CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-414

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

New Drug Application
Clinical Pharmacology and Biopharmaceutics Review

NDA:	20-414
Types of Submissions:	N-BC
Generic Name:	Pyridostigmine Bromide
Formulation:	Immediate Release Tablets
Strengths:	30 mg
Route:	PO
Sponsor:	Department of the Army Fort Detrick, MD
Submission Dates:	January 16, 2003
Reviewer:	Ronald Evan Kavanagh, B.S. Pharm., Pharm.D., Ph.D.

I. EXECUTIVE SUMMARY

In the OCPB review dated January 8, 2003 an interim dissolution method and specification was set for pyridostigmine 30 mg tablets.[1] It was also documented that the sponsor had made a phase IV commitment to provide additional dissolution information so that a permanent regulatory dissolution specification could be set.

In the present submission, dissolution profiles in various media have been provided and a final regulatory dissolution specification is recommended.

II. RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics / Division of Pharmaceutical Evaluation I (OCPB/DPE-1) has reviewed NDA #20-414 N-BC submitted January 16, 2003.

OCPB finds this application acceptable. Comments should be communicated to the sponsor as appropriate (see Section III Comments to the Sponsor on page 2).

[1] See DFS folder N020414 N 000 BX 07-Jan-2003; File C:\Kavanagh Data\Reviews\DNPDP\NDA\20-414 Pyridostigmine-Soman Prophylaxis\Review\N20414.doc

III. COMMENTS TO THE SPONSOR

A. Dissolution

Please adopt the following dissolution method and specification for pyridostigmine 30 mg tablets.

Table 1 Final Regulatory Dissolution Method and Specification

Parameter	Description	
Apparatus type	USP Apparatus II (paddle)	
Media	Water	
Volume (ml)	900 ml	
Temperature	37 ± 0.5 °C	
Speed of rotation (rpm)	50 rpm.	
Sample times (hours)*	60 minutes	
Specification* (% of Label Claim)	Q = 80% at 60 minutes	Acceptance criteria as per USP XXV – NF 20 <711> Dissolution Acceptance Table

* Changed from interim method and specification

B. Phase IV Commitments

The sponsor is advised that the phase IV commitment to provide data to support a final regulatory dissolution method have been fulfilled.

IV. SIGNATURES

/S/

Ronald Evan Kavanagh, B.S. Pharm., Pharm.D., Ph.D. Date

Division of Pharmaceutical Evaluation I
Office of Clinical Pharmacology and Biopharmaceutics

/S/

Ray Baweja, Ph.D. Date

Team Leader
Division of Pharmaceutical Evaluation I
Office of Clinical Pharmacology and Biopharmaceutics

CC: NDA 20-414 (orig., 1 copy)
HFD-120 (Rzeszotarski, Katz, Nighswander)
HFD-860 (Kavanagh, Baweja, Mehta, Sawajwalla)
Central Document Room (Barbara Murphy)

V. REVIEW

The sponsor has provided dissolution profiles for pyridostigmine 30 mg tablets manufactured by ICN Pharmaceuticals packaging lot number 1A8682 (bulk tablet biobatch lot number 1A8675) in pH 1.2 Hydrochloric Acid, pH 4.5 Acetate Buffer, pH 6.5 Phosphate Buffer, and Water.

All dissolution experiments were carried out with 12 individual tablets, each in 900 ml of media at 37 °C using USP Apparatus II (paddle) at 50 rpm. Samples were collected at 15, 30, 45, 60 and 120 minutes.

At 15 minutes mean percent dissolved ranged from 51% to 69% with the dissolution rate increasing as pH increases. At 30 minutes mean dissolution was essentially complete in both buffers, (pH 4.5 and 6.5), and water. However, in Hydrochloric Acid mean dissolution was 88% ± 8.0% (CV = 9.1%) and the range was with 6 of the 12 tablets dissolving 85% or less. Consequently, pyridostigmine cannot be considered rapidly dissolving according to the BCS classification system as dissolution is frequently less than 85% at 30 minutes at pH 1.2.

Usually pH 1.2 media would be the appropriate medium for dissolution testing for this formulation. However, there is only data for a single batch and this one batch only passed at level 2 testing for a Q of — at 30 minutes at pH 1.2.

In addition, certificates of analysis (COA) were provided for 23 lots with dissolution data (n = 12) generated per the current USP method, i.e. Q = — at 60 minutes in water, (see Table 2).

Table 2 Summary Dissolution Data for USP Method from COAs

Lot #	% Dissolved		
	Mean	Minimum	Maximum
1A8675	92		
1H9991	97		
1H9992	90		
1J0623	89		
1J0624	88		
1J0625	97		
1J0626	99		
1J0627	96		
1J0628	98		
1J0630	92		
1J0632	89		
1J0633	88		
1J0634	88		
1K0934	88		
2F2641	90		
2F2642	92		
2F2643	93		
2F2644	89		
2F2645	95		
2I3360	88		
2I3361 [a]	86		
2I3362 [a]	83		
2I3363 [a]	88		

a Lots in bold required level 2 dissolution testing

Since there is limited data at pH 1.2, and since 4 of 23 production batches (13.0%) required level 2 testing, the current dissolution method in water is appropriate for this formulation. Additional reasons to recommend this method is that it is the same as the current USP method in use for higher strengths.

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/s/

Ron Kavanagh
1/29/03 11:50:51 AM
BIOPHARMACEUTICS

Raman Baweja
1/29/03 04:20:46 PM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 20-414

Drug :	Pyridostigmine Bromide
Generic name:	Pyridostigmine
Formulation:	30 mg tablets
Indications:	protection against nerve agents
Applicant:	U.S. Department of Defense
OCPB Division:	Division of Pharmaceutical Evaluation I (HFD-860)
OND Division:	Division of Neuropharmacological Drug Products (HFD-120)
Submission Dates:	3/4/94; 5/24/96
Reviewer:	Brian Booth Ph.D.
Team Leader:	Joga Gobburu, Ph.D.
Type of Submission:	NDA-original

I. Executive Summary

The U.S. Department of Defense (DOD) is seeking marketing approval of 30 mg pyridostigmine bromide tablets as a preventative pretreatment against exposure to nerve agents. DOD submitted studies of Rhesus monkeys treated with a pyridostigmine, atropine and 2-pralidoxime that were challenged with soman nerve agent. Using the monkey dose-survival curve and the human and simian pharmacokinetics, a dose that produces an equivalent exposure in humans was calculated.

RECOMMENDATIONS:

A 0.6 mg/kg dose of pyridostigmine appears to equate to a 29 mg dose in humans. The safety and effectiveness of this dose in humans needs to be assessed by the Medical division.

/S/

/S/

Brian Booth, Ph.D.
Pharmacokinetic Reviewer
Division of Pharmaceutical Evaluation I

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Team Leader, Pharmacometrics
Division of Pharmaceutical Evaluation I

Clinical Pharmacology and Biopharmaceutics Briefing: November 7, 2002 (PKLN 6A19).

cc: NDA 21-414(original file)
HFD-120/Division File
HFD-850/LLesko, SMHuang, PILee
HFD-880/ Pelsor F
HFD-860/ MMehta, CSahajwalla, JGobburu, RBaweja, RKavanagh, BBooth
CDR Biopharm

Abbreviations:

- GD: soman
- I.M.: intramuscular
- I.G.: intra gastric
- MD: multiple dose
- N.G.: nasogastric; I.G. and N.G. are essentially equivalent oral delivery
- PYR: pyrisdostigmine
- SD: single dose

Introduction

Pyridostigmine (PYR) is a reversible acetylcholine esterase inhibitor, that in combination with atropine (ATR) and 2-pralidoxime (2-PAM) is proposed as a preventative/rescue treatment against nerve agents. PYR at doses beginning with 60 mg is used to treat myasthenia gravis. PYR is characterized by an elimination half-life of approximately 3 hours. To support their application, the sponsor submitted a number of studies in Rhesus monkeys. These data consisted of pharmacokinetic and survival studies. These data were used in conjunction with the human pharmacokinetic studies of PYR to predict doses that produce an equivalent exposure in humans.

The sponsor conducted a series of in vivo pyridostigmine studies (Task 92-30) in male Rhesus monkeys (—— The objectives of these studies were

Phase I: to determine the 48-hr LD_{50} of intramuscular (i.m.) Soman (GD) with or without ATR and 2-PAM.

Phase II: to determine the PYR dose and Tmax that produces a 23% inhibition of RBC acetylcholine esterase (ACHE).

Phase III: to determine the dose-response relationship of PYR (with and without ATR/2-PAM) in monkeys challenged with 5 x 48-hr LD_{50} GD (32.5 µg/kg i.m.)

Phase IV: to determine the intragastric dose of PYR that produced the same level of RBC ACHE inhibition as an i.m. PYR and to determine if an equivalent dose of i.g. PYR would be effective against a 5 x 48-hr- LD_{50} GD (32.5 µg/kg i.m.) challenge.

Phase V: to evaluate whether 0.1 mg/kg diazepam (DZM) added to PYR/ATR/2-PAM would improve treatment of GD.

The sponsor also conducted additional studies (Task 85-18) in Rhesus monkeys —— to evaluate the pharmacokinetics, RBC-ACHE inhibition and survival of PYR, ATR and 2-PAM (NDA 21-414, volumes 8-14 of Section 5). These data were used to assess the clinical pharmacology of pyridiostigmine, and extrapolate to the human setting.

1. Pyridostigmine Bioavailability-*MONKEY*

In order to utilize the maximum amount of survival data (vida infra), it was necessary to determine the bioavailability of PYR. This determination is based on the comparison of the amount of drug absorbed following oral administration to the amount of drug administered intravenously (which is deemed to be 100 % bioavailable). However, a specific study to assess simian bioavailability was not formally conducted. Instead, relative bioavailability of PYR was informally assessed by comparing naso- or intra-gastric PYR (pseudo-per oral delivery) to intramuscular PYR administration. Generally, this comparison is made using AUC, which reflects the amount of drug absorbed. However, AUC data for Task 92-30 was not located. Therefore, relative bioavailability was based a comparison of Cmax values. This comparison is

based on the assumption that the pharmacokinetics of the two formulations are the same. Therefore, the value determined (F_{rel} = 30.6%) should be considered an approximate value.

