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APPLICATION NUMBER:

203629Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

CLINICAL PHARMACOLOGY MEMO

NDA: 203629 Original Submission Date: 12/28/12
Re-submission Date: 7/11/2014

Submission Type; Code: 505(b)(2)

Generic Name: Neostigmine Methylsulfate Injection, USP

Primary Reviewer: David Lee, Ph.D.

Team Leader: Yun Xu, Ph.D.

OCP Division: DCP 2

OND Division: Division of Anesthesia, Analgesia, and Addiction Products

Sponsor: APP Pharmaceuticals, LLC

Relevant NDA(s) -

Relevant IND(s): 106574

Formulation; Strength(s): 0.5 and 1 mg/mL

Proposed Indication: For reversal of non-depolarizing neuromuscular blocking agents, (b) (4)
(b) (4)

Proposed Dosage (b) (4)

Regimen: [Additionally, anticholinergic agents, atropine sulfate (b) (4) or glycopyrrolate (b) (4) also be administered intravenously using separate syringes].

Summary

The Sponsor submitted on 12/28/11, a New Drug Application (NDA) 203629, Neostigmine Methylsulfate Injection, USP, 0.5 and 1.0 mg/mL, accordance with 505(b)(2) provisions of the Food, Drug and Cosmetic Act for the use of "neostigmine" for reversal of non-depolarizing neuromuscular blocking agents (b) (4)

The Office of Clinical Pharmacology / Division of Clinical Pharmacology II (OCP/DCP-II) reviewed the application and found the information submitted in the NDA acceptable, pending agreement on the labeling language. The clinical pharmacology review can be found in DARRTS dated 8/24/2012. However, the original submission was not approved and received a complete response letter on 1/29/2013 mainly due to deficiencies on facility inspection.

The Sponsor re-submitted the application on 7/11/2014 to address the deficiencies. No additional clinical pharmacology information was submitted in this submission. As stated in the review for the original submission, the Office of Clinical Pharmacology / Division of Clinical Pharmacology II (OCP/DCP-II) found the NDA acceptable from clinical pharmacology perspective. Since labeling negotiation with the Sponsor was not completed in the original submission cycle, we will continue to work with the Sponsor on the labeling language, such as dosing recommendations in specific population such as elderly, patients with renal or hepatic impairment.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DAVID J LEE
12/23/2014

YUN XU
12/23/2014

**BIOPHARMACEUTICS
GENERAL APPLICATION REVIEW
Office of New Drug Quality Assessment**

Application No.:	203-629	Reviewer: Minerva Hughes, Ph.D.	
Submission Date:	27 January 2014 11 July 2014		
Division:	Division of Anesthesia, Analgesia, and Addiction Products	Team Leader: Angelica Dorantes, Ph.D. Secondary: Okpo Eradiri, Ph.D. (acting)	
Sponsor:	Fresenius Kabi	Supervisor: Paul Seo, Ph.D.	
Trade Name:	Neostigmine Methylsulfate Injection, USP	Date Assigned:	4 March 2014
Generic Name:	Neostigmine Methylsulfate Injection	Date of Review:	3 December 2014
Indication:	Reversal of non-depolarizing neuromuscular blocking agents.	Type of Submission: Class 2 Resubmission NDA – Marketed Unapproved Drug	
Formulation/strengths	Injection/ 0.5 mg and 1.0 mg		
Route of Administration	Injection		

SUBMISSION: NDA 203-629 was originally submitted on 28 December 2011 in accordance with section 505(b)(2) of the FDC Act for the use of neostigmine methylsulfate as a reversal agent to the neuromuscular blocking effects of nondepolarizing muscle relaxants. A complete response (CR) action was issued on 29 January 2013.

On 27 January 2014, the Applicant submitted a complete response (resubmitted on 11 July 2014) to the 29 January 2014 CR letter.

BIOPHARMACEUTICS REVIEW:

The complete response submission did not propose any changes to the biopharmaceutics information previously found acceptable under the original NDA. There were no outstanding biopharmaceutics issues under the original NDA. Reference is made to the Biopharmaceutics Quality review dated 23 August 2012 in DARRTS for full details.

APPROVAL RECOMMENDATION:

NDA 203-629 for the use of neostigmine methylsulfate is recommended for approval from the perspective of Biopharmaceutics.

Signature Block

Minerva A. Hughes -S
Digitally signed by Minerva A. Hughes -S
DN: c=US, o=U.S. Government,
ou=HHS, ou=FDA, ou=People,
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Date: 2014.12.03 11:07:03 -05'00'

Minerva Hughes, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

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Okpo Eradiri, Ph.D.
Biopharmaceutics Team Leader (Acting)
Office of New Drug Quality Assessment

CLINICAL PHARMACOLOGY REVIEW

NDA: 21-330	Submission Date: 12/28/12
Submission Type; Code:	505(b)(2)
Brand/Code Name:	To-be-determined
Generic Name:	Neostigmine Methylsulfate Injection, USP
Primary Reviewer:	David Lee, Ph.D.
Team Leader:	Yun Xu, Ph.D.
OCP Division:	DCP 2
OND Division:	Division of Anesthesia, Analgesia, and Addiction Products
Sponsor:	APP Pharmaceuticals, LLC
Relevant NDA(s)	-
Relevant IND(s):	106574
Formulation; Strength(s):	0.5 and 1 mg/mL
Proposed Indication:	For reversal of non-depolarizing neuromuscular blocking agents, (b) (4) (b) (4) (b) (4)
Proposed Dosage Regimen:	(b) (4) [Additionally, anticholinergic agents, atropine sulfate (~15 µg/kg) or glycopyrrolate (~10 µg/kg), also be administered intravenously using separate syringes].

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1 Executive Summary

1.1 Recommendations

The Office of Clinical Pharmacology / Division of Clinical Pharmacology II (OCP/DCP-II) has reviewed the information submitted in the current application for neostigmine methylsulfate intravenous injection. From a clinical pharmacology perspective, the information submitted in the NDA is acceptable, pending agreement on the labeling language.

1.2 Phase IV Commitments

Not applicable.

1.3 Summary of CP Findings

APP Pharmaceutical LLC submitted on 12/28/11, a New Drug Application (NDA) 203629, Neostigmine Methylsulfate Injection, USP, 0.5 and 1.0 mg/mL, accordance with 505(b)(2) provisions of the Food, Drug and Cosmetic Act for the use of “neostigmine”

for reversal of non-depolarizing neuromuscular blocking agents

(b) (4)

The proposed doses for neostigmine in adults and pediatrics are (b) (4). The Package Insert recommends that anticholinergic agents, atropine sulfate (~15 µg/kg) or glycopyrrolate (~10 µg/kg), also be administered intravenously using separate syringes. It should be noted that atropine and glycopyrrolate have been used in clinical practice for at least a couple of decades as an adjunct to reversal of neuromuscular blockade. Atropine undergoes enzymatic hydrolysis. The majority of glycopyrrolate dose administered intravenously has been reported to be eliminated in urine as unchanged moiety. The pharmacokinetic interactions between neostigmine and atropine or glycopyrrolate are not expected. With respect to dosing, the Labeling stated that “the

(b) (4)

As dictated by the indication, neostigmine usage in reversal of non-depolarizing neuromuscular blocking agents may be considered as a single administration with a titration scheme.

