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Population Pharmacokinetics and Pharmacodynamics of Pyridostigmine Bromide for Prophylaxis against Nerve Agents in Humans

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This study was conducted to determine the pharmacokinetics and pharmacodynamics of pyridostigmine given as 30 mg of pyridostigmine bromide every 8 hours in healthy subjects. Plasma pyridostigmine concentration and red blood cell acetylcholinesterase activity were measured in blood samples collected during a 3-week period. Population analysis was performed using standard pharmacokinetic and pharmacodynamic models with the nonlinear mixed-effect modeling software (NONMEM). The pharmacokinetic model that best fit the pyridostigmine plasma levels was a two-compartment open model with first-order absorption, a lag time, and first-order elimination from the central compartment. The pharmacodynamic model that best fit red blood cell acetylcholinesterase activity was an inhibitory $E_{\text{max}}$ model with an effect compartment linked to the central compartment. The results showed that the pharmacokinetics of pyridostigmine bromide are both gender and weight dependent. The pharmacodynamic effect does not lag significantly from the plasma concentration and returns to near normal within 8 hours. With the present dosage regimen of 30 mg every 8 hours, 30% of individuals may not have red blood cell acetylcholinesterase inhibition >10% at the time of the trough. J Clin Pharmacol 1998;38:227-235.

Pyridostigmine is a reversible inhibitor of acetylcholinesterase and is used for the symptomatic treatment of myasthenia gravis.\(^1\) It has recently been shown to be an adjunct to the therapy of organophosphorus cholinesterase inhibitors in animal studies.\(^2\)

Pyridostigmine is thought to act by preventing the irreversible binding of organophosphorus agents to acetylcholinesterase. The ability to prevent irreversible binding of organophosphorus agents to acetylcholinesterase would make pyridostigmine useful as a pretreatment to minimize the effects of “nerve gas” if used in conjunction with the standard treatment of atropine and pralidoxime chloride (2-PAM).\(^3\) The concern for the use of nerve agents in modern warfare was high enough that the coalition forces issued and used pyridostigmine bromide during the war against Iraq.\(^4\)

This study was designed to determine the population pharmacokinetics and pharmacodynamics of pyridostigmine bromide when given as a 30-mg tablet every 8 hours. Red blood cell acetylcholinesterase activity was chosen as a pharmacodynamic endpoint because it has been shown to correlate with survival in nerve agent exposure in some animal models.\(^5\) The standard dose that was prescribed to United States
army personnel was tested in order to develop an understanding of the population variability of the drug and explore any covariates that could be used to optimize the dosage regimen.

SUBJECTS AND METHODS

Clinical Protocol

The study was approved by the First Foundation for the Protection of Human Subjects in Research, Inc., of Miami, Florida. Ninety healthy male and female volunteers between 18 and 45 years of age were included in this placebo-controlled, randomized, double-blind study. All subjects signed the approved informed consent form.

In all, 60 subjects (30 men and 30 women) received the study drug, with 30 subjects (15 men and 15 women) receiving placebo. The average age for the group receiving the study drug was 32 years (range for men and women, 19–44 years). Weight ranged from 50 to 105 kg for the men and 43 to 91 kg for the women.

Baseline examination of all subjects was conducted within 21 days before admission to the experimental unit (Clinical Pharmacology Associates, Miami, FL) and included a medical history, physical examination, and laboratory screening, including electrocardiogram, chest x-ray (not taken if normal within 12 months), urinalysis, complete blood count and differential, electrolytes, liver associated enzymes, antibody to human immunodeficiency virus, and red blood cell acetylcholinesterase. Female subjects were admitted to the study if they had a negative serum beta human chorionic gonadotrophin (β-HCG) test result within 24 hours before admission. β-HCG was also measured on discharge from the experimental unit.

All subjects were confined to the experimental unit for the full 25 days of the study, which included 1 day before administration and 3 days after the last dose. The subjects were followed as outpatients for 1 year after the treatment regimen.

Pyridostigmine Regimen

The regimen was designed to study the multiple dose pharmacokinetics and pharmacodynamics of pyridostigmine when given orally as a 30-mg tablet (Mestinon; LaRoche, Nutley, NJ) every 8 hours for 21 days. Subjects were given the test medication or placebo under observation. All subjects fasted from midnight until 2 hours after the 8:00 AM dose and also fasted for 1 hour before and after the 4:00 PM and 12:00 AM doses. Vital signs (blood pressure, pulse, and temperature) were taken daily throughout the study before 9:00 AM. Physical examinations were performed on days 1, 8, 15, 22, and before discharge on day 25. Laboratory examinations and electrocardiograms were performed on days 8, 15, and 23.