Relative Bioavailability Calculation:

a. **Data:**

NDA 21-414: Study TASK 92-30

Dose-ug/kg	Study Phase	Route	Cmax (ng/ml*)
8.4	II*	Intramuscular	11.4
25-27	II*	intramuscular	25.8
50	IV!	intragastric	15.8

*-data listed in NDA 21-414, vol. 2.83, Task 92-30, page 27 Table 3.
!- data listed in NDA 21-414, vol. 2.83, Task 92-30, page 33, Table 5

b. $F = Cmax_{intragastric}/Cmax_{intramuscular}*(Dose_{intramuscular}/Dose_{intragastric})* 100$
$F= (15.8/25.8)*(25/50)*100$

F= 30.6%

2. **Dose-Survival Relationship of PYR in Monkeys**

The relationship between PYR dose and 48-hr survival was derived from data generated in Tasks 85-18 and 92-30 of the NDA. The data used were monkey subjects that also typically received ATR/2-PAM, and were challenged with 5 x 48-hr LD_{50} GD (32.5 µg/kg i.m.). However, there were exceptions to these conditions, which are described for each set of experiments below. Some of the differences were the timing of the GD challenges, which differed among studies. Most of the data was derived from the i.m. administration of PYR, but some data were derived from i.g. administration of PYR. In the case of the i.g. PYR administration, doses were adjusted using the relative bioavailability to approximate the i.m. dose.

The standard approach in Tasks 85-18 and 92-30 was to use 48-hr survival as the criterion for judging the outcome. This criterion appears to be the standard in this area (literature sources use this standard as well). For Task 85-18, all data refers to 48-hr survival. For Task 92-30, **only** phase V (effect of diazepam on PYR/ATR/2-PAM) studies refer to 48-hr **and** 10-day survival (vol 2.83). In this study, animals treated with PYR/ATR/2-PAM, the 48-hr survival was 50%, and 10-day survival was 20%. In the databases for phases III and IV, several animals were listed as dying post-48 hours; these animals are not in the PYR/ATR/2-PAM groups. No other references to 10-day survival was located in the vol. 8-14 02 2.83 in Section 5 of NDA 21-414.

Single Dose PYR data (SD-open symbols)

O
From Task 92-30, phase V studies. The purpose of these studies was to determine the effect of adding diazepam (DIA) to the PYR/ATR/2-PAM regimen. Four groups were tested

1. ATR/2-PAM (n=8)
2. ATR/2-PAM/DIA (n=10)
3. PYR/ATR/2-PAM (n=10)
4. PYR/ATR/2-PAM/DIA (n=10)

The PYR/ATR/2-PAM group data is presented in the figure. The dose of each drug used in each of the treatment regimens was 0.4 mg/kg ATR, 25.7 mg/kg 2-PAM, 0.1 mg/kg DIA and 4 ug/kg PYR.

Eighty to 260 ug/kg GD was administered i.m. 45 minutes post PYR administration. Survivors were dosed with 80, 130, 160, 175 and 210 ug/kg GD.

Δ
From Task 92-30, phase III studies. The purpose of these studies was to determine the dose-survival response of PYR with ATR (0.4 mg/kg)/2-PAM (25.7 mg/kg) when challenged with 5 x 48-hr LD50 of GD (32.5 ug/kg).

In this group, PYR was dosed at 4 ug/kg i.m. (n=10). GD 32.5 was administered i.m. 45 minutes post PYR administration.

□
From Task 92-30, phase III studies. The purpose of these studies was to determine the dose-survival response of PYR with ATR (0.4 mg/kg)/2-PAM (25.7 mg/kg) when challenged with 5 x 48-hr LD50 of GD (32.5 ug/kg).

In this group, PYR was dosed at 8.4 ug/kg i.m. (n=10). GD was administered i.m. 45 minutes post PYR administration.

◊
From Task 92-30, phase IV studies. The purpose of these studies was to determine an intragastric (i.g.) dose of PYR that provided a similar level of RBC ACHE inhibition and survival as a known i.m. PYR dose. PYR was administered with ATR (0.4 mg/kg)/2-PAM (25.7 mg/kg) and challenged with 5 x 48-hr LD50 of GD (32.5 ug/kg).

In this group, PYR was dosed at 40 ug/kg i.g. (n=10). Accounting for bioavailability (~30%), the equivalent i.m. dose of PYR was approximately 12.2 ug/kg. GD was administered i.m. 150 minutes post PYR administration.

∇
From Task 92-30, phase III studies. The purpose of these studies was to determine the dose-survival response of PYR with ATR (0.4 mg/kg)/2-PAM (25.7 mg/kg) when challenged with 5 x 48-hr LD50 of GD (32.5 ug/kg).

In this group, PYR was dosed at 24 ug/kg i.m. (n=10). GD was administered i.m. 45 minutes post PYR administration.

Multiple Dose data (closed symbols)

From Task 85-18, the purpose of these studies was to determine the RBC ACHE inhibition and survival of 1.2 and 2.4 mg/kg PYR N.G. with ATR (0.4 mg/kg)/2-PAM(25.7

mg/kg) compared to ATR/2-PAM alone or no treatment. Based on relative bioavailability, the equivalent i.m. PYR doses would correspond to 367 and 734 ug/kg. Animals were dosed t.i.d. for a total of six PYR doses. Animals were then challenged with GD doses that ranged from 15 to 617 ug/kg i.m., 300 minutes post PYR.

Two groups of 48 animals each were studied at low and high PYR dosages. The standard drug regimen used throughout the experiments was to use 0.4 mg/kg ATR, and 25.7 mg/kg 2-PAM. In task 85-18, in early studies some monkeys (n=8) were treated with 0.095 mg/kg of ATR instead of the standard 0.4 mg/kg used in all other experiments (4 from each group: low PYR: 3f1, 906t 3ke, 388d; Hi PYR B910, 2y5, 810t, 889c;). Therefore, these animals were excluded from the analysis. One animal 187D was removed from the study, but no reason is apparent (vol.11). Therefore, the number of evaluable monkeys was 44 and 43, in the low-dose and high-dose PYR groups respectively. The sponsor reported data for 36 monkeys per group (the reasoning for this is unclear). The data is presented as n=48 and n=44/43. Additionally, data from animals challenged with 50 µg/kg of GD or less are presented for the low and high PYR groups.

●
Low dose PYR (1.2 mg/kg N.G./364 ug/kg i.m.). N=48

▲
Low dose PYR (1.2 mg/kg N.G./364 ug/kg i.m.). N=44. Four animals were removed because they were dosed with 0.095 mg/kg ATR instead of 0.4 mg/kg.

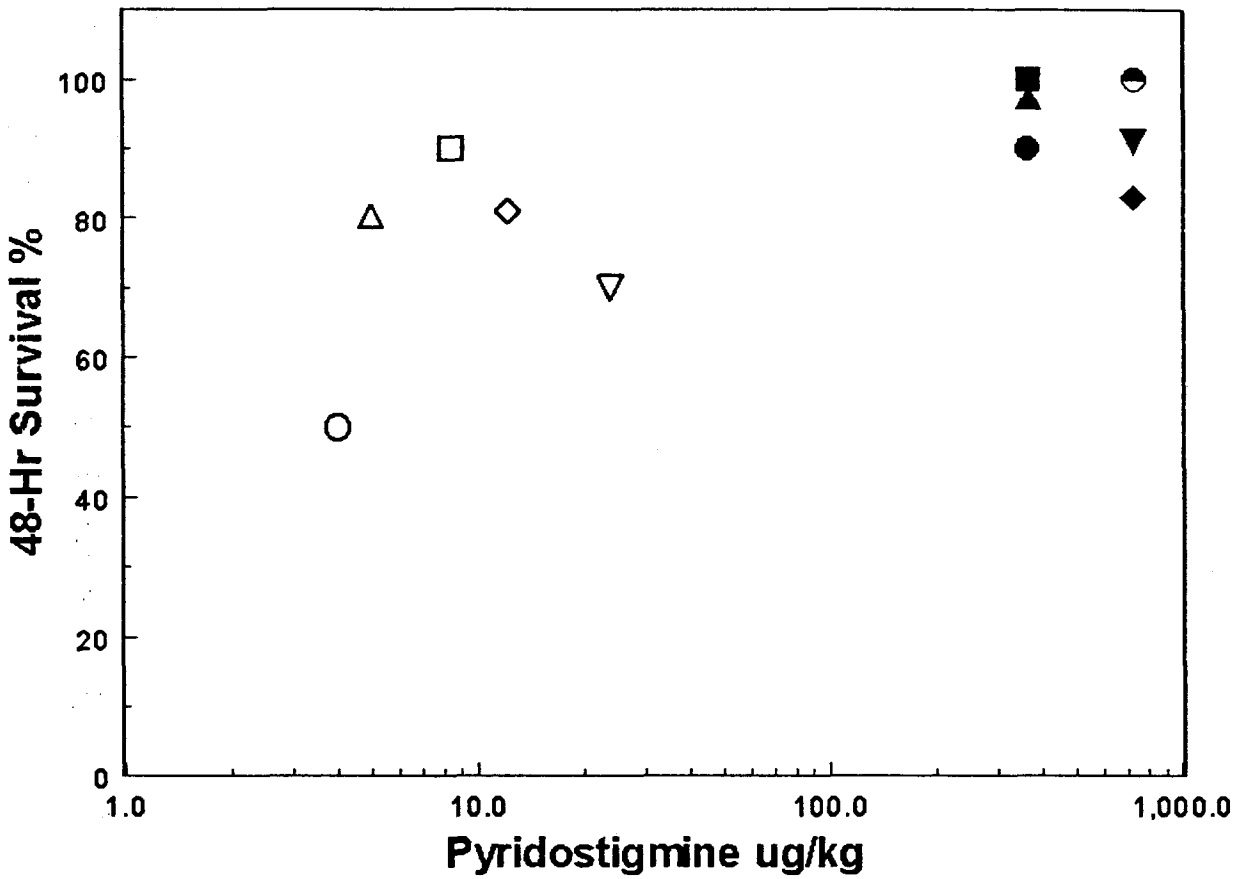
■
Low dose PYR (1.2 mg/kg N.G./364 ug/kg i.m.). N=15. Only animals challenged with 50 ug/kg or less of GD are presented in this group.

◆
High dose PYR (2.4 mg/kg N.G/728 ug/kg i.m.). N=48

▼
High dose PYR (2.4 mg/kg N.G/728 ug/kg i.m.). N=43 Four animals were removed because they were dosed with 0.095 mg/kg ATR instead of 0.4 mg/kg. One additional animal was removed from the study.

⬬

High dose PYR (2.4 mg/kg N.G/728 ug/kg i.m.). N=7. Only animals challenged with 50 ug/kg or less of GD are presented in this group.



A logistic regression analysis (SAS ver 8.2) indicated that no relationship existed between dose and survival (p=0.16). However, as most of the data appears to be at the approximate Emax, there may be insufficient data at lower doses to adequately describe the relationship with survival. The data suggest survival of 80 % or greater may be achieved in monkeys at doses ranging from approximately 6 to 1000 μg/kg.