Neostigmine has been used since the late 1930s with extensive clinical experience most likely as an unapproved drug. It should be noted that, according to the published Federal Register Notice Vol. 61, No 151, Monday, August 5, 1996, Docket No. 96N-0257, Progstigmin (neostigmine bromide solution) Ophthalmic Solution 5%, NDA 6-54, was withdrawn by Hoffmann-La Roche. Therefore, neostigmine is not a new molecular entity or new chemical entity. Since there are no approved neostigmine products on the market, there is no reference listed drug for this NDA. As well, the relative bioavailability comparison is not feasible. For this application, the Applicant did not conduct any clinical trials in this submission. Instead, the Applicant submitted supporting information, including the proposed dosing regimen, from the literature for approval. During a pre-IND meeting held on 12/22/09 the Agency stated that the Applicant may submit their NDA based the literature information, including to support a biowaiver request, pending the formulations used in the literature are appropriate for reference. Specifically, the Agency stated that “the formal review of submitted information in the NDA application will determine the adequacy of literature to support a request to waive pharmacokinetic/bioavailability studies for the proposed adult and pediatric subjects.” Therefore, the Applicant requested to waive in vivo pharmacokinetic/bioavailability studies.

With respect to bioavailability/bioequivalence requirement as per the 21 CFR320, there are no concerns due to the fact that 1) the bioavailability is “self-evidence” since the Applicant’s formulation is for intravenous use; and, 2) that the Applicant and intravenous formulations described in the literature (based on the descriptions provided in the publications, e.g., neostigmine, preservatives (phenol) and saline) appear to be simple solutions. Although the intravenous solutions submitted in the literature appear to use three different drug substances, neostigmine methylsulfate, neostigmine bromide and neostigmine, the main active ingredient in the formulations is neostigmine. Therefore, the formulations used in the literature seem to be appropriate for comparison from a clinical pharmacology perspective.

The Applicant submitted 8 and 5 publications under clinical pharmacology and biopharmaceutics (assay methodology), respectively. Of the submitted publications, Chan et al. (1976) and De Ruyter et al. (1980) publications were submitted in both clinical pharmacology and biopharmaceutics sections. All submitted publications in this NDA submission were reviewed comprehensively, except the following publications, Davison et al (1980), Varin et al. (1999) and Somani et al (1980). The aforementioned biopharmaceutics publications were not used by other authors and it was considered not need-to-know methodology publications; however, a cursory review was conducted and presented below in a table format. All publications were reviewed based on the current review practice. In particular, study design, dosage administration, blood sampling scheme, and analytical methodology information were focused during the review.

Overall findings

The following two tables contain a quick overview of the study design, treatments, and analytical methodology information submitted in the clinical pharmacology and biopharmaceutical sections, respectively.

Clinical pharmacology publications

Author	Study objectives	# of patients	Treatment		PK Assay information presented			Reviewer's Comments
			Neo	Other meds	Stand. curve	Q.C.	Assay Validation	
Williams, Br.J. Anaesth. (1978) 50, 1065	Neo PK after neuromuscular (NM) block	Healthy Female: 5 Age: 22- 62 range WT: 63.1 – 72.6 kg	5 mg iv	Atropine sulfate 1.2 mg iv	No	No	No	1. Refers to Chan et al, 1976 2. No within analytical methods presented in the paper 3. Not useful to overall PK information due to missing assay information
Chan, 1976 J. of Chrom. (also in Biopharm section)	Neo bioassay human plasma after NM block	1 (sex not reported) Not reported	5 mg iv	Not reported	50-1000 ng/mL; no data provided	No	No	1. Used neostigmine bromide as analyte 2. Not optimal, the information presented in the paper is good enough to accept the analytical methodology 3. Not useful to overall PK information due to missing assay information
De Ruyter, 1980 J. of Chrom. (also in Biopharm section)	Neo bioassay human plasma after NM block	Not reported	0.05 mg/kg iv	Not reported	0-1000 ng/mL; no data provided	No	No	1. Not optimal, the information presented in the paper is good enough to accept the analytical methodology 2. Not useful to overall PK information due to missing assay information

Fisher, 1983 Anesth.	Neo PK in infants, children and adults after NM block	Infant: n=5 2-10mon; Children: n=5 1-6 y Adults: n=5 29-48 y	Infant: 100 µg/kg iv; Children and adults: 70 µg/kg iv	Atropine 30 µg/kg iv	No	No	No	1. Refers to De Ruyter et al, 1980 2. No within analytical methods presented in the paper
Calvey 1979 Brit.J. Clin. Pharm.	Neo PK after NM block with tubocurarine	Female: n=6 Age not reported;	Neo Methyl 68.9-103 µg/kg iv	Atropine sulfate (1.2 mg iv)	No	No	No	1. Refers to Chan et al, 1976 2. No within analytical methods presented in the paper 3. Not useful to overall PK information due to missing assay information
Morris, 1981; Anesth.	Neo PK after NM block with tubocurarine	Male: 6 Age not reported	Neo Methylsulfate (0.07 mg/kg iv)	Atropine sulfate (1 mg iv)	No	No	No	1. Refers to De Ruyter et al, 1980 2. No within analytical methods presented in the paper
Broggini 1991; Meth Find Exp Clinical Pharm.	Neo SD PK post intranasal and IV	Male: 3 Female: 3 Age: 25.5 y (23-28y)	0.5 mg	Not reported	No	No	No	1. Authors have their own HPLC method 2. However, no assay information presented in the paper 3. Not useful to overall PK information due to missing assay information
Cronnelly 1979, Anesth.	Neo PK in healthy, transplant and anephric patients	Healthy: n=8 patients Anephric: 4 patients Transplant: 6 patients Age: 23-52 y range	0.07 mg/kg iv	Atropine (0.03 mg/mg iv)	No	No	No	1. Refers to Chan et al, 1976 2. No within analytical methods presented in the paper 3. Not useful to overall PK information due to missing assay information

Biopharmaceutics publications

	Matrix	Assay Methodology	Analyte	Calibration / Assay Range	Analytical Sensitivity
Chan (1976) <i>J Chrom.</i> 120: 349-358	Human plasma	Gas-liquid chrom with nitrogen detection, followed by MS	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 50 – 1000 ng/mL	5 ng/mL
DE RUYTER (1980) <i>J of Chrom.</i> 183: 193-201	Human plasma	Reverse phase, liquid chrom	Neostigmine	Calibration curves not described. Assay range 0 – 1000 ng/mL	5 ng/mL
DAVISON (1980) <i>Methods and Findings Ex Clin Pharm</i> , 2: 77-82 Cursory review only	Human plasma	Gas chrom with nitrogen detection	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 5 – 100 ng/mL	4.7 ng/mL

VARIN ET AL., (1999) <i>J of Chrom.(B)</i> , 723: 319-323 Cursory review only	Human plasma and CSF	High performance liquid chrom with UV detection	Neostigmine methylsulfate	Drug free plasma was spiked with neostigmine methylsulfate and serial dilutions between 2.6 – 167 ng/mL were prepared for calibration curves	2.6 ng/mL for plasma 6.9 ng/mL for CSF
SOMANI ET AL. (1980) <i>Clin Pharm Thera</i> , 28: 66-68 Cursory review only	Human plasma and urine	Plasma: per Chan et al. Urine: Scintillation spect. of labeled drug	Neostigmine methylsulfate	Per method of Chan et al.	5-7 ng/mL for plasma

Clinical Pharmacology findings presented by the Applicant

The following table summarizes the overall findings as presented by the Applicant.

