. By the same reasoning, the inconsistency between the present NONMEM analysis and Marino *et al*.[14] in the *CL/F*–bodyweight covariate relationship might be attributed to the comparatively narrower weight distribution of our study population. Overall, the lack of an influence of subject covariates on the pharmacokinetic and pharmacodynamic profiles in this study lends support to unit-based dose administration in young, healthy Chinese males found, for example, in the military. However, before our findings could be applied to the broader population, further population analyses considering larger sample sizes as well as richer and more diverse/representative covariate information should be undertaken.

When normalised by the subjects' bodyweights, the mean individual estimates for *CL/F* and *Vd/F* were higher and lower, respectively, with estimates reported by Marino *et al*.[14] (3.19 vs 3.02 l/h per kg and 2.19 vs 2.09 l/kg for *CL/F* and *Vd/F*, respectively). To this end, the average value (77.5 kg) of the reported weight range (50–105 kg) for the male volunteers was used to normalise the mean estimates of *CL/F* and *Vd/F* reported in Marino *et al*.[14] Additionally, the average elimination half-life of pyridostigmine bromide in Chinese subjects was appreciably lower compared with that reported from pharmacokinetic studies involving Caucasian subjects.[11,12]

In the literature, the recommended dosing schedule for nerve agent pretreatment is 30 mg orally every eight hours. This is so as to maintain the percentage of RBC AChE inhibition over time above the recommended threshold of 10%.[25] (Golomb[24] also reported a more conservative threshold of 30%.) However, when this dosage regimen was implemented in Caucasian subjects, the variability in the study population suggested that 30% of individuals may have been below 10% RBC AChE inhibition at the time of the plasma pyridostigmine trough.[14] Indeed, following this recommended dosing strategy, it was reported that the percentage of RBC AChE inhibition may fall below 20% six hours after dosing, leading to no less than five hours a day in which RBC AChE inhibition falls below 20%.[24]

Due to these observations and the differing pyridostigmine pharmacokinetics/pharmacodynamics between Caucasians and Chinese (this study), our final model was subjected to stochastic simulations to separately predict the percentage inhibition of RBC AChE activity under dosing regimens of 30 mg every 4, 6 or 8 h over a hypothetical time period of 48 h.[14] The plasma pyridostigmine concentrations and RBC

Table 3 Number of simulated subjects with percentage inhibition of steady-state trough red blood cell acetylcholinesterase activity below 10%, expressed as a percentage, in the pyridostigmine study

	Percentage of population with % AChE inhibition < 10	Steady-state peak plasma pyridostigmine concn (ng/ml)
This study		
30 mg every 4 h	0	70.3
30 mg every 6 h	29	59.5
30 mg every 8 h	78	53.1
Marino *et al*.[14]		
30 mg every 8 h	30	na

The simulations were performed for 500 Chinese males who each received 3 × 10 mg pyridostigmine bromide tablets every 4, 6 or 8 h over 48 h. Figure 6 shows the associated histograms of individual percentage inhibition of steady-state trough red blood cell (RBC) acetylcholinesterase (AChE) activity for these dosage regimens. The recommended threshold of 10% for percentage inhibition of RBC AChE activity is based on Golomb.[24] na, not available.

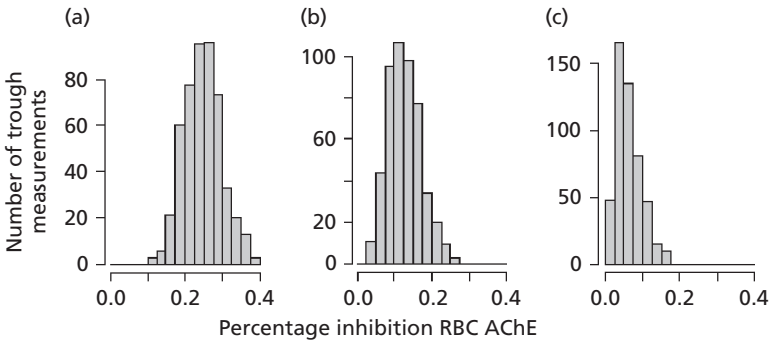

Figure 6 Histograms of individual red blood cell acetylcholinesterase percentage inhibition. The values were at steady-state trough based on 500 simulated individuals after multiple oral doses of 3 × 10 mg pyridostigmine bromide tablets every 4 (a), 6 (b) and 8 (c) h. RBC AChE, red blood cell acetylcholinesterase.

AChE activity levels were simulated in 500 healthy Chinese males based on the values of the fixed-effects parameters and the variances in the final model for pyridostigmine. The target was to achieve efficacy in terms of the steady-state trough percentage inhibition of RBC AChE activity greater than 10%.

The dosing simulations showed that an oral pyridostigmine dosage regimen of 30 mg repeated every 4 h reached the set target in the Chinese male population (Table 3 and Figure 6). As anticipated, owing to a larger mean pyridostigmine *CL/F* in Chinese compared with Caucasians, an adjunctive therapy of 30 mg thrice daily, i.e. 30 mg every 8 h, resulted in a high 78% of the population with steady-state trough percentage inhibition below 10%. Although a dosage regimen of 30 mg repeated every 6 h resulted in 29% of the population with steady-state trough percentage inhibition below 10%, the predicted 50th percentile RBC AChE inhibition profile fell on the safe side of the 'therapeutic' threshold. This regimen may be more appealing to the military as it may be more practical for soldiers to administer doses every 6 h as opposed to every 4 h during battlefield conditions. Furthermore, dosing every 4 h will result in higher plasma pyridostigmine concentration levels, which may be a clinical concern since heightened exposure has been shown to cause reduced ACh release, withdrawal of nerve fibres from junctional folds and reduced sensitivity of ACh receptors.[24]

There are several important caveats to this study. First of all, as the intended application is for the military setting, the study considered only healthy young male subjects with narrow ranges of covariates. As such, the data collected from this study would not be ideal for population analysis. A larger data set, incorporating richer covariate information (including genetic polymorphisms of enzymes linked to pyridostigmine bromide metabolism) across more diverse ranges, would have been desirable for population analysis and for deriving more definitive assessments of covariate effects. Finally, modelling and simulation can advise but not supplant clinical data. The findings from this study need to be confirmed in further clinical or pharmacokinetic/pharmacodynamic studies.

Conclusions

A one-compartment model was successfully fitted to the plasma pyridostigmine concentration–time data, while a saturable pharmacodynamic model adequately described the inhibition of AChE activity in RBC after drug administration. The lack of correlation between subject covariates and the model parameters appeared to support unit-based dose administration of pyridostigmine in young healthy Chinese males. A suitable oral dosage strategy may be 30 mg pyridostigmine bromide repeated every six hours. Pending the availability of more data, we are cautious about extrapolating any proposed dosage regimen to the wider population with different sociodemographics (e.g. age, ethnicity, sex, lifestyle factors).

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

Financial support for the study was provided by the Singapore Ministry of Defence.

Acknowledgements

The authors would like thank all the test volunteers who participated in this study and for providing blood samples. The assistance of Donna Tan, Mui-Hoon Lai, Yong-Teng Tan, Lay-Keng Priscilla Lim and Wenda Douglas Goh in the complex task of data collection and archival is acknowledged. The authors would like to thank Dr Wing-Yuen Eli Chan for helpful discussions during the preparation of the manuscript.

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