There are a couple of confounding issues which should be considered. First, subjects that survived certain phases of the experiments were sometimes re-used in later experiments after an un-described period of washout. Which subjects were re-used is unclear. The effect of previous treatment on survival to a later challenge of GD is unknown. Secondly, data from different study designs were used. Some data was from single dose data, whereas some was multiple-dose administration. The comparison between the two cases may be hampered by drug accumulation (if any) during multiple dosing. Lastly, the intragastic data has been adjusted for bioavailability. The bioavailability is only an approximation. Therefore, the oral doses that produce this survival following multiple doses may be higher or lower than those used here.

3. PYR Pharmacokinetics in Monkeys

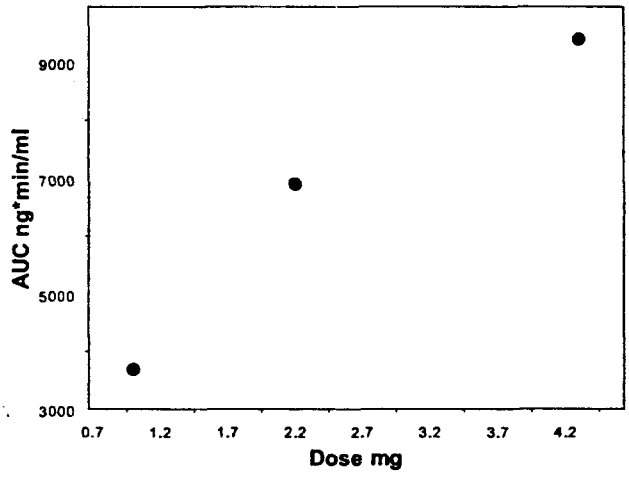
In Task 85-18, the pharmacokinetics of PYR were assessed. Monkeys were fasted for 12 hr and then dosed with PYR, ATR and 2-PAM. PYR was administered by nasogastric tube at approximately 0.3, 0.6 and 1.2 mg/kg. The pharmacokinetic modeling used by the sponsor was not clearly described, and the sponsor assigned either "0" or an LOQ value ([redacted] ng/ml) to missing data. For these reasons, the data were re-fit by FDA using WinNonLin Professional ver. 3.2. Data were fit noncompartmentally using extravascular input and assuming first order elimination. The results of this modeling are listed in the table below

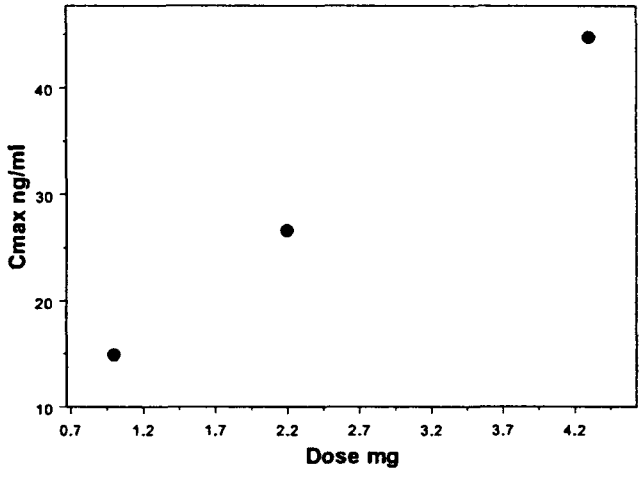
Monkey PK (Task 85-18)

Route	N	Ave Dose mg	Ave Dosage mg/kg	AUC ng*hr/ml	Cmax ng/ml	CL ml/min	CL L/hr/kg	CL L/hr/kg (-outliers)*
intragastric	12	1.0	0.303	61.3 ± 24.7 (40%cv)	14.9 ± 9.6	351.7 ± 188	8.4 ± 7.41	6.1 ± 2.78
intragastric	12	2.2	0.604	114.9 ± 54 (47%cv)	26.6 ± 11.2	396.5 ± 274	6.65 ± 4.67	5.44 ± 1.88
intragastric	12	4.3	1.18	156.5 ± 66 (42%cv)	44.7 ± 22.8	491.6 ± 140.8	8.29 ± 2.89	7.29 ± 1.79

* CL values greater than 3 x the SD were deemed outliers.

The variability in these data vary between 40 to 50%. Removal of apparent outliers (on a visual basis) reduced the variability. Cmax and AUC appear to increase proportionately to dose, as shown in the figures below. The CL of PYR using all animals is 7.66 ± 5.22 L/hr/kg.





4. Human Pharmacokinetics of PYR.

The sponsor studied the pharmacokinetics of PYR in 30 male and 30 females. Pyridostigmine was administered orally every 8hrs for 21 days (total of 64 doses). Patients were fasted for the first and last doses. PYR pharmacokinetics were assessed with dense sampling on Day 1 and Day 22. FDA re-analyzed the pharmacokinetics of PYR using WinNonLin Professional Ver 3.2, Noncompartmental modeling with extravascular input and assuming first order elimination. These data are listed in the Table below.

Human PK of PYR-All data

Route	N	Dose mg	Ave Dosage mg/kg	Cmax ng/ml	AUC ng*hr/ml	CL L/hr/kg
SD day 1, p.o.	60	30	0.43	21.0 ± 8.9	89.1 ± 32.0	5.35 ± 1.9
MD day 22, p.o.	60	30	0.43	27.9 ± 10.2	118.2 ± 42.3	4.06 ± 1.5

Effect of Sex and Time on Human PYR PK

Subject/Regimen	Dosage mg/kg	Cmax ng/ml	AUC ng*hr/ml	CL L/hr/kg
Males SD	0.43	19.5 ± 5.8	87.3 ± 24.8	4.88 ± 1.85
Females SD	0.43	22.5 ± 11.1	91.1 ± 38.2	5.8 ± 1.9
Males MD	0.43	26.0 ± 8.83	113.6 ± 39.7	3.81 ± 1.40
Females MD	0.43	29.9 ± 11.1	122.7 ± 45.0	4.31 ± 1.58

A preliminary statistical analysis of the effect of sex on the pharmacokinetics of PYR indicated that no significant differences were observed for Cmax, AUC or CL ($p>0.05$; unpaired t-test).

A preliminary statistical analysis also indicated that the AUC increased and CL decreased significantly ($p<0.001$; paired t-test; power $>>0.80$) both for male and female subjects on Day 22 compared to Day 1. The CL decreased (males and females combined) decreased by 34% by Day 22.

5. PREDICTED HUMAN DOSES

The monkey PYR dose-survival curve, and the pharmacokinetic characterization of PYR in monkeys and humans allow for the prediction of doses that provide an equivalent human exposure. This extrapolation is based on several assumptions, namely

1. The monkey PK of pyridostigmine are reflective of the PK in humans
2. The survival (PD) in monkeys is reflective of the survival (PD endpoint) in humans.
3. Linear pharmacokinetics of pyridsotgmine in monkeys and humans

a. Target AUCs derived from monkeys

The dose-survival curve and the pharmacokinetics of PYR in monkeys can be used to calculate target AUCs in humans. The pharmacokinetic equation

$$CL = Dose/AUC \qquad (1)$$

Can be algebraically manipulated to calculate CL, dose or AUC given knowledge of the other parameters. Using an average monkey weight of 3.54 kg CL and Dose can calculated and then AUC can be determined.

$$CL = 7.66 \text{ L/hr/kg} * 3.54 \text{ kg} = 27.1 \text{ L/hr} \quad (2)$$

$$\text{Dose} = \text{Dosage}* \text{ wt} \quad (3)$$

Then AUC is determined as

$$\text{AUC} = \text{Dose/CL} \quad (4)$$

Tabulated Target AUCs

Dosage mg/kg*	Dose mg !	CL L/hr!	AUC ng*hr/ml	AUC mg*hr/L
0.05	0.177	27.1	**6.531**	**0.006531**
0.1	0.354	27.1	**13.1**	**0.013063**
0.2	0.708	27.1	**26.1**	**0.026126**
0.3	1.062	27.1	**39.2**	**0.039188**
0.4	1.416	27.1	**52.2**	**0.052251**
0.5	1.77	27.1	**65.3**	**0.065314**
0.6	2.12	27.1	**78.2**	**0.078228**

*-from 48-hr survival curve; ! determined using 3.54 kg

b. Calculation of human doses based on target AUC's in monkey

Using the target AUC determined with the monkey data, human doses can be calculated by rearrangement of equation (1). An average human weight of 70 kg was used for all calculations.

$$CL \text{ (L/hr/kg)} = 5.35 \text{ L/hr/kg}*70 \text{ kg} = 374.5 \text{ L/hr} \quad (5)$$

$$\text{Dose} = CL* AUC_{(monkey)} \quad (6)$$

Extrapolated Human Doses

CL L/hr	$Target_{monkey}$ AUC (mg*hr/L)	Dose mg	Dosage mg/kg (using 70 kg)
374.5	0.006531	**2.45**	**0.035**
374.5	0.013063	**4.89**	**0.070**
374.5	0.026126	**9.78**	**0.140**
374.5	0.039188	**14.7**	**0.21**
374.5	0.052251	**19.6**	**0.28**
374.5	0.065314	**24.5**	**0.35**
374.5	0.078228	**29.3**	**0.419**

Conclusions:

Providing the underlying assumptions hold true, the results indicate doses from 2.5 to 29 mg should provide exposures similar to the PYR dose-survival curve for monkey. Marino et al reported 31% variability for clearance, which is likely a more accurate assessment than that determined in this case (35-37%), due to the methodology employed. Therefore, some individuals in this population may have a CL value up to 3 x SD higher than the average. In these cases, the corresponding PYR exposure (AUC) will be substantially lower. Doses less than 14.7 mg of PYR would equate to dosages less than 10 µg/kg.

APPEARS THIS WAY
ON ORIGINAL

Appendices

Volumes reviewed
Item 5. Vol 8-14; 2.83;
Item 6: vol 2.2, 2.57, 2.85, 2.86, 2.88

Attended: 11/07/02 PKLN 6A19: Bbooth, Jgobburu; Rkavanagh: Mmehta; SMHuang; PILee; Fpelsor; Llesko(t-con)

References

1. Marino MT, Schuster BG, Bruekner RP, Lin E, Kaminski A, Lasseter KC. 1998. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans J Clin Pharmacol 38:227-235.