Author	Treatment		PK parameters			
	Neo	Other meds	Cmax, T _{ax} , AUC	T _{1/2} (min)	CL (mL/min/kg)	Vd (L/kg)
Williams	Neo methylsulfate 5 mg iv	Atropine sulfate 1.2 mg iv	Conc. profile	24	Not reported	6.2
Chan (Analytical method)	Neo bromide 5 mg iv	Not reported	Conc. profile	Not reported	Not reported	Not reported
De Ruyter (Analytical method)	Neo 0.05 mg/kg	Not reported	Conc. profile	Not reported	Not reported	Not reported
Fisher De Ruyter method	Infants 100 µg/kg iv Children and adults 70 µg/kg iv	Atropine 30 µg/kg iv	Conc. profile	Infants: 39 Children: 48 Adults: 67	Infants: 13.6 mL/min/kg Children: 11.1 Adults: 9.6	Infants: 0.08 Children: 0.09 Adults: 0.04
Calvey	Neo methyl 68.9-103 µg/kg iv	Atropine sulfate 1.2 mg iv	Conc. profile	25.4	5.72	0.12
Morris De Ruyter method	Neo methyl 70 µg/kg iv	Atropine sulfate 1.0 mg iv	Not reported	a=3.4 b=77	9.2	0.74
Broggini Authors' own HPLC method	Neo 0.5 mg iv	Not reported	Cmax: 8.84 Tmax: 0.08 h AUC=126.8 (ng.h/mL)	1.88 h or (112.8 min)	0.7 L/h/kg	0.18 MRT 2.83 h
Cronnelly Chan method	Neo 70 µg/kg iv	Atropine 0.03 mg/kg iv	Conc. profile	Normal: 79.8 Anephric: 181.1 Transplant: 104.7	Normal: 16.7 Anephric: 7.8 Transplant: 18.8	Normal: 1.4 Anephric: 1.6 Transplant: 2.1

Adequacy of the neostigmine clinical pharmacology information from the publication

It was determined that all of the publications submitted in the application do not have adequate analytical information (e.g., QCs, recovery, stability, validations, etc.). Based on the current clinical pharmacology standards, none of the publications are adequate and are not optimal in constructing the information for the Labeling purpose. However, it appears that the following information is consistent through out the publication regardless which analytical methods used.

Single-dose

Neostigmine half life ranged from 77 to 113 minutes after a single intravenous administration.

Metabolism

Nonclinical information suggested that neostigmine is eliminated in the urine and feces (unabsorbed material given by routes other than IV) unchanged and undergoes hepatic metabolism in the liver microsomes. 3-Hydroxyphenytrimethyl ammonium (PTMA) is the primary metabolite, which then becomes glucuronide conjugated PTMA.

Pediatric

Fisher et al. determined the pharmacokinetics of neostigmine, five subjects per group, in infants (2-10 months), children (1-6 years) and adults (29-48 years). Neostigmine was administered as a 2-min intravenous infusion. Infants' dose was 100 µg/kg; children and adults doses were 70 µg/kg. Atropine was also administered as 30 µg/kg. The plasma conc vs. time data were fitted to a three-compartment pharmacokinetic model. Elimination half-life for infants, children and adults were 39 ± 5 min, 48 ± 16 min, and 67 ± 8 min (mean ± SD), respectively. Clearance for infants, children and adults were 13.6 ± 2.8, 11.1 ± 2.7 and 9.6 ± 2.3 mL/min/kg (mean ± SD), respectively.

Hepatic

The pharmacokinetics of neostigmine in patients with hepatic impairment has not been studied. Neostigmine is metabolized by microsomal enzymes in the liver. Use with caution in patients with impaired hepatic functions.

Renal

Cronnelly et al, determined the pharmacokinetics of neostigmine in patients with normal renal function (n = 8), undergoing renal transplantation (n = 6) or bilateral nephrectomy (n = 4). Neostigmine, 0.07 mg/kg, and atropine, 0.03 mg/kg, were given by infusion over a 2-min period. Blood samples were obtained at pre-, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min following neostigmine administration. Plasma conc vs time data was fitted to a two-compartment pharmacokinetic model. Elimination half

life for normal, transplant and anephric patients were 79.8 ± 48.6 , 104.7 ± 64 and 181 ± 54 min (mean \pm SD), respectively. Clearances for normal, transplant and anephric patients were 16.7 ± 5.4 , 18.8 ± 5.8 and 7.8 ± 2.6 mL/min/kg (mean \pm SD), respectively. The clearance in patients with impaired renal function is lower compared to patients with normal renal functions. Use with caution in patients with impaired renal functions.

Elderly

Considering the elderly patients will have decreased renal function which will lead to decreased neostigmine clearance, neostigmine should be used with caution in elderly patients.

Drug Interaction

The pharmacokinetic interaction between neostigmine and other drugs has not been studied. Neostigmine is metabolized by microsomal enzymes in the liver. Use with caution when using neostigmine with other drugs which may alter the activity of metabolizing enzymes or transporters.

Gender, Race

No information was submitted.

Analytical Methodology

As stated above, the Applicant submitted 5 publications under the biopharmaceutics section, for an analytical method assessment. Of the submitted publications, two publications, Chan et al. (1976) and De Ruyter et al. (1980), were mostly used by the publications submitted under the clinical pharmacology section. Chan et al., and De Ruyter et al., developed gas-liquid chromatography with nitrogen detection followed by mass spectroscopy and a reverse phase liquid chromatography, respectively, to analyze neostigmine in plasma. The concentration ranges were 50-1000 and 0-1000 ng/mL, respectively. As stated above, both publications did not contain the optimal information (e.g., quality control samples), and, thus, the data obtained using these analytical methods should be carefully interpreted.

2 QBR

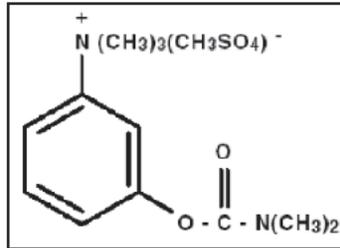
2.1 General Attributes of the Drug and Drug Product

2.1.1 What are known properties of neostigmine?

Neostigmine is an anticholinesterase agent. Neostigmine was first synthesized by Aeschlimann and Reinert in 1931 and was subsequently reported to be effective in the symptomatic treatment of myasthenia gravis.

Its molecular formula is C₁₃H₂₂N₂O₆S. It has a molecular weight of 334.39 g/mol. Neostigmine is soluble in water and sparingly soluble in acetone.

Neostigmine methylsulfate structure:



2.1.2 What is neostigmine to-be-marketed formulation?

The proposed neostigmine formulation is presented below. It is a simple solution for intravenous use.

Ingredients	APP Pharmaceuticals, LLC	
	Neostigmine Methylsulfate Injection, USP	
	Product Code 38210	Product Code 38310
Neostigmine Methylsulfate, USP	0.5 mg/mL	1.0 mg/mL
Liquified Phenol, USP	4.5 mg/mL	4.5 mg/mL
Sodium Acetate, USP (trihydrate)	0.2 mg/mL	0.2 mg/mL
Water for Injection, USP	q.s. to 1 mL	
(b) (4) Acetic Acid, USP	As required to adjust pH	
Sodium Hydroxide, NF	As required to adjust pH	

2.1.3 What is the proposed mechanism of action?

Neostigmine inhibits the hydrolysis of acetylcholine by competing with acetylcholine for binding to acetylcholinesterase at sites of cholinergic transmission. By reducing the breakdown of acetylcholine, neuromuscular transmission is facilitated. Neostigmine also has direct postsynaptic cholinomimetic effects which can be managed clinically by the co-administration of atropine or glycopyrrolate. Neostigmine inhibition of acetylcholinesterase is fully reversible.