Pyridostigmine Plasma Levels and Red Blood Cell Acetylcholinesterase Activity

Blood samples (5 mL) were obtained for plasma pyridostigmine levels and red blood cell acetylcholinesterase activity before and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours after the first dose. Levels were drawn 5 minutes before the daily 8:00 AM dose on days 4, 7, 9, 11, 14, 16, 18, and 21. During the last dose on day 22, samples were drawn 5 minutes before and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 14, 18, 24, 28, 36, 40, 48, 52, 56, 60, 66, and 72 hours after administration.

The samples were collected in heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for analysis of plasma pyridostigmine levels and in EDTA tubes for analysis of red blood cell acetylcholinesterase activity. They were placed on wet ice before transfer to the clinical laboratory. The blood samples used for the determination of red blood cell acetylcholinesterase were diluted 1:10 with 1% trismex 100 (Triton X-100; Sigma, St. Louis, MO) before analysis. The blood samples used for the determination of pyridostigmine were centrifuged at 4°C for 10 minutes before separation of the plasma from the red blood cells. All samples were stored at −80°C until analysis was performed.

Plasma was analyzed for pyridostigmine using high-performance liquid chromatography (HPLC). The assay required 0.5 mL of plasma for the determination of pyridostigmine concentration. The HPLC system included an LC-600 pump with an SPD 10A ultraviolet detector with absorbance at 208 nm (Shimadzu, Kyoto, Japan) and an Intelligent Sample processor 710 B (Waters, Milford, MA). Separation was carried out on an Axiom Silica column (4.6 × 250 mm, 5 mm particle size; Richard Scientific, Novato, CA).

The mobile phase consisted of acetonitrile/water (1:1, v:v) with final concentrations of 0.05% tetramethylammonium chloride and 5 mmol/L dibasic ammonium phosphate (final apparent pH 7.2). Acetonitrile, phosphoric acid, and dibasic ammonium phosphate were obtained from Fischer Scientific (Fairlawn, NJ); tetramethylammonium chloride was obtained from Fluka Chemika (Buchs, Switzerland); pyridostigmine bromide (lot no. 8950426) was obtained from Sigma Chemical (St. Louis, MO); and
neostigmine bromide (lot no. KT05130J) was obtained from Aldrich Chemical (Milwaukee, WI).

Assay samples were prepared by spiking known volumes of plasma with a known amount of internal standard (neostigmine bromide). Standard curve samples were generated by spiking known amounts of interference-free human plasma (Irwin Memorial Blood Bank, San Francisco, CA) with known amounts of pyridostigmine and internal standard. Samples were prepared by adding 18.75 mg of neostigmine bromide (internal standard) and 1 mL of acetonitrile to each 0.5-mL plasma sample. The mixture was centrifuged and the supernatant was placed on a Varian C8 Bond Elut SPE cartridge (Harbor City, CA). The cartridge was rinsed with 2 mL of water, 4 mL of CH₃CN/H₂O (50:50), 2 mL of CH₃CN, and 0.5 mL of CH₃CN/H₂O (85:15) containing 1 mmol/L (NH₄)₂HPO₄ at pH 3.6. The fraction containing pyridostigmine was eluted by adding 2 mL of CH₃CN/H₂O (85:15) containing 1 mmol/L (NH₄)₂HPO₄ at pH 3.6. The eluates were concentrated by evaporation to approximately 200 mL under nitrogen at room temperature, transferred to WISP vials (Waters, Milford, MA) and 25- to 50-mL aliquots were injected on column.

The peak height ratios of pyridostigmine (retention time 14 min) and internal standard (retention time 16 min) were calculated for each sample. Standard curve concentrations and peak height ratios were fit by weighted linear least squares regression to the equation \( y = mx + b \) (\( x \) equals known concentration and \( y \) equals peak height ratio). Drug concentrations in the unknown samples were calculated from the standard curve for each sample run.