Route	Study	Regimen	Dose ug/kg	N	Deaths	Survival	Adjusted Dose	GD minutes	Survival Period
NG	85-18	MD	1200	48	5	90	367	300	48 hr
NG*	85-18	MD	1200	44	1	98	367	300	48 hr
NG**	85-18	MD	1200	15	0	100	367	300	48 hr
NG	85-18	MD	2400	48	7	85	734	300	48 hr
NG*	85-18	MD	2400	43	3	93	734	300	48 hr
NG**	85-18	MD	2400	7	0	100	734	300	48 hr
IM	92-30/phase3	SD	4	10	2	80	NA	45	48 hr
IM	92-30/phase3	SD	8.4	10	1	90	NA	45	48 hr
IM	92-30/phase3	SD	24	10	3	70	NA	45	48 hr
IG	92-30/phase4	SD	40	10	2	80	12.2	150	48 hr
IM	92-30/phase5	SD	4	10	5	50	NA	45	48 hr

NG-naso-gastric; IG-intra-gastric; MD-multiple dose; SD single-dose. * Animals treated with low ATR removed from study. **Animals treated with 50 ug/ml or less GD. NA-not applicable.

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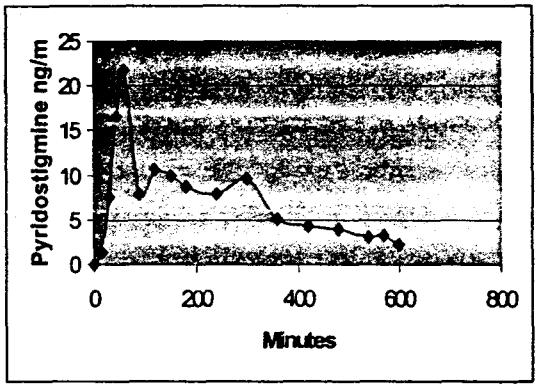
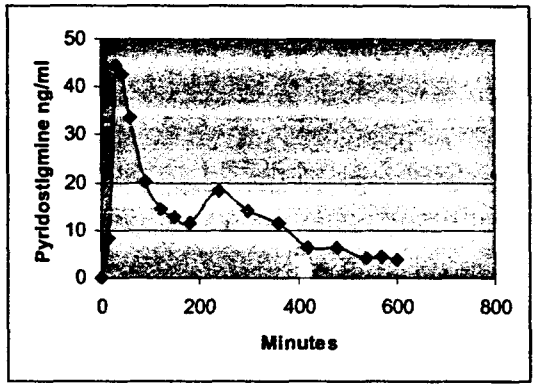
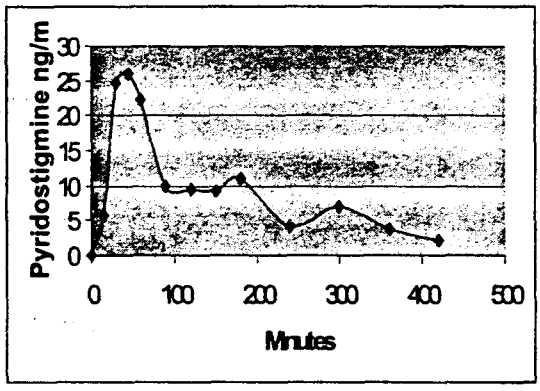
Monkey PK Profiles (N=36) from Task 85-18



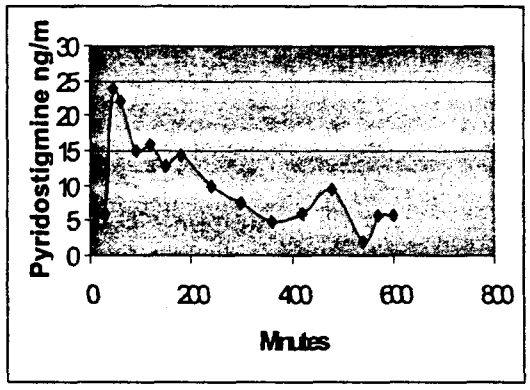
246D. 1.171 mg/kg



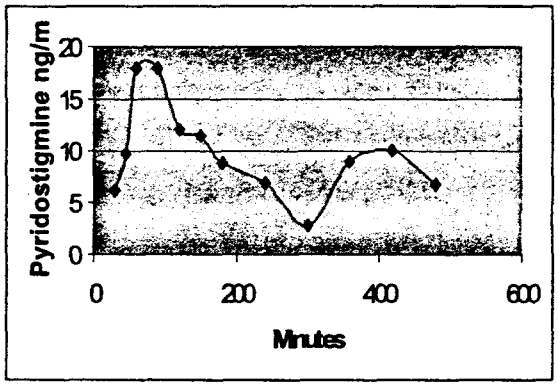
025D: 1.188 mg/kg



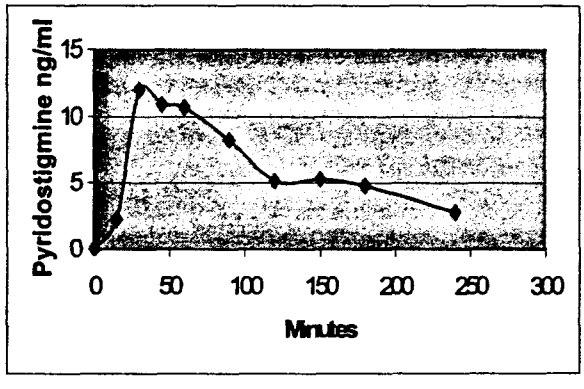
33D: 0.614 mg/kg



400D: 0.602 mg/kg

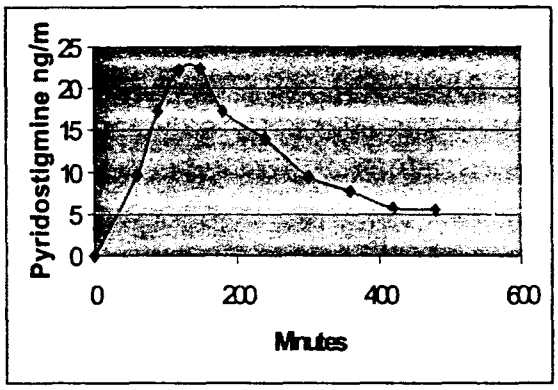


937C: 0.301 mg/kg

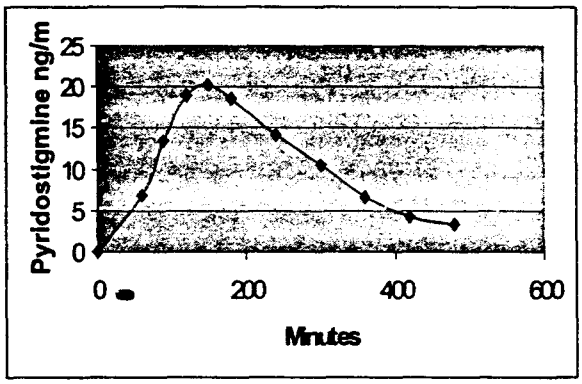


245D: 0.326 mg/kg

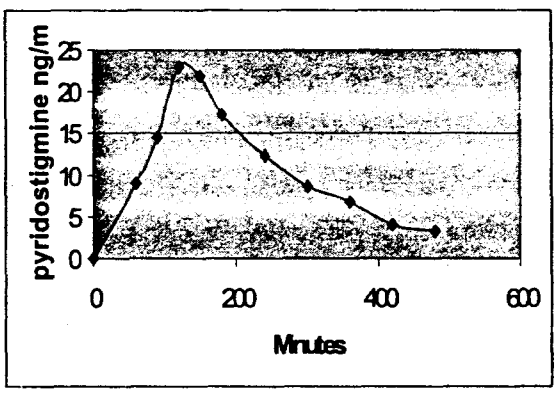
Human PK Profiles from Study Protocol 94-09 (30 mg dose)



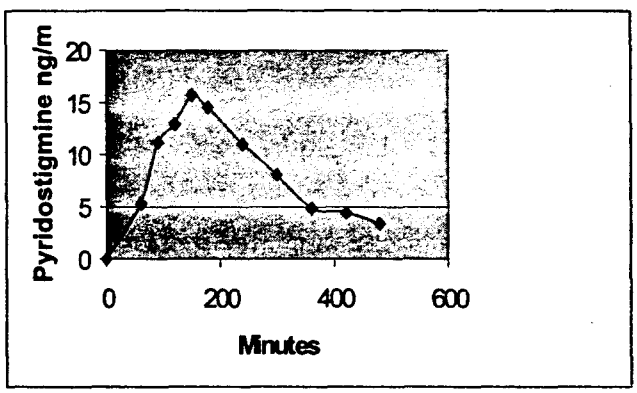
Subject 3, male-low weight



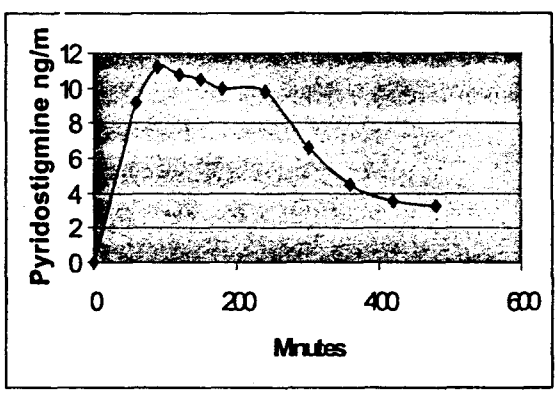
Subject 57: male-low weight



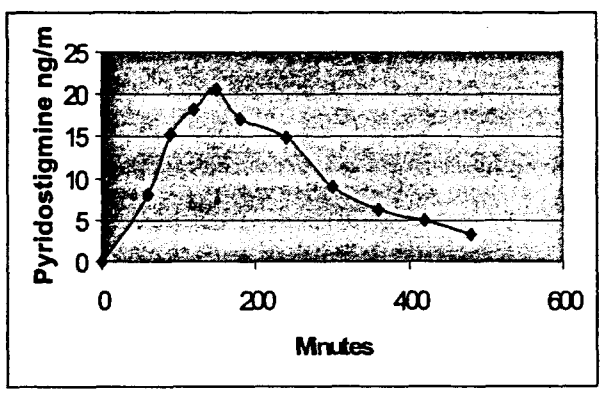
Subject 47: mid weight



Subject 10: mid-weight



Subject 13: hi-weight



Subject 58: Hi-weight

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/s/

Brian Booth
1/8/03 02:37:39 PM
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Lee and Lesko not on CC list; don't know how to add them.

Jogarao Gobburu
1/8/03 03:38:15 PM
BIOPHARMACEUTICS

New Drug Application
Clinical Pharmacology and Biopharmaceutics Review

NDA:	20-414
Types of Submissions:	N-AZ N-BZ
Generic Name:	Pyridostigmine Bromide
Formulation:	Immediate Release Tablets
Strengths:	30 mg
Route:	PO
Sponsor:	Department of the Army Fort Detrick, MD
Submission Dates:	January 3, 2003 January 7, 2003 20-414 May 24, 1996 N(RS) C2.1 pg 444
Related NDA	09-829
Related IND	———
Related DMF	——— Dated June 4, 2001
Reviewer:	Ronald Evan Kavanagh, B.S. Pharm., Pharm.D., Ph.D.