2.1.4 What are the proposed dosage and route of administration?

The proposed route of administration is via the intravenous route for the use of “neostigmine” for reversal of non-depolarizing neuromuscular blocking agents

(b) (4)
 The proposed doses in the
 Package Insert for neostigmine in adults and pediatrics are (b) (4)

(b) (4) Additionally, the Package Insert recommends that anticholinergic agents, atropine sulfate (~15 µg/kg) or glycopyrrolate (~10 µg/kg), also be administered intravenously using separate syringes.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the pivotal clinical trials and efficacy measurements?

There were no clinical studies conducted under the application. However, the Applicant submitted literature information to support for the approval. The discussion regarding the efficacy and safety of neostigmine is beyond the scope of this review, as the Medical Reviewer is fully committed to review the submitted literature information. The reader is prompted to see Medical Officer's Review by Dr. Arthur Simone for additional information.

2.2.2 Does neostigmine prolong the QT interval?

No information was submitted to characterize neostigmine.

2.2.3 Protein binding, metabolism, enzyme induction/inhibition

The following information was obtained from the literature.

Protein Binding:

Protein binding to human serum albumin ranges from 15 to 25%.

Metabolism:

Nonclinical studies demonstrate that Neostigmine is eliminated in the urine and feces (unabsorbed material given by routes other than IV) unchanged, and also undergoes hepatic metabolism in the liver microsomes. 3-Hydroxyphenytrimethyl ammonium (PTMA) is the primary metabolite, which then becomes glucuronide conjugated PTMA. Up to 5 metabolites of neostigmine have been reported as excreted in the urine.

2.2.4 What are the single dose PK parameters?

Neostigmine half life ranged from 77 to 113 minutes after a single intravenous administration.

2.3 Intrinsic Factors

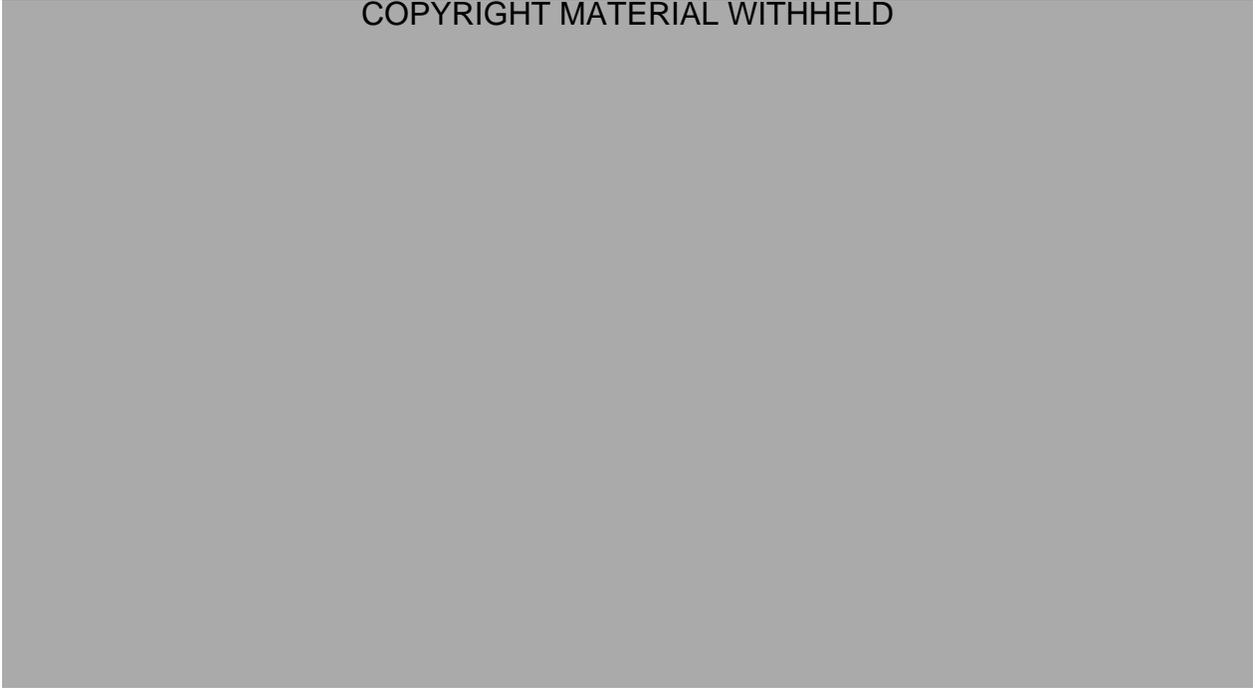
No information was submitted to characterize neostigmine in race and gender.

2.3.1 What is the neostigmine exposure in pediatric subjects?

Fisher et al. determined the pharmacokinetics of neostigmine in infants, children and adults. Three groups of five patients (infants, 2-10 months; children, 1-6 years; and adults, 29-48 years) were administered neostigmine as a 2-min intravenous infusion. Infants' dose was 100 µg/kg; children and adults doses were 70 µg/kg. Atropine dose was 30 µg/kg. Blood samples were obtained intermittently for 4 h (pre-, 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min. post drug administration), and concentrations of neostigmine were determined using a high-pressure liquid chromatographic technique (analytical method described by De Ruyter, et al, 1980; sensitivity: 3.0 ng/ml; coefficient of variation of 5%). The plasma conc vs. time data were fitted to a three-compartment pharmacokinetic model. Distribution half-lives and distribution volumes were similar for infants, children, and adults. Elimination half-life for infants, children and adults were 39 ± 5 min, 48 ± 16 min, and 67 ± 8 min (mean \pm SD), respectively. Clearance for infants, children and adults were 13.6 ± 2.8 , 11.1 ± 2.7 and 9.6 ± 2.3 mL/min/kg (mean \pm SD), respectively. The following plasma profiles were presented in the publication.

Neostigmine conc. vs. time profiles for infants, children and adults

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No individual parameters are presented. Additionally, no subject information was given (e.g., body weight, dose administered, etc.). It also should be noted that the publication did not contain adequate analytical information. However, by looking at the presented neostigmine profiles, there may be a reasonable assurance that the presented PK parameters are acceptable.

2.3.1.1 Renal impairment

Cronnelly et al, determined the pharmacokinetics of neostigmine in patients with normal renal function (n = 8), undergoing renal transplantation (n = 6) or bilateral nephrectomy (n = 4). Neostigmine, 0.07 mg/kg, and atropine, 0.03 mg/kg, were given by infusion over a 2-min period. Blood samples were obtained at pre-, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min following neostigmine administration. Plasma conc vs time data was fitted to a two-compartment pharmacokinetic model. Elimination half life for normal, transplant and anephric patients were 79.8 ± 48.6 , 104.7 ± 64 and 181 ± 54 min (mean \pm SD), respectively. Clearances for normal, transplant and anephric patients were 16.7 ± 5.4 , 18.8 ± 5.8 and 7.8 ± 2.6 mL/min/kg (mean \pm SD), respectively.

Mean plasma conc. vs time profiles for normal, immediate renal transplantation and anephric patients, respectively, are presented below.