The assay was linear within the range of the standard curve (1.53–76.3 ng/mL pyridostigmine free base). The lower limit of quantitation was 1.53 ng/mL, with an intraday coefficient of variation (CV) of 14.6% (\( n = 6 \)) and an interday CV of 12% (\( n = 6 \)). No significant degradation of standard samples were noted over 4 months when kept at −80°C, and all samples were analyzed within that time period. The red blood cell acetylcholinesterase levels were measured by standard enzymatic techniques with a lower limit of quantitation of 0.46 U/mL with a CV of 6.5% and linearity to 20 U/mL. The CV was less than 2% between 5 and 15 U/mL.¹²

**Pharmacokinetic/Pharmacodynamic Population Analysis**

The pharmacokinetic and pharmacodynamic analysis was performed with NONMEN IV, version 2.¹¹ on a Silicon Graphics Indigo 2 UNIX workstation (Mountain View, CA). A two compartment model with first-order absorption with a lag time and first-order elimination from the central compartment was used to fit the plasma pyridostigmine concentrations. A standard two-step procedure was followed to do the population pharmacokinetic analysis.² The pharmacokinetic model was first fitted to the pharmacokinetic data for the entire population. A relationship between the pharmacokinetic parameters and covariates such as weight and gender was explored using residual analysis and linear regression.¹⁰ Relationships between volume of the central compartment and weight and clearance and gender were found to be significant. Volume of the central compartment and clearance were then both modeled as being dependent on weight and gender, respectively (equations 1 and 2).

\[
\frac{V_c}{F} = \Theta_1 \times Kg
\]  
\[
\text{Clearance} = \Theta_{\text{men}}
\]  
\[
\Theta_{\text{women}}
\]

Analysis on the models was done by comparing the objective functions (differences in the full and reduced model’s objective function using \( \chi^2 \) with the number of parameters in the reduced model as the degrees of freedom).¹¹ Parameter estimates for different covariates (gender and weight) were compared using the \( t \) test.

In the second step the Bayesian estimates of the pharmacokinetic parameters for each individual were used in equation 3 to generate the effect site concentration. The effect compartment was linked to the central compartment via a standard effect compartment equation. The effect (red blood cell acetylcholinesterase activity) was modeled with an inhibitory \( E_{\text{max}} \) model:

Red blood cell acetylcholinesterase activity

\[
= \text{Baseline} - \frac{E_{\text{max}} \times C_e}{(E_{\text{50}} + C_e)}
\]  
(3)

The NONMEM model for the red blood cell acetylcholinesterase data is the same general model as used for the plasma concentration (equation 4). The NONMEM model for the \( j \)th concentration in the \( i \)th individual is given by equation 4:

\[
C_{ij} = f(p_i, t_{ij}) + H(f, \theta) \times \epsilon_{ij}
\]  
(4)

where \( f \) is the predicted concentration and \( p_i \) is the pharmacokinetic parameter estimates for the \( i \)th individual. The second term in equation 4 represents the departure of the model from the observations where \( \epsilon_{ij} \) was assumed to be a random gaussian vari-

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**PHARMACOKINETICS AND PHARMACODYNAMICS**

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TABLE I
Pharmacokinetic and Pharmacodynamic Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SEM)</th>
<th>Variability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacokinetic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl/F (men), L/hr</td>
<td>221 (13.4)</td>
<td>31</td>
</tr>
<tr>
<td>Cl/F (women), L/hr</td>
<td>172 (11.4)</td>
<td>31</td>
</tr>
<tr>
<td>Vc/F (L/kg)</td>
<td>2.09 (0.12)</td>
<td>51</td>
</tr>
<tr>
<td>Vp/F (L)</td>
<td>4240 (570)</td>
<td>431</td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>29 (3.4)</td>
<td>9.7</td>
</tr>
<tr>
<td>Ka (hr')</td>
<td>0.32 (0.027)</td>
<td>9.7</td>
</tr>
<tr>
<td><strong>Pharmacodynamic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keo (hrs^-1)</td>
<td>1.32 (0.07)</td>
<td>22.2</td>
</tr>
<tr>
<td>EC50 (ng/mL)</td>
<td>69 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Emax/Baseline (U/mL)</td>
<td>10.4 (0.14)</td>
<td>9.7</td>
</tr>
<tr>
<td><strong>Intraindividual Error</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ1 (ng/mL)</td>
<td>1.04 (0.84)</td>
<td></td>
</tr>
<tr>
<td>θ2 (%)</td>
<td>26 (3)</td>
<td></td>
</tr>
<tr>
<td>θ1 (U/mL)</td>
<td>0.62 (0.15)</td>
<td></td>
</tr>
<tr>
<td>θ2 (%)</td>
<td>9.0 (0.20)</td>
<td></td>
</tr>
</tbody>
</table>

able with a mean of zero and variance \( \sigma^2 \). \( H(f, \theta) \) is
given by

\[
H(f, \theta) = \theta_1^2 + \theta_2^2 \times f \tag{5}
\]

represents a combination of an additive \( \theta_1^2 \) and proportional \( \theta_2^2 \) error model.