I. EXECUTIVE SUMMARY

Previous OCPB reviews for NDA 20-414 pyridostigmine bromide 30 mg tablets include:

A) NDA 20-414 Submission Dates March 4, 1994, May 17, 1996 and July 19, 1996. OCPB Review by Iftekhar Mahmood dated 8/8/96

B) IND ——— Serial Number 070 Submitted October 2, 2001. OCPB Review by Hong Zhao dated 10/16/01 and may be found in DFS Folder I ——— N 070 IM 02-Oct-2001, Filename IND ——— .doc.

OCPB review A) above included clinical pharmacology data generated with formulations from ——— Roche in addition to literature references. In the future the only 30 mg formulation that will be marketed by the Army is a different formulation from ICN Pharmaceuticals, (ICN-Canada). OCPB review B) above evaluates a pivotal bioavailability/bioequivalence study with the ICN-Canada and Roche-UK formulations.

The present review updates the labeling proposed in the initial OCPB review (review A above) with data from the to-be-marketed ICN-Canada formulation (review B above) and also the reviews pivotal dissolution data for the Roche-UK and ICN-Canada products used in the pivotal bioavailability study.

Based upon the limited dissolution data provided an interim dissolution method and specification has been set.

The sponsor has made a phase IV commitment to provide additional dissolution information so that a permanent regulatory dissolution specification may be set.

II. RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics / Division of Pharmaceutical Evaluation I (OCPB/DPE-1) has reviewed NDA #20-414 — submitted January 3, 2003, January 7, 2003, May 24, 1996 N(RS) C2.1 pg 444 and DMF — submitted June 4, 2001.

OCPB finds this application acceptable provided that currently outstanding issues are adequately resolved. Comments should be communicated to the sponsor as appropriate (see Section III Comments to the Sponsor on page 2). Labeling comments should also be communicated to the sponsor as appropriate (see Section V.A.2.a)

Labeling - Professional Labeling – Package Insert - Current OCPB Proposal - Previous OCPB Proposal with Edits on page 6).

III. COMMENTS TO THE SPONSOR

A. Dissolution

Please adopt the following interim dissolution method and specifications for pyridostigmine 30 mg tablets.

Table 1 Proposed Interim Regulatory Dissolution Method and Specifications

Parameter	**Description**	
Apparatus type	USP Apparatus II (paddle)	
Media	Water	
Volume (ml)	900 ml	
Temperature	37 ± 0.5 °C	
Speed of rotation (rpm)	50 rpm.	
Sample times (hours):	— minutes	
Specifications (% of Label Claim)	Q = — at — minutes	Acceptance criteria as per USP XXV – NF 20 <711> Dissolution Acceptance Table

B. Labeling Comments

The sponsor is requested to adopt OCPB proposed labeling as outlined in Section 0 Labeling on page 6

C. Phase IV Commitments

The sponsor should commit to providing complete dissolution profile data in various media within 6 months so that a final dissolution method and specification can be established. A written request that included the phase IV commitment proposal was provide to the sponsor at a meeting in December 2002. The phase IV commitment is as follows:

Batch information and complete dissolution profiles in 4 media, (pH 1.2, pH 4.5, pH 6.8 and water), for the two pyridostigmine bromide 30 mg tablet batches used in the pivotal bioavailability study (see Table 2), using 900 ml of media and USP type II (paddle) apparatus at 50 RPM.

Table 2 Batches used in Pivotal Bioavailability Study 205-01-11618

Formulation	Pyridostigmine Bromide 30 mg tablets	Pyridostigmine Bromide 30 mg tablets
Manufacturer	Roche – UK	ICN – Canada
Lot Number	PYA 569	1A8682

Twelve individual units each of the test and reference batches should be tested for each dissolution medium, and the similarity factor (f2) should be provided for each pair-wise comparison. From the results obtained, the agency will be able to select one medium as the official regulatory dissolution medium for the tablets.

If batches PYA 569 (Roche UK) or 1A8682 (ICN Canada), have reached their expiration date, then

a) The sponsor is requested to submit all available dissolution data for these batches.

b) If the sponsor does not have dissolution information or has partial information on the expired biobatches, then you may generate additional dissolution information on these batches if you can show that content uniformity is at least 90% of the label claim for the two biobatches.

c) Alternatively, the requested dissolution data may be generated for batches identical to the biobatches, (i.e. identical with regard to manufacturing site, method of manufacture, composition, and batch size), that have not yet reached their expiration date.

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IV. SIGNATURES

/S/

Ronald Evan Kavanagh, B.S. Pharm., Pharm.D., Ph.D. — Date

Division of Pharmaceutical Evaluation I
Office of Clinical Pharmacology and Biopharmaceutics

/S/

Ray Baweja, Ph.D. — Date

Team Leader
Division of Pharmaceutical Evaluation I
Office of Clinical Pharmacology and Biopharmaceutics

An informal discussion of the labeling and dissolution was held between Kavanagh, Baweja, and Mehta on Tuesday, January 7, 2003.

CC: NDA 20-414 (orig., 1 copy)
HFD-120 (Prohaska, Feeney, Katz, Rzeszotarski, Rosloff, Nighswander)
HFD-860 (Kavanagh, Baweja, Mehta, Sawajwalla)
Central Document Room (Barbara Murphy)

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2 page(s) of revised draft labeling has been redacted from this portion of the review.

5. Qualitative / Quantitative Composition

Table 3 Qualitative /Quantitative Composition of Pyridostigmine Bromide 30 mg Tablets from Roche UK Including Overage

Ingredients	Specification Reference	Function	Manufacturer	Tablet Amount (mg / tablet)	Batch Amount (kg)		% (w/w)
					Sub-batch	Full Batch	
Pyridostigmine bromide	USP						17.9
Lactose	NF						17.4
Starch	NF						45.6
Silica Precipitated	FCC‡						17.1
Talc	USP						1.8
Magnesium Stearate	NF						0.2
Purified Water*	USP						0.0
Total				**175.0**			**100.0**

* Removed during manufacture

‡ - Food and Chemical Codex

Table 4 **OCPB Predicted Qualitative /Quantitative Composition of Pyridostigmine Bromide 30 mg Tablets from ~~Excluding~~ Excluding Overage**

Ingredients	Specification Reference	Function	Manufacturer	Tablet Amount (mg / tablet)	Batch Amount (kg)		% (w/w)
					Sub-batch	Full Batch	
Pyridostigmine bromide	USP						17.3
Lactose	NF						17.5
Starch	NF						46.0
Silica Precipitated	FCC‡						17.3
Talc	USP						1.8
Magnesium Stearate	NF						0.2
Purified Water*	USP						0.0
Total				**173.75**			**100.0**

b) ICN – Canada

Table 5 **Qualitative /Quantitative Composition of Pyridostigmine Bromide 30 mg Tablets from ICN - Canada Including Overage**

Ingredients	Specification Reference	Function	Tablet Amount (mg / tablet)	Batch Amount (kg)	% (w/w)
Pyridostigmine Bromide	Active	Active			16.3
Lactose, Anhydrous	NF				72.5
Silicon Dioxide, Colloidal	NF				8.0
Stearic Acid	NF	Lubricant			3.2
Total			**187.5**	**375.0**	**100.0**

* Currently a 2.0% overage is included in the manufacturing process formulation.

6. Method of Manufacture

a) Roche

The manufacturing process for Pyridostigmine Bromide Tablets USP 30mg is based on the process for a similar product, Mestinon (pyridostigmine bromide tablets 60mg), which has been manufactured by Roche since 1954.

b) ICN - Canada

7. Dissolution Methods

For the single point dissolution data for the pyridostigmine bromide 30 mg tablets provided by Roche-UK and ICN-Canada for the pivotal bioavailability study, The Department of Defense contracted the dissolution experiment to The method used by varied slightly from the method used by Roche-UK and the USP method in that it used a higher quality of water (Milli-Q) and a temperature that is slightly below the USP specification of 37 ± 0.05 °C.

A summary of the method is shown in Table 6.

Table 6 Dissolution Method Used by for Interim Specifications

Parameter	Description
Apparatus	USP Apparatus II (paddle)
Media	Water
Volume	900 ml
Temperature	
Speed of Rotation	50 rpm
Sampling Time	minutes

8. Dissolution Data – 45 minutes

Table 7 Single Point Dissolution Data
February 15, 2002

Manufacturer	Lot Numbers			Date of Manufacturer	n	% Labeled Content at 45 minutes	Comments
	—[a]	—[a]	Manufacturer's Lot #				
Roche	WR250710	BP18555	PYA569[b]	Feb 1998	6	94.3 ± 2.3 (2.4) 91.2 – 97.4	Burst like disintegration into powdery clumps after 10- 15 minutes. Powder then remained at the bottom of the vessel for the duration of the 45 minute experiment.
ICN Canada	WR250710	BP21838	1A8682[b,c]	Jan 2001	6	98.3 ±1.8 (1.8) 95.7 – 101.0	Dissolved slightly faster. Complete dissolution within 10 – 15 minutes. Almost all of the excipients as a fine powdery substance floated to the top of the medium for the remainder of the 45 minutes.

a – Basis of these lot numbers were not identified
b – These lot numbers are for the batches used in the pivotal bioavailability/bioequivalence study.
c - It has been determined that ICN-Canada lot number 1A8682 refers to packaging. The lot number for the tablet drug product batch is 1A8675

3 page(s) of revised draft labeling has been redacted from this portion of the review.

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/s/

Ron Kavanagh
1/8/03 11:57:47 AM
BIOPHARMACEUTICS

Raman Baweja
1/8/03 01:55:20 PM
BIOPHARMACEUTICS

An Exposure-Response Model for Identifying Covariates That Account for Interspecies Difference in Pyridostigmine Protection Against Soman Toxicity

Prepared by
Peter I. Lee, Ph.D.
Associate Director, Pharmacometrics
Office of Clinical Pharmacology and Biopharmaceutics
January 3, 2002

Objectives

The objective of this modeling analysis was to identify the covariates that account for interspecies differences in pyridostigmine (PYR) protection against soman toxicity expressed as LD50. The covariates investigated in the model included (1) PYR dose, (2) basal carboxylesterase (CaE) activity, (3) basal acetylcholinerase (AChE) activity, (4) AChE inhibition by PYR, (5) decarbamoylation rate, and (6) cresylbenzodioxaphosphorin oxide (CBDP) administration. Soman mortality data and information on the covariates in mouse, rat, guinea pig, rabbit, marmoset, and rhesus monkey were collected from the literature.