Normal:

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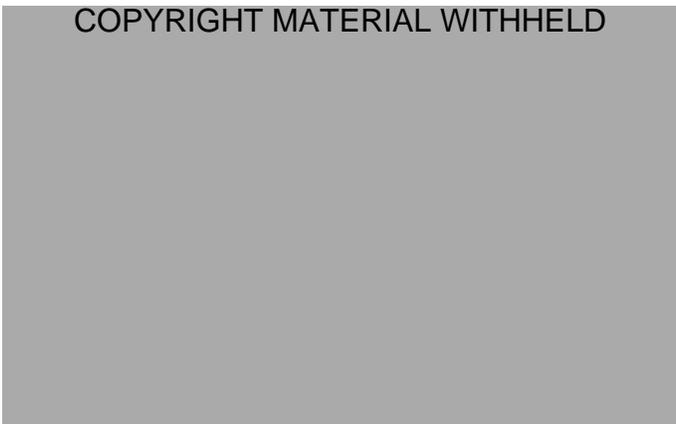
Immediate renal transplantation:

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Anephric patients:

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No individual parameters were presented in the publication. Additionally, no subject information was given (e.g., body weight, dose administered, etc.). It also should be noted that the publication did not contain adequate analytical information. However, by looking at the presented neostigmine profiles, there may be a reasonable assurance that

the presented PK parameters are acceptable. The clearance in patients with impaired renal function is lower compared to patients with normal renal functions. Use with caution in patients with impaired renal functions.

2.3.1.2 Hepatic impairment

The pharmacokinetics of neostigmine in patients with hepatic impairment has not been studied. Neostigmine is metabolized by microsomal enzymes in the liver. Use with caution in patients with impaired hepatic functions.

2.3.1.3 Elderly

According to an abstract published (American Society of Anesthesiologists (ASA) meeting), Young et al. (1984) compared the neostigmine pharmacokinetics of five elderly patients (ages 71-80) and seven younger patients (ages 34-56). A bolus of 70 µg/kg of neostigmine and 20 µg/kg of atropine were administered intravenously. The only significant difference between the young and elderly was initial volume of distribution (V₁), which was lower in the elderly. Numerically the clearance in elderly (23.4 ± 4 mL/kg/min) is also lower compared to younger patients (33.5 ± 4 mL/kg/min). Overall the duration of maximum response to neostigmine was significantly prolonged in the elderly (42 ± 10 minutes) compared to the younger group (13.14 ± 2.4 minutes). A caution should be exercised in interpreting the data since the fact that this abstract is not a fully peer reviewed article. However, considering the elderly patients will have decreased renal function which will lead to decreased neostigmine clearance, neostigmine should be used with caution in patients with impaired renal functions.

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2.4 Extrinsic Factors

No information was submitted to characterize neostigmine. The pharmacokinetic interaction between neostigmine and other drugs has not been studied. Neostigmine is metabolized by microsomal enzymes in the liver. Use with caution when using neostigmine with other drugs which may alter the activity of metabolizing enzymes or transporters.

2.5 General Biopharmaceutics – Not applicable

2.6 Analytical Section

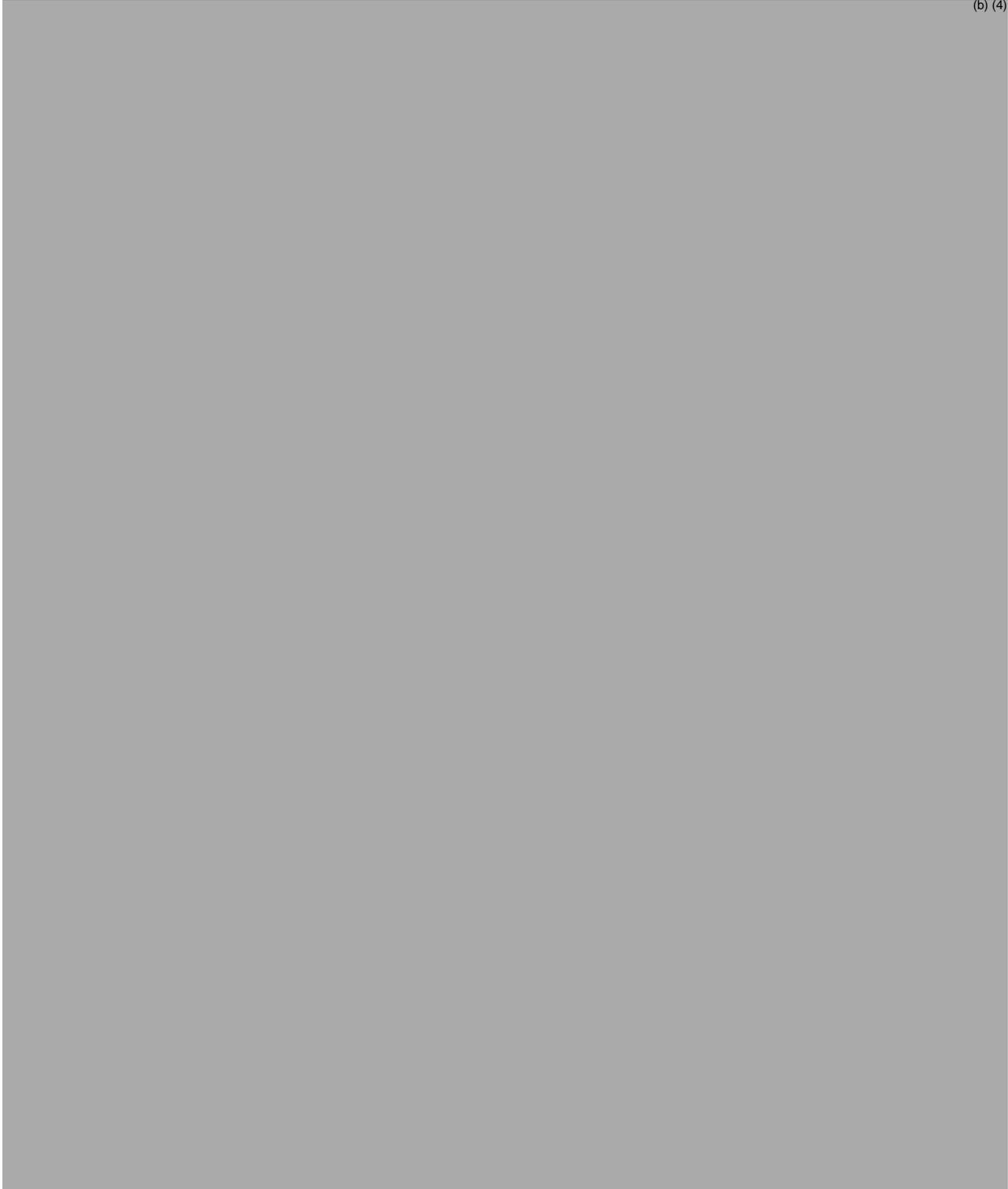
2.6.1 How are neostigmine and its metabolites measured in plasma?

The Applicant submitted 5 publications under the biopharmaceutics section, for an analytical method assessment. Of the submitted publications, two publications, Chan et al. (1976) and De Ruyter et al. (1980), were mostly used by the publications submitted under the clinical pharmacology section. Chan et al., and De Ruyter et al., developed gas-liquid chromatography with nitrogen detection followed by mass spectroscopy and a reverse phase liquid chromatography, respectively, to analyze neostigmine in plasma. The concentration ranges were 50-1000 and 0-1000 ng/mL, respectively. As stated above, both publications did not contain the optimal information (e.g., quality control samples), and, thus, the data obtained using these analytical methods should be carefully interpreted.