Both the pharmacokinetic and pharmacodynamic parameters were modeled with the assumption of log
normal distribution as follows:

\[
p_i = p_{pop} \times \exp(\eta_{pi}) \tag{6}
\]

where \( p_{pop} \) is the population mean and \( \eta_{pi} \) is the differ-
ence between the population mean and the param-
eter of the \( j \)th subject. \( \eta_{pi} \) was assumed to be a
gaussian variable with a mean of zero and variance
\( \sigma^2 \). The population parameters were assumed to be
independently distributed without any covariance.

RESULTS
Pharmacokinetics

The pharmacokinetic parameters estimated were ab-
sorption rate constant \( K_a \), volume of the central
compartment \( Vc/F \), volume of the peripheral com-
partment \( Vp/F \), oral clearance \( Cl/F \), and lag time.
Population means and standard errors as well as es-
imates of the intersubject variances were obtained
and are shown in Table I. The population and Bayes-
ian predictions are plotted against the measured val-
ues in Figure 1. The log10 pyridostigmine plasma
concentrations are plotted against time for the last
dose interval for each individual in Figure 2. The
majority of subjects demonstrate a distribution and
elimination phase. Relationships between the in-
dividual Bayesian predicted pharmacokinetic parame-
ters and the covariates of body weight and gender
were used in the model as previously stated (equa-
tions 1 and 2).

The relationship between \( Vc/F \) and weight shown in Figure 3
and clearance and gender is shown in Table I. The high variability in \( Vp/F \) is
secondary to six individuals having values well
above and below the mean value.

Pharmacodynamics

The pharmacodynamic parameters estimated were
elimination rate constant from the effect compart-
ment \( K_{eo} \), baseline (where baseline is measured in
units of RBC acetylcholinesterase activity), and con-
centration at steady state giving half maximal effect
\( (EC_{50}) \). To simplify the model, maximal effect \( E_{max} \)
was set to baseline. This was done because inhibition
could not exceed baseline red blood cell acetylcho-
linesterase activity, and with increasing pyridostig-
mine plasma levels high degrees of inhibition
\((>70\%) \) are possible.9 Population means and their
standard deviations, along with intersubject vari-
ces, were obtained and are shown in Table I. Transfer rate constant into the effect compart-
ment \( K_{eo} \) was fixed at 0.001 of elimination rate constant
from the central compartment \( K_a \) so that the amount of
pyridostigmine modeled in the effect compart-
Figure 1. Population predictions (A) and Bayesian predictions (B) plotted versus the individual values.

Figure 2. Pyridostigmine plasma levels for all individuals and population predicted mean versus time for the average male subject of 77 kg (dotted line) and the average female subject of 68 kg (solid line) during the first dosing interval.
The red blood cell acetylcholinesterase activity during the first dose interval for each individual is shown in Figure 4. No relationship was found between the pharmacodynamic parameters $K_{\infty}$, $EC_{50}$, and baseline and the covariates of weight and gender.

Figure 3. Volume of the central compartment/F plotted versus weight. Solid line is the population predicted volume of the central compartment/F and the dotted lines are the 95% confidence limits for the population prediction.

Figure 4. Red blood cell acetylcholinesterase activity for all individuals and population predicted mean versus time for the average male subject of 77 kg (dotted line) and the average female subject of 68 kg (solid line) during the first dosing interval.
DISCUSSION

This report describes the first population pharmacokinetic/pharmacodynamic analysis of pyridostigmine bromide. It also evaluates the relationship of the covariates gender and weight with basic kinetic and dynamic parameters. The kinetic model that best fit our data was one with a first-order input and a lag time into a two-compartment model with first-order elimination from the central compartment.

A previous study of pyridostigmine has shown it to have a two-compartment kinetic profile with intravenous infusion or intravenous bolus with t1/2 of 7 to 9 minutes and 97 to 112 minutes for the distribution and elimination phases, respectively.10,12 One report describes a three-compartment model when given as an intravenous bolus to anesthetized patients, although the method of analysis was based on a radioimmunoassay for pyridostigmine.13 Previous studies using oral administration demonstrated a one-compartment kinetic profile with an elimination t1/2 of 200 minutes, which the authors felt was due to absorption proceeding slower than elimination.12

In our study, we were unable to detect the rapid distribution phase as described in the intravenous studies because of the slower absorption phase after oral administration. We were able to estimate this slow elimination phase, because the assay used was more sensitive and plasma samples were measured for longer periods after administration than in previous studies.12 This suggests that intravenously administered pyridostigmine would follow a three-compartment model if plasma levels were taken for a sufficiently long period of the elimination phase, as confirmed by Stone et al.13