Methods

The model, proposed in Maxwell's paper [1] and later formalized in Sweeney's paper [2], for competition between pyridostimine and soman for binding with acetylcholineesterase (AChE) and carboxylesterase (CaE), can be illustrated in Figure 1. The numbers shown in the figure correspond to the covariates (numbered 1 to 6) above.

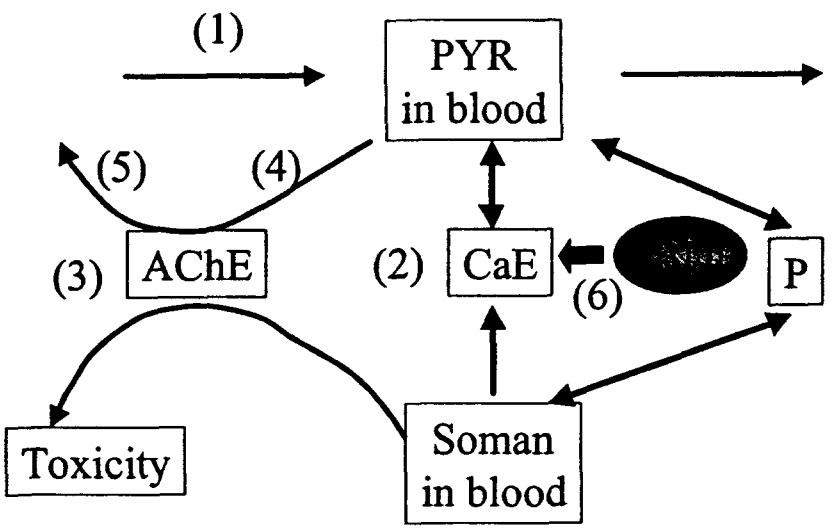


Figure 1. A model illustrating the competition between pyridostimine and soman for bindings with AChE and CaE. *P* represents other proteins in the blood. Covariates: (1)

PYR dose, (2) basal CaE activities, (3) basal AChE activities, (4) AChE inhibition by PYR, (5) decarbamoylation rate, and (6) CBDP administration.

As shown in Figure 1, soman irreversibly binds to AChE, inhibits its activity, and this results in soman toxicity. Soman also irreversibly binds to CaE, and because of this binding, active soman is removed from the blood. In theory, pretreatment with PYR, which reversibly binds to AChE, protects AChE from soman binding. Pretreatment of CBDP inhibits CaE activity, and thus leads to higher active soman concentration in the blood. In the Mawell's paper [1], four specific experimental conditions were studied, and the inhibition of AChE and CaE by PYR and/or CBDP are shown in Figure 2.

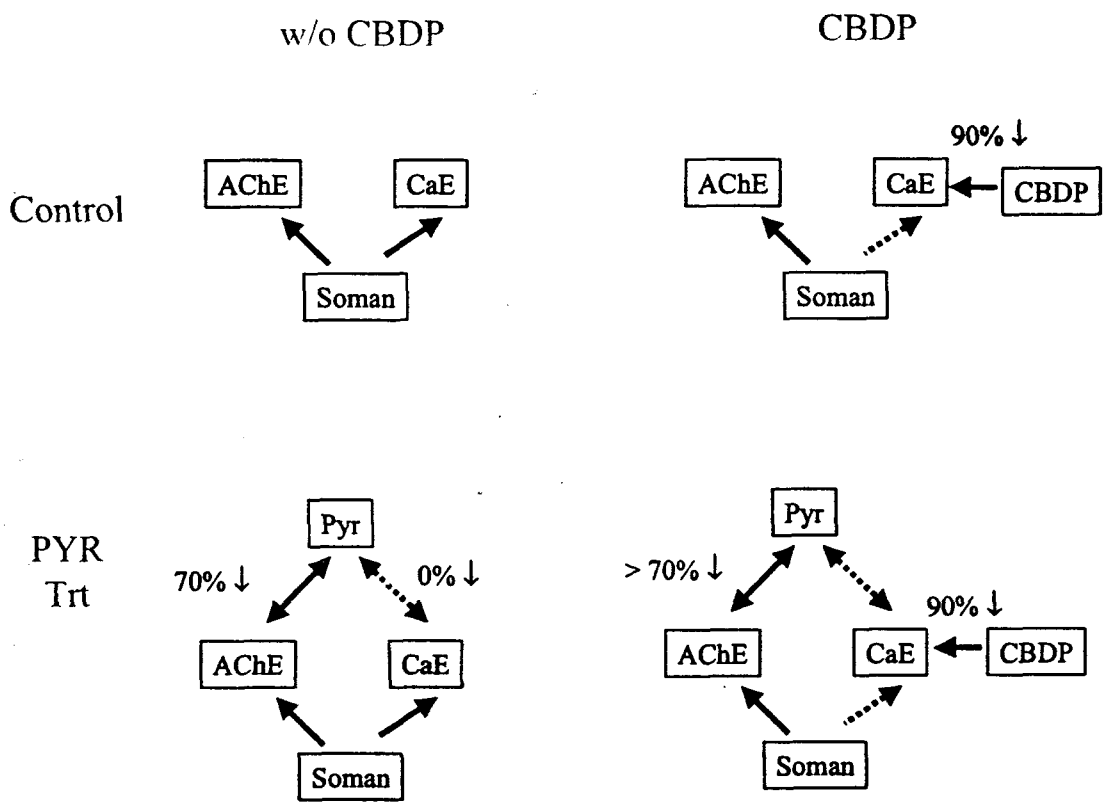
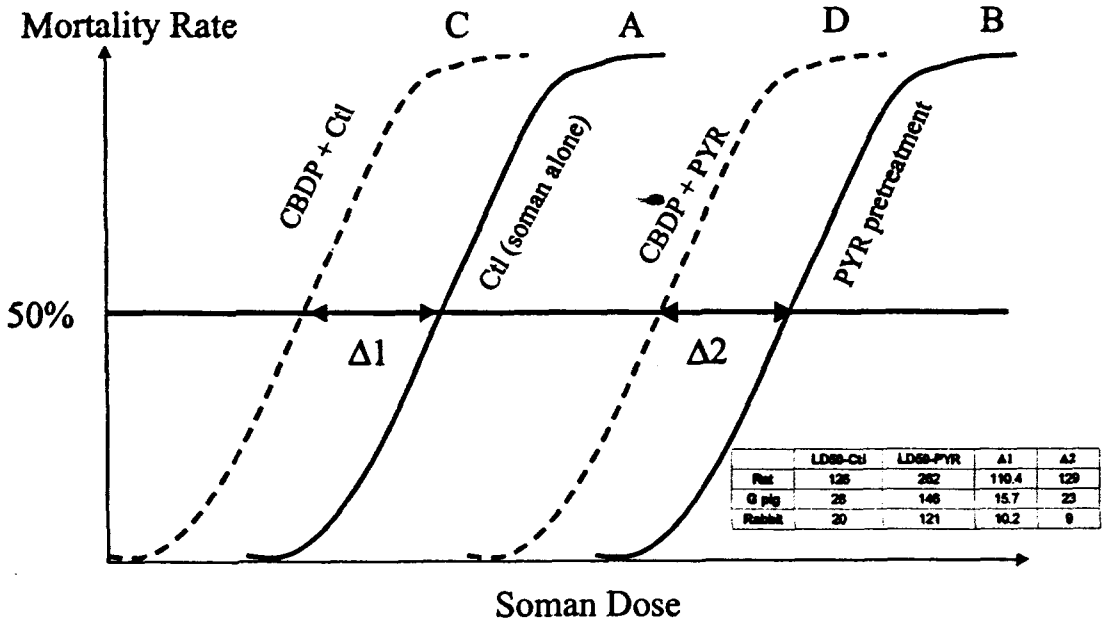


Figure 2. AChE and CaE inhibitions by PYR and CBDP in three animal species under 4 experimental conditions conducted in Maxwell's paper [1].

Based on the mechanism proposed by Maxwell, CBDP increases the active unbound soman. The influence of CBDP on the dose-toxicity relationship of soman for the control and PRY-treated group can be illustrated in Figure 3. The PYR treatment protects the animals against soman, and shifts the dose-toxicity relationship (curve B) to the right relative to that of the control group (curve A). The increased active soman due to CBDP pre-treatment shifts the dose-toxicity curve of both control and PYR groups to the left. As the net result of more active soman being available, the LD50 of soman decreases and the protection ratio increases with CBDP pre-treatment. This has been shown by the Maxwell data for rat, guinea pig, and rabbit (Figure 3). The changes in LD50 due to

CBDP pre-treatment for the control (Δ1) and PYR-treated (Δ2) groups are similar within each species (shown in the insert table of Figure 3).



	LD50-Ctl	LD50-PYR	Δ1	Δ2
Rat	126	262	110.4	129
G pig	28	146	15.7	23
Rabbit	20	121	10.2	9

Figure 3. A schematic to demonstrate the influence of CBDP on the dose-toxicity relationships of soman for the control and PRY-treated groups. (A) control group with soman alone, (B) PRY-treated group, (C) control group pretreated with CBDP, and (D) PYR group pre-treated with CBDP.

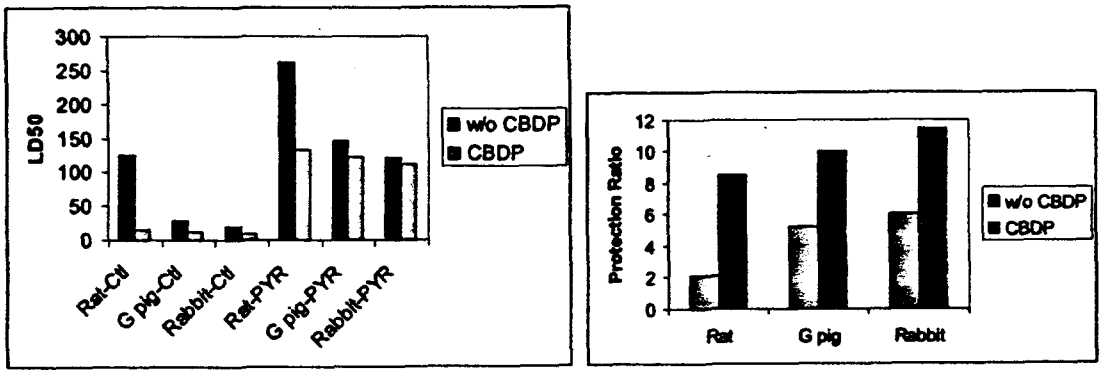


Figure 4. As predicted by the proposed MOA depicted in Figure 3, LD50 decreases and protection ratio (PR) increases in CBDP-pretreated animals for all species [1].