	Matrix	Assay Methodology	Analyte	Calibration / Assay Range	Analytical Sensitivity
Chan (1976) <i>J Chrom.</i> 120: 349-358	Human plasma	Gas-liquid chrom with nitrogen detection, followed by MS	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 50 – 1000 ng/mL	5 ng/mL
DE RUYTER (1980) <i>J of Chrom.</i> 183: 193-201	Human plasma	Reverse phase, liquid chrom	Neostigmine	Calibration curves not described. Assay range 0 – 1000 ng/mL	5 ng/mL
DAVISON (1980) <i>Methods and Findings Ex Clin Pharm</i> , 2: 77-82 Cursory review only	Human plasma	Gas chrom with nitrogen detection	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 5 – 100 ng/mL	4.7 ng/mL
VARIN ET AL., (1999) <i>J of Chrom.(B)</i> , 723: 319-323 Cursory review only	Human plasma and CSF	High performance liquid chrom with UV detection	Neostigmine methylsulfate	Drug free plasma was spiked with neostigmine methylsulfate and serial dilutions between 2.6 – 167 ng/mL were prepared for calibration curves	2.6 ng/mL for plasma 6.9 ng/mL for CSF
SOMANI ET AL. (1980) <i>Clin Pharm Thera</i> , 28: 66-68 Cursory review only	Human plasma and urine	Plasma: per Chan et al. Urine: Scintillation spect. of labeled drug	Neostigmine methylsulfate	Per method of Chan et al.	5-7 ng/mL for plasma

3 Detailed Labeling Recommendations

There are changes recommended for the Clinical Pharmacology section of the label, as below. The package insert is modified by strikeouts of the existing texts and addition of new texts, in **RED** fonts, where appropriate.



(b) (4)

8 Pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page.

4.2 Individual study review

Summary table:

Author	Study objectives	# of patients	Age (mean, range)	Treatment		PK Assay		
				Neost	other meds	Standard curve	Q.C.	Validation
Willams, <i>Br.J. Anaesth.</i> (1978) 50, 1065	Neo PK after neuromuscular (NM) block	Healthy Female: 5	22- 62 WT: 63.1 – 72.6 kg	5 mg iv	Atropine sulfate 1.2 mg iv	No	No	No
Chan, 1976 J. of Chrom	Neo bioassay human plasma after NM block	1 (sex not reported)	Not reported	5 mg iv	Not reported	50-1000 ng/mL; no data provided	No	No
De Ruyter, 1980 J.of Chrom	Neo bioassay human plasma after NM block	Not reported	Not reported	0.05 mg/kg iv	Not reported	0-1000 ng/mL; no data provided	No	No
Fisher, 1983 Anesthesio.	Neo PK in infants, children and adults after NM block	Infant:5 Children: 5 Adults: 5	Infant: 2-10 mon.; Children: 1-6 yrs; Adults: 29-48 yrs;	Infant: 100 µg/kg iv; Children and adults: 70 µg/kg iv	Atropine 30 µg/kg iv	No	No	No
Calvey 1979, <i>Brit.J. of Clinical Pharm.</i>	Neo PK after NM block with tubocurarine	Female: 6	Not reported; 22- 62?	Neo Methyl 68.9-103 µg/kg iv	Atropine sulfate (1.2 mg iv)	No	No	No
Morris, 1981; Anesthesio.	Neo PK after NM block with tubocurarine	Male: 6	Not reported	Neo Methylsulfate (0.07 mg/kg iv)	Atropine sulfate (1 mg iv)	No	No	No
Broggini 1991; <i>Meth Find Exp Clinical Pharm.</i>	Neo SD PK post intranasal and IV	Male: 3 Female: 3	25.5 (23-28)	0.5 mg	Not reported	No STD curve mentioned but no info	No	No
Cronnelly 1979, Anesthesio.	Neo PK in healthy, transplant and anephric patients	8 patients Anephric: 4 patients Transplant: 6 patients	Range (23-52)	0.07 mg/kg iv	Atropine (0.03 mg/mg iv)	No	No	No

	Matrix	Assay Methodology	Analyte	Calibration / Assay Range	Analytical Sensitivity
Chan (1976) <i>J Chrom.</i> 120: 349-358	Human plasma	Gas-liquid chromatography with nitrogen detection, followed by MS	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 5 – 1000 ng/mL	5 ng/mL
DE RUYTER (1980) <i>J of Chrom.</i> 183 193-201	Human plasma	Reverse phase, liquid chromatography	Neostigmine	Calibration curves not described. Assay range 5 – 200 ng/mL	5 ng/mL
DAVISON (1980) <i>Methods and Findings Ex Clin Pharm.</i> 2: 77-82 Cursory review only	Human plasma	Gas chromatography with nitrogen detection	Neostigmine bromide	Neostigmine was dissolved in sterile water and a series of 3 mL solutions in plasma were prepared covering the range 5 – 100 ng/mL	4.7 ng/mL
VARIN ET AL., (1999) <i>J of Chrom.(B)</i> , 723: 319-323 Cursory review only	Human plasma and CSF	High performance liquid chromatography with UV detection	Neostigmine methylsulfate	Drug free plasma was spiked with neostigmine methylsulfate and serial dilutions between 2.6 – 167 ng/mL were prepared for calibration curves	2.6 ng/mL for plasma 6.9 ng/mL for CSF
SOMANI ET AL (1980)	Human	Plasma: per method of	Neostigmine	Per method of Chan et al (4)	5-7 ng/mL for

<i>Clin Pharm Thera</i> , 28: 66-68 Cursory review only	plasma and urine	Chan et al (4) Urine: Scintillation spectroscopy of labeled drug	methylsulfate		plasma
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Note: Are neostigmine profiles different in Neostigmine-Br spectrum and Neostigmine Methylsulfate?

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Clearance of neostigmine from the circulation during the antagonism of neuromuscular block

N. E. Williams¹, T. N. Calvey¹ and K. Chan²

¹Whiston Hospital, Prescot, Lancashire, and Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX. ²B.PHARM., M.SC., PH.D., School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF. *Br.J. Anaesth.* (1978) 50, 1065

Notes:

1. Drug: neo methylsulfate 5 mg and atropine sulfate 1.2 mg
2. Assay:
 - Authors stated that they used Chan et al., 1976, gas-liq chromatography method, which used neostigmine bromide an analyte, not neo Methylsulfate
 - No assay information at all presented in the paper
3. Since there is no assay information, the plasma conc information is questionable.

Objective:

To investigate the clearance of neostigmine from the circulation during the antagonism of neuromuscular block induced by tubocurarine.

SUMMARY

The plasma concentration of neostigmine was measured in five patients during the antagonism of neuromuscular block. The concentration of neostigmine decreased drastically between 2 and 5 min after administration. Neostigmine was detected at 60 minute sample, which was the last blood sample collection point. In the five patients the distribution half-life of neostigmine was less than 1 min; the elimination half-life ranged from 15.4 to 30.1 min.

Subjects:

Five females who were undergoing plastic surgical procedures were studied. None was suffering from renal or hepatic disease. The ages of four patients were in the range 22-36 yr; one patient was aged 62 yr. Their body weights ranged from 63.1 to 72.6 kg.