Bioavailability was not measured in this study. Our value for VC/F suggests a bioavailability of 12% using published values for VC (21 ± 7 L).10 This value is consistent with a previously published value of 14%.12 The variability in the estimate of VC/F may be due to the intrasubject variability from dose to dose within subject. Our value for oral clearance is compatible with those in previous reports and supports the idea that either poor absorption or significant first-pass metabolism occurs.14 Oral clearance, which was calculated using the area under the concentration–time curve (AUC) by the trapezoidal rule from the average of the first and last dose for each subject, was 311 ± 120 L/hr, which is much higher than clearance calculated from intravenous administration (36 ± 7 L/hr).13 Results of human metabolic studies using 14C-labeled pyridostigmine are consistent with first-pass metabolism.15 Human metabolites of pyridostigmine have been defined in small studies,14,15 but the enzyme(s) responsible for pyridostigmine metabolism is unknown. Whether metabolite(s) have any intrinsic ability to inhibit acetylcholinesterase is also not known.

The pharmacodynamic model that best fit the red blood cell acetylcholinesterase activity was an inhibitory Emax model linked to an effect compartment. Kie was fixed to a small value so as not to influence the kinetic parameters.16 The baseline red blood cell acetylcholinesterase activity was modeled as the Emax because maximal inhibition could not reduce values below zero and 100% inhibition is pharmacologically possible with several of the inhibitors.17 This also reduced the number of parameters that needed to be estimated. Kie was found to be 1.32 hrs⁻¹, giving a t1/2 effect of 32 minutes.

In conjunction with the kinetic parameters governing absorption and distribution, this explains the rapid approach to pharmacodynamic equilibrium and the return to baseline within several hours of the final dose. With this rather short Kie t1/2, a slow-release formulation or an infusion would be required to maintain a more consistent level of red blood cell acetylcholinesterase inhibition.

The EC50 is the steady-state plasma concentration of pyridostigmine needed to produce 50% of the baseline red blood cell acetylcholinesterase activity. In previously published studies of other acetylcholinesterase inhibitors, red blood cell acetylcholinesterase percent inhibition was used as the pharmacodynamic endpoint.18 This is the difference between the baseline red blood cell acetylcholinesterase activity and the measured red blood cell acetylcholinesterase activity at any time point expressed as a percentage of the baseline red blood cell acetylcholinesterase activity. We did not use this in our analysis, because our placebos showed a ±16% intrasubject variation from their mean baseline measurement over the course of the study, and we had only one predose red blood cell acetylcholinesterase activity measurement for each subject. Rather, we estimated the baseline red blood cell acetylcholinesterase activity (using NONMEM) in our model.

In animal studies, 10% red blood cell acetylcholinesterase activity inhibition provides protection against organophosphorus agents.8 Based on the pharmacodynamic model the mean plasma concentration needed to obtain 10% inhibition from this study is 7.7 ng/mL. With the dosage regimen tested in this study, the mean level of inhibition was greater than 10% throughout the study. The population variability, however, would suggest that 30% of individuals may be below this level at the time of the pyridostigmine trough (Figure 5).

Despite tremendous variability in both the kinetic and dynamic effects of pyridostigmine, we have
shown that it is possible to produce an effective model to describe both the pharmacokinetics and pharmacodynamics of orally administered pyridostigmine bromide. In this study of pyridostigmine population pharmacokinetics and pharmacodynamics, we found that when pyridostigmine bromide is given orally as a 30-mg tablet every 8 hours for 21 days the drug demonstrates an "intermediate" distribution phase and a "slow" elimination phase. This pharmacokinetic profile has not been previously described for the oral administration of pyridostigmine, probably because most studies did not take plasma samples into the terminal elimination phase.

We also found significant interindividual variation in Vc/F. The covariates of body weight and gender did appear to have a significant relationship with the kinetic parameters Vc/F and Cl/F. With the possibility of a significant first-pass effect for this compound, tissue esterases and perhaps cytochrome P450 status may be more important factors in determining clearance or bioavailability.

Population pharmacodynamic parameters governing red blood cell acetylcholinesterase inhibition have not been previously described. The analysis shows that an E_max model with an effect compartment fits the data well. Previously published work on another acetylcholinesterase inhibitors has shown the utility of the E_max model.18 The majority of the variability in the pharmacodynamic response is secondary to the variability in the kinetic profile. The t1/2 for the effect compartment is relatively "fast," and thus the delay between peak plasma levels and peak effect is brief, as is the delay between trough plasma levels and effect. Although the mean response is greater than 10% inhibition during any dose interval, 30% of subjects had inhibition lower than this value around the plasma trough level (Figure 5). Based on the results of this study, it appears that this dosage regimen has utility in the possible protection of soldiers against organophosphorus nerve agents.

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REFERENCES


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