Based on the model shown in Figure 1, the total amount of soman in the blood (S_{total}) can be accounted for as the sum of CaE-free soman ($S_{[CaE\text{-}free]}$) plus the soman bound to CaE ($n \cdot CaE$).

$$S_{Total} = S_{[CaE-free]} + n \cdot CaE \quad (1)$$

where n is the number of soman molecules bound to each CaE molecule. If the CaE-free soman ($S_{[CaE\text{-}free]}$) is the active fraction of the total soman dose causing toxicity, then the LD50 of soman can be expressed as the following, derived from Equation 1:

$$\begin{aligned} LD50_{total\,S} &= LD50_{[CaE-free\,S]} + k \cdot CaE \\ &= f([AChE - PYR]) + k \cdot CaE \end{aligned} \quad (2)$$

where $LD50_{total\,S}$ is the LD50 based on total soman, $LD50_{[caE\text{-}free\,S]}$ is the LD50 based on CaE-free soman, and k is equal to $n \cdot V_{soman}$, where V_{soman} is the volume distribution of soman. The $LD50_{[caE\text{-}free\,S]}$ can be determined in each animal species with a pre-treatment of CBDP, a CaE inhibitor, before the soman dose. Data also implies that $LD50_{[caE\text{-}free\,S]}$ is correlated to AChE inhibition by PYR, wherein the relationship is denoted by a function $f([AChE\text{-}PYR])$.

Results

Based on Equation (2), if $f([AChE\text{-}PYR])$ is similar between animal species, i.e. by inhibiting the same percentages of AChE with PYR pretreatment, $LD50_{total\,S}$ should be linearly correlated to CaE basal activity. To test this theory, the relationships between $LD50_{total\,S}$ and CaE activity in various species with 0% and 70% inhibition of red blood cell (RBC) AChE activity are plotted in Figure 5. The relationship between $LD50_{total\,S}$ and CaE activity at 0% and 70% inhibition of RBC AChE appear to be linear among mouse, rat, guinea pig, rabbit, and marmoset. The two linear relationships also appear to be parallel to each other, indicating the value of k in Equation (2) remains relatively constant regardless of PYR dose. In addition, when the CaE activities in the mouse, rat, guinea pig, and rabbit are inhibited by CBDP (i.e. CaE=0) the LD50 in these species become similar to that of marmoset, which has little CaE basal activity.

The only species that does not fall on the linear relationships shown in Figure 5 is the monkey. Specifically, by controlling CaE activity at 0 and [AChE-PYR] inhibition at 70%, the LD50 of soman, denoted as $f(70\%)$, is 4-fold higher in the monkey compared to those in marmoset (w/o CBDP) and in other species (pre-treated with CBDP). Therefore, additional covariates other than CaE activity and RBC AChE inhibition (%) must be also responsible for the interspecies difference in LD50. Several covariates, including RBC AChE basal activity and RBC AChE decarboylation rate, have been explored to determine if these factors are correlated to the interspecies difference in $f(70\%)$. The results are shown in Figure 6. It appears that $f(70\%)$ is well correlated to basal RBC AChE activity between species, where a 4-folds higher RBC AChE activity corresponds to a 4-fold higher $f(70\%)$ in monkey compared to other species.

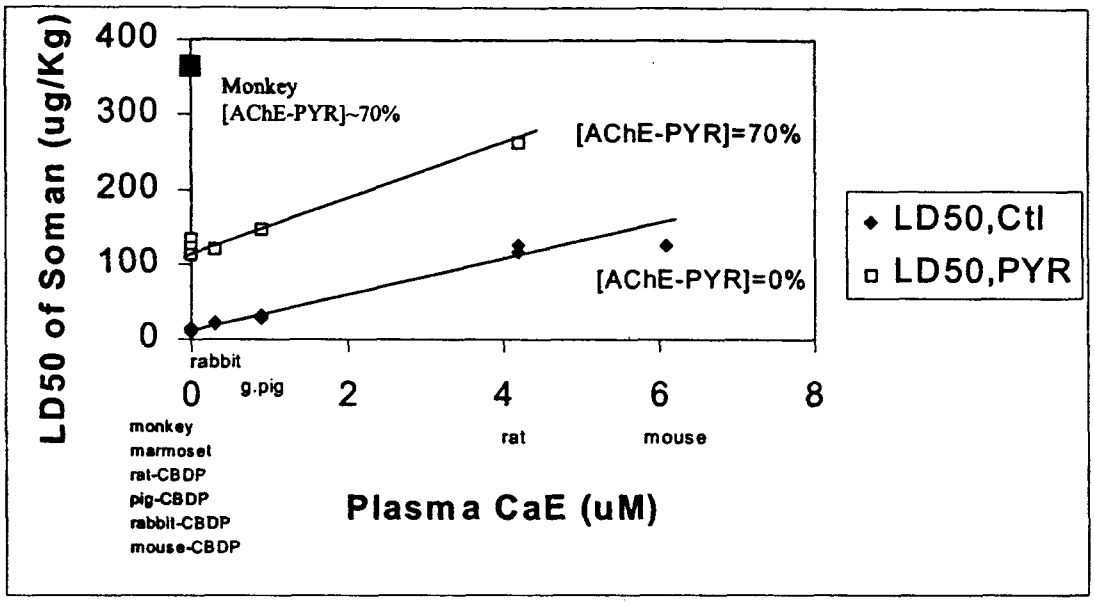


Figure 5. Correlations between LD50 of soman [1,3,4,5] vs plasma CaE activities [6,7] among different species at various levels of RBC AChE inhibition by PYR.

Discussions and Conclusions

1. The LD50 across species is correlated to plasma CaE basal activities, RBC AChE basal activities, and AChE inhibition by pyridostigmine as shown in the following equation:

$$LD50_{\text{total S}} = f([AChE - PYR\%], AChE) + k \cdot CaE \qquad (3)$$

The data demonstrate (Figure 5) that the LD50 is linearly correlated to CaE when both [AChE-PYR%] and AChE remain similar among species. It is also shown (Figure 6) that the LD50 is correlated to AChE when both [AChE-PYR%] and CaE remain similar among the species.

2. Human and monkey are relatively similar in the basal activities of plasma CaE and RBC AChE, with the following ratio:

CaE:	human (0) : monkey (0)	
AChE:	human (4.98) : monkey (4.14)	(4)

Therefore, if the interspecies correlation (Equation 3) can be extended to human, then the LD50 of soman should be similar between man and monkey.

3. The LD50 of soman in monkey following the pretreatment of PYR and post-soman treatment of atropine is correlated to blood ChE inhibition (%) [5]:

f(0%) = 13 ug/Kg
f(30%) = 176 ug/Kg
f(54%) = 378 ug/Kg (5)

In rat and guinea pig, LD50 of soman has also been shown to linearly correlate to blood AChE inhibition (%) by PYR [12]. In human, 30 mg PYR 3 times a day regimen inhibits 30-40% blood ChE within 5 hr postdose [UK data]. As shown above (Equation 5), with the same level (30-40%) of blood ChE inhibition in monkey, a protection ratio of 13 or greater against soman can be achieved.

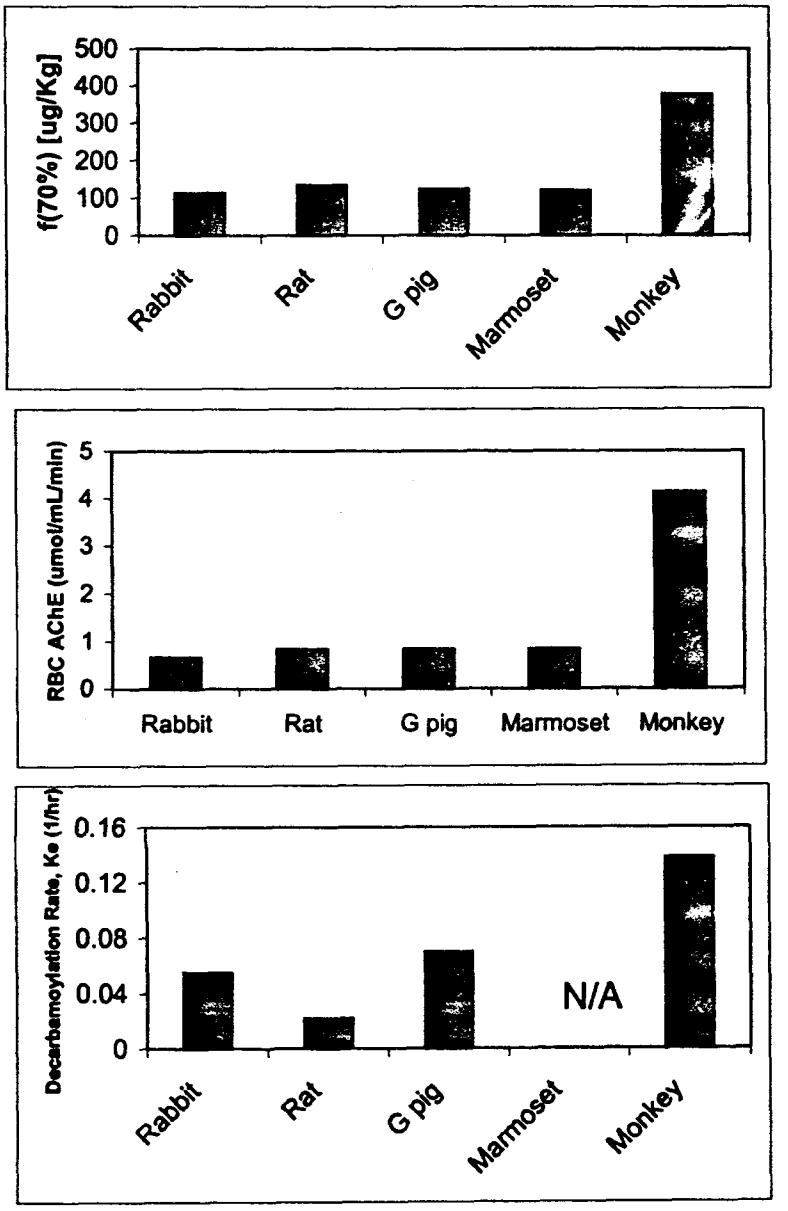


Figure 6. Upper panel: *f(70%)* is the LD50 of soman with CaE=0 and [AChE-PYR]=70% in different species; the LD50 are extracted from Figure 5 at CaE=0. Middle panel: basal RBC AChE activities in different animals [1,8,9,10]; data from difference sources are normalized to the same unit. In human, RBC AChE=4.91 umol/mL/min. Lower panel: decarbomoylation rate constant (*Ke*) of RBC AChE in different species [11]. N/A: data in marmoset is not reported in the cited paper. In human, *Ke*=0.245 1/hr.

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I concur with the report.