Procedures:

All were premedicated with nitrazepam 10 mg orally, given on the previous night, and diazepam 5-10 mg orally on the day of operation. Following the induction of anaesthesia with thiopentone 250-400 mg, tubocurarine chloride 45 mg was administered. After tracheal intubation, anaesthesia was maintained with nitrous oxide 60-70% and halothane

0.5% in oxygen, utilizing IPPV. The duration of surgery was usually 60-90 min, and there was little or no fluid loss or replacement.

At the end of the operation, an i.v. cannula was placed in a superficial vein. Its patency was maintained by the intermittent infusion of small volumes of heparinized saline. Residual neuromuscular block was antagonized with neostigmine methylsulphate 5 mg and atropine sulphate 1.2 mg.

Blood samples:

At 2, 3, 4, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min post application, and placed in tubes containing lithium heparin. As soon as was feasible, plasma was obtained by centrifugation and stored at - 20°C.

Neostigmine was extracted from plasma as an iodide complex, and its concentration was estimated by gas-liquid chromatography using a nitrogen detector (Chan et al., 1976).

No assay QC, standard curve information.

Results:

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Fig 1. Plasma concentration of neostigmine after i.v. injection. Each point and vertical bar represents the mean and standard error of five observations.

The plasma concentration of neostigmine was invariably expressed as a biexponential equation of the form:

$$C_t = A e^{-\alpha t} + B e^{-\beta t}$$

TABLE I. Relation between the plasma concentration of neostigmine and time. Plasma concentration at time t (C^t) = $A e^{-\alpha t} + B e^{-\beta t}$, where A , B , α and β are constants
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Calculation parameters:

$$\begin{aligned} \text{distribution half-life} &= 0.693/\alpha \\ \text{elimination half-life} &= 0.693/\beta \\ \text{total apparent volume} &= \text{dose}/\beta[(A/\alpha) + (B/\beta)] \\ \text{of distribution} & \end{aligned}$$

PK parameters:

Patient #2 is 62 yrs old

Table 2.7.2- 2 Half-Life and V_d of Neostigmine after IV Injection (Williams et al.)
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A quantitative gas-liquid chromatographic method for the determination of neostigmine and pyridostigmine in human plasma

K. Chan, N. E. Williams, J. D. Baty and T. N. Calvey
Departments of Pharmacology and Medicine, University of Liverpool, Liverpool L69 3BX (Great Britain) (Received November 21st, 1975). *Journal Of Chromatography*, 120 (1976) 349-358

Notes:

For analysis part which other papers use this method:

1. No QC samples were measured; however, it appears that recovery values were presented from the conc. used in standard curves.
2. It appears that the method may consistently reproduce the standard curve; the paper stated that the linear response was observed in the range of 50-1000 ng/mL (six different times)
3. Overall looking at the analytical information, it is less than optimal; however, this reviewer felt that analytical information presented in the paper is sufficient enough to conclude that the employed gas-liquid chromatographic method is adequate.

For one patient study with neostigmine bromide 5 mg or pyridostigmine bromide (20 mg):

1. One patient only – no info on the patient
2. Assay:
 - No assay information at all presented in the paper, when this patient's blood samples (2, 5, 10, 15, 20, 30, 40, 50 and 60 min) were analyzed.
3. N=1 data is not sufficient; this information is not optimal and thus, consider not useful to overall information.

SUMMARY

The analytical procedure involved preliminary ion-pair extraction of the drugs into dichloromethane, followed by concentration and analysis of the ion-pair complex using a gas-liquid chromatographic system fitted with a nitrogen detector. Using the peak area ratio technique, pyridostigmine bromide was used as the internal standard for the quantitation of neostigmine in plasma; neostigmine bromide was the internal marker for the determination of pyridostigmine. The method depends on the thermal dequaternization of the quaternary amines, and, can be used to detect 5 ng/ml in a 3-ml plasma sample in the range of 50 - 1000 ng/mL. This assay procedure has been applied to the separate determination of the plasma concentration of neostigmine and pyridostigmine after single administration of intravenous doses in anaesthetized patients.

Equipments:

Perkin-Elmer Model 17 [Diatomite CQ (100-120 mesh) coated with 3% (w/w) OV-17, 2 m x ¼ in. 0.0. glass] and Pye 104 gas chromatograph coupled to an MS 12 were used for gas chromatography and mass spectrometer, respectively.

Calibration graphs

Standard solutions of neostigmine bromide and pyridostigmine bromide were prepared by dissolving the salts in distilled water and diluted to give a series of solutions in plasma (3 ml) covering the concentration range 50-1000 ng/ml.

Recovery

The mean relative recoveries of neostigmine and pyridostigmine from plasma were 85.7% and 88.7%, respectively. Recovery of neostigmine was lowest at 50 ng/mL (78.1 %) and highest at 800 ng/mL (94.8 %).

Studies using radioactive neostigmine and pyridostigmine indicated that only 30% of the original radioactivity was recovered after extraction from plasma. The authors speculated that the loss of drug complexes during transfer procedures may account for the low recovery; alternatively, color or chemical quenching may be produced by the test samples. Using a similar procedure, the recovery of tubocurarine from tissue extracts was approx. 89 %. In the present experiments, when the radioactive extracts were analyzed by the GLC system, 95% recovery was achieved at a concentration of 1.6 nmole/mL of pyridostigmine.

RECOVERY STUDIES

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Selectivity

There were no chromatographic peaks from a normal plasma extract which interfered with the measurement of peaks corresponding to neostigmine and pyridostigmine.

Reproducibility

When plasma samples containing neostigmine (using pyridostigmine as the internal marker) were assayed on six different occasions, the reproducibility of the peak area ratio was $100 \pm 9\%$ at 50 ng/mL, and, $100 \pm 7\%$ at 1000 ng/mL. The corresponding values for pyridostigmine were $100 \pm 10\%$ (50 ng/mL) and $100 \pm 6\%$ (1000 ng/mL). The standard curves were linear over the range 50 – 1000 ng/mL (reproducible when repeated six times during the studies).

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Fig. 4. Calibration graphs of neostigmine using pyridostigmine as the internal marker. ● Methanolic solutions of the bromides of neostigmine and pyridostigmine; ○, plasma extracts of potassium iodide-glycine complexes of neostigmine and pyridostigmine. Each point represents the mean of six experiments.

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Fig. 5. Calibration graphs of pyridostigmine using neostigmine as the internal marker. ● Methanolic solutions of the bromides of neostigmine and pyridostigmine; ○, plasma extracts of potassium iodide-glycine complexes of neostigmine and pyridostigmine. Each point represents the mean of six experiments.

Storage.

Samples of plasma from healthy volunteers or from the Blood Bank (either freshly separated, or stored at -20° for 24 h or 7 days) did not interfere with the measurement of analytical peaks corresponding to the dequaternized neostigmine or pyridostigmine. There was no increase or decrease in peak area ratios of drug to internal standard in extracts stored at -20° for 7 days.

Application Patient DATA (N=1) This patient received neostigmine bromide, not methylsulfate

Tubocurarine was used to produce neuromuscular block in anaesthetized patients, and the anticholinesterase drugs were used to reverse the effects of the muscle relaxant on voluntary muscle function. After intravenous injection of neostigmine bromide (5 mg) or pyridostigmine bromide (20 mg), blood samples were collected at 2, 5, 10, 15, 20, 30, 40, 50 and 60 min.