AUG 8 1996

AUG 8

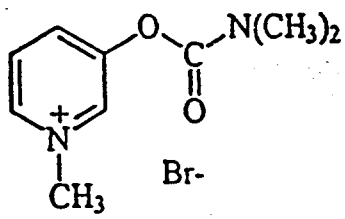
Pyridostigmine Bromide 30 mg tablet
NDA # 20-414
Reviewer: Iftekhar Mahmood, Ph. D.
Dates of submission: March 4, 1994; May 17, 1996 and July 19, 1996.

Office of the Surgeon General
Dept of the Army
Falls Church, VA 22041-3258

DETIRN
AUG 0 9 1996

INTRODUCTION

Pyridostigmine bromide is an orally active reversible cholinestrase inhibitor. Pyridostigmine bromide has been in use in the treatment of myasthenia gravis for almost a quarter century and produces its beneficial effect by reversibly inhibiting the enzyme acetylcholinesterase in the brain. Commercially available dosage forms include tablets, syrup and injectable with an average daily dose of 600 mg. However, pyridostigmine bromide can also be useful as a prophylactic against cholinesterase poisoning, especially in the case of nerve agent which binds acetylcholinesterase irreversibly. Administration of an appropriate dose of pyridostigmine bromide will covalently bind acetylcholinesterase and cannot be replaced by nerve agent.



$C_9H_{13}BrN_2O_2$ M.W. = 261.14

PYRIDOSTIGMINE BROMIDE

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SUMMARY

Pharmacokinetics:

Absorption, Distribution and Elimination:

Following a six-hour IV infusion of 6 mg pyridostigmine bromide the volume of distribution of the central compartment and at steady state was about 0.3 ± 0.1 L/kg and 1 ± 0.4 L/kg, respectively. The systemic clearance of pyridostigmine was 830 mL/min. The elimination half-life was 3.2 ± 3.4 hours (Study #1).

Following a single oral dose of 30 mg pyridostigmine bromide under fasting state, the C_{max} of pyridostigmine bromide was 22.4 ± 6.9 ng/mL and the T_{max} was 1.3 ± 0.3 hours. The $AUC_{(0\text{-}24\ h)}$ was 108 ± 52 ng.hr/mL. The absolute bioavailability of pyridostigmine bromide tablet when compared to an intravenous infusion was approximately 17 ± 6% in fasting state.

No information on protein binding of pyridostigmine is available.

Effect of Food:

Following a single oral dose of 30 mg pyridostigmine bromide with food both C_{max} and AUC of pyridostigmine bromide decreased approximately by 20% compared to fasting state. However, food increased the T_{max} of pyridostigmine bromide by 100%. The absolute bioavailability of pyridostigmine bromide tablet compared to an intravenous infusion was 12 ± 2% in fed state (Study #1).

Multiple Dosing:

Following multiple dosing of pyridostigmine bromide (30 mg, q8h for 21 days) the C_{max} increased approximately by 30%, 42% and 30% in males of low (134 ± 7 lbs), medium (169 ± 6 lbs) and high (212 ± 9 lbs) weight, respectively, as compared to a single dose. The C_{max} increased by 18%, 25% and 74% in low (111 ± 7 lbs), medium (143 ± 6 lbs) and high (184 ± 10) weight females upon multiple dosing (Study #2).

The oral clearance was 324 ± 121 l/hr and 339 ± 138 l/hr in males and females, respectively. The clearance was independent of gender and weight in low and medium weight groups. However, in both male and female high weight groups, the clearance was higher by 26% and 68%, respectively (Study #2).

The mean steady-state concentration of pyridostigmine during Days 4-22 was between 5 to 10 ng/mL. The accumulation ratio of pyridostigmine bromide was 1.6 on day 22 (Study #2).

Dose Proportionality:

The pharmackinetics of single dose of pyridostigmine bromide given as syrup was linear over the dose range of 0.40 mg/kg to 0.90 mg/kg (30-60 mg dose) (Study #3).

Gender:

The clearance of pyridostigmine bromide was independent of gender and weight (Study #2).

Pharmacokinetic data obtained from the literature:

Metabolism:

Pyridostigmine undergoes hydrolysis by cholinesterases and is also metabolized in the liver. It is excreted in the urine both as unchanged drug and metabolites (Martindale Pharmacopeia, Vol 31, 1996). In the urine of myasthenia gravis patients, 3-Hydroxy-N-methylpyridinium has been identified as one of the three metabolites (Somani et al. Clin Pharmacol. Ther, 1972, 13: 393).

Renal Dysfunction:

Compared to healthy subjects, in anephric patients pyridostigmine elimination half-life increased (112 vs 379 minutes) and systemic plasma clearance decreased from 8.6 to 2.1 ml/kg/min. (Cronnelly et al. Clin Pharmacol. Ther, 1980, 28: 78).

Age:

In the elderly (71-85 years) the elimination half-life, volume of distribution of the central compartment and volume of distribution at steady state of pyridostigmine was comparable with the young (21-51 years). However, the systemic plasma clearance was significantly lower in the elderly as compared to younger group (6.7 ± 2.2 vs 9.5 ± 2.7 ml/kg/min, $p < 0.05$). (Stone et al. Anesth Analg, 81:773, 1995).

Pharmacodynamics:

The maximum inhibition of RBC acetylcholinesterase activity following a single 30 mg pyridostigmine bromide oral dose was $38 \pm 9\%$ and the time to reach maximum inhibition was 2.9 ± 0.6 hours (Study #1).

In a study conducted in males and females over broad weight categories who received 30 mg pyridostigmine bromide as a single and multiple dose (30 mg q8h for 3 weeks), there was a considerable variability of AChE activity among subjects. Following a

single dose the maximum inhibition of acetylcholinesterase activity ranged from 29-45% in different weight groups and this inhibition was achieved in 2-3 hours post dose. Following a single dose the targeted inhibition of 20-40% lasted up to 4-5 hours post dose. Following multiple dosing the maximum inhibition of acetylcholinesterase activity ranged from 41-49% in different weight groups and the targeted inhibition of 20-40% lasted up to 7-8 hours post dose. The mean percentage inhibition of acetylcholinesterase activity during days 4-22 of the study (trough levels) was 18-26% in various groups (Study #2).

Rationale For Selection of Dose:

The basis for the suggested dosage regimen of a 30 mg tablet every 8 hours in man is related to the animal studies which demonstrated that 20-40% inhibition of RBC acetylcholinesterase activity protects against soman poisoning while minimizing the side effects that may occur above 40% AChE inhibition. Although levels below 20% provide some protection, these target levels may not be reached consistently and maintained long enough to provide reliable protection at lower dosage regimens. Thus, the 20-40% target inhibition was selected to develop the indicated dose and dosage regimen.

Comparative Human and Monkey Pharmacokinetic and Pharmacodynamic Parameters:

The following table compares the pharmacokinetic and pharmacodynamic parameters in monkeys and in humans following a single oral dose of 0.57 mg/kg of pyridostigmine bromide syrup (40 mg dose for human).

Parameters	Humans	Monkey
T_{max} (hrs)	2.1 ± 1.1	1.1 ± 0.53
C_{max} (ng/ml)	22.8 ± 7.5	26.6 ± 11.2
AUC (0-24) (ng.hr/ml)	110.6 ± 40.8	87.5 ± 50
$T_{inhibition}$ (hr)	2.5 ± 1.0	1.43 ± 0.4
I_{max} (%)	46.2 ± 8.0	43 ± 14.7

In monkeys the protective effect of pyridostigmine bromide was found with RBC AChE inhibition as low as 10%, and there is no apparent change in the protective effect when RBC AChE is inhibited by as much as 51%. The I_{max} values of pyridostigmine bromide in man and monkey at different doses have been summarized in the table on page 6a.

Analytical Methods:

(i) Determination of pyridostigmine bromide Plasma Concentration:

Pyridostigmine plasma concentrations were analyzed by a

The assay requires ml plasma to determine the cation concentrations of pyridostigmine. The method involves precipitation of plasma proteins, a with acidified % acetonitrile, separation on a column with an aqueous mobile phase in an isocratic elution, and ultraviolet absorbance detection nm). Neostigmine bromide was used as internal standard. The assay was linear within the range of the standard curve, ng/ml pyridostigmine cation.

(ii) Determination of RBC AChE:

The RBC acetylcholinesterase (AChE) activity was determined using standard enzymatic techniques at the laboratory of the U.S. Army Medical Research Institute for Chemical Defense. AChE catalyzes the hydrolysis of acetylthiocholine (ATCh), resulting in the formation of thiocholine and acetic acid. Thiocholine reacts with the color reagent 5, 5'-Dithio-bis (2-nitrobenzoic acid), forming 5-thio-2-nitro-benzoic acid. Thiocholine formation is directly proportional to AChE activity and is detected at spectrophotometrically. The lower limit of quantitation is U/mL with a C.V. of 6.5%. The linearity range of the calibration curve is up to 20 U/mL (this information could not be found in the analytical method section). The recovery of the method is 100% irrespective of the concentration tested (2.98 to 12.11 U/mL).

Dissolution:

Tablet dissolution was performed as outlined by USP XXIII (official monograph for pyriodostigmine bromide tablets). Six dissolution chambers (Apparatus 2) were filled with 900 mL of water and equilibrated to One tablet (30 mg) was added to each chamber. After the mixture had been stirred for minutes at 50 rpm, an approximately 10 mL sample of each was collected and filtered through a

In the alloted minutes, an average of 93.5 ± 1.1% of the labeled 30 mg of pyridostigmine bromide in the tablets dissolved into the solution. This was well above the minutes) required by the USP.

Table 6.2.3.2 **Comparative Human and Monkey Pharmacodynamic Parameters at Single, Oral Doses of Pyridostigmine Bromide**

	Pharmacodynamic Parameter Estimates RBC AChEI Activity		
Dose mg/kg	**Human**	**Monkey**	**Ratio Monkey to Human**
0.286[a]		I_{max} = 30%	
0.29 - 0.69[d]	I_{max} = 28.7 - 44.7%		
0.40[a,c]	I_{max} = 39.0 - 39.4%	I_{max} = 25 - 30%	0.63 - 0.76
0.57[a,b]	I_{max} = 46.2%	I_{max} = 43%	0.93
0.73[a]	I_{max} = 52.2%		
0.90[a]	I_{max} = 56.6%		
1.14[b]		I_{max} = 55%	

[a]Altstatt, *et al.* (Vol. 1.37, p.000001).
[b]Joiner and Kluwe (Vol. 1.42, p. 000022).
[c]Avlonitou and Elizondo (Vol. 1.39, p. 000023).
[d]Lasseter, K., and Garg, D. (Vol. 2.84, p. 000099).

6a