In the one patient studied, the concentration of neostigmine in plasma declined from 4.47 $\mu\text{g/mL}$ at 2 min to 0.61 $\mu\text{g/mL}$ at 10 min. The concentration decreased to 0.11 $\mu\text{g/mL}$ at 60 min after intravenous administration of the drug. After intravenous pyridostigmine, the initial concentration of the quaternary amine in plasma was lower (1.39 $\mu\text{g/mL}$ at 2 min); the concentration then declined relatively slowly.

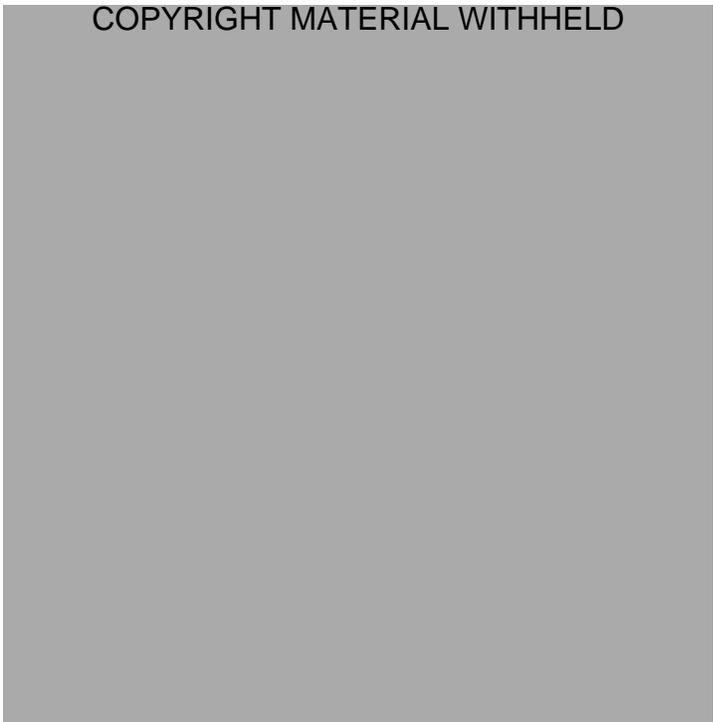


Fig. 6. Plasma concentration of neostigmine and pyridostigmine after intravenous administration of the quaternary amines. \circ , Neostigmine bromide (5 mg); \bullet , pyridostigmine bromide (20 mg).

Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds
determination of pyridostigmine, neostigmine and edrophonium in biological fluids

M.G.M. De Ruyter¹ and R. Cronelly¹, N. Castagnoli, Jr.²

¹Department of Anesthesia, University of California Medical Center, San Francisco, CA 94143 and ²Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143. *Journal of Chromatography*.183 (1980) 193-201

Notes:

Assay:

1. Extraction recovery: the authors presented a table indicating that 96.4 % and 99.1% were recovered (n=9) for pyridostigmine and neostigmine, respectively, at 80 ng/mL and 100 ng/mL; however, the authors do not show the 'raw' numbers.
2. Linearity: authors stated that linearity was observed over the range of 0-400 ng/mL for neostigmine, 0-1000 ng/mL for pyridostigmine and 0-1500 ng/mL for edrophonium. However, there are no figures that represent the findings, e.g., a plot of peak ratio vs. concentrations.
3. Chromatograms: It appears that the method adequately separates the species, as presented by several chromatograms in the paper.
4. Within-day precision: no 'raw' data is presented in the paper, although the authors claim that CV is low, indicating that the analytical method is stable between runs.
5. Overall, although the information presented in the paper is less than optimal, the analytical method described in the paper is acceptable.

An example of the procedure in PK assessment:

1. Authors presented a paragraph indicating that the procedure was employed to examine the pharmacokinetics (concentration versus time curves) of neostigmine (0.05 mg/kg) and edrophonium (0.5 mg/kg). However, authors do not give any information other than the dose administered. According to the plot, serum concentrations could be followed up to 4 h after administration for neostigmine and beyond for edrophonium. This information is considered less than optimal and will not be used in the overall assessment of neostigmine in this review.

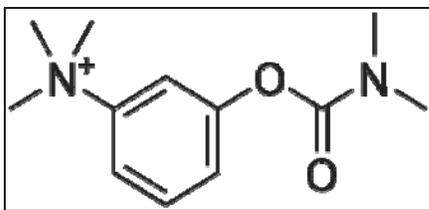
Summary:

A reversed-phase, ion-pair liquid chromatographic method for the quantitative determination of quaternary acetylcholinesterase inhibitors (e.g., pyridostigmine, neostigmine, edrophonium) was used to assay for pyridostigmine and neostigmine. The method uses an ion-pair extraction to isolate the drugs from biological material prior to liquid chromatographic separation and online UV detection at 214 nm. Quantitation down to 5 ng/ml and within-day precision with coefficient of Variation (C.V.) of 1.5% ($n=10$. $x = 100$ ng/mL) for neostigmine, C.V., 1.7% ($n=10$. $x = 80$ ng/mL) for pyridostigmine and C.V., 1.5% ($n=10$. $x=100$ ng/mL) for edrophonium have been achieved. The assay was designed for pharmacokinetic studies of these drugs in anesthetized patients.

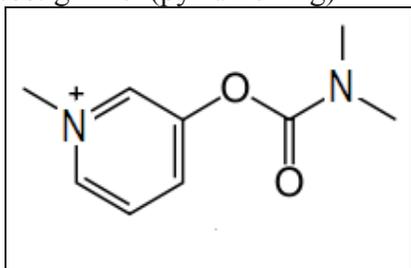
Design:

The assay was used to determine pharmacokinetic parameters of edrophonium, neostigmine and pyridostigmine. These results will be published in forthcoming papers.

Neostigmine (benzene ring)



Pyridostigmine (pyridine ring)



Procedures for bioanalysis:

Extraction from biological fluids

The internal standard used for the determination of pyridostigmine and neostigmine was edrophonium; neostigmine served as internal standard for the edrophonium assay. Standard solutions of these compounds ($0.5 \mu\text{g/mL}$) were prepared in water.

Chromatography of biological extracts

The Altex 5-1 μm ultrasphere Octyl column was used. The assays were performed at ambient temperature. Detection was at 214 Dm.

Standard curve/Quantitation

Blank serum samples were spiked with known amounts of the analytes. Peak height ratios of analytes vs. internal standard were used to establish calibration curves. Serum concentrations in the unknown samples were determined using these calibration curves.

Recovery/Reproducibility

Spiked samples with known quantities were used. Within-day precision was determined by performing ten replicate analyses of spiked serum samples.

Results:

1. Extraction recovery:

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Fig. 4. Liquid chromatographic trace from the injection of an extract from a serum sample containing 53 ng/ml neostigmine. Eluent: 0.01 M $C_7H_7SO_3^-Na^+$, 0.01 M NaH_2PO_4 and 0.0025 M TMA^+Cl^- in acetonitrile-water (20:80, v/v), pH 3.0. Column: 5- μ m Ultrasphere Octyl (15 \times 0.46 cm). Flow-rate, 2 ml/min. Detection at 214 nm 0.004 a.u.f.s. Peaks: I = interference, E = edrophonium (internal standard), N = neostigmine and CH_2Cl_2 = dichloromethane present in the injection solution.

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Fig. 5. Separation possibilities of analytes (N, neostigmine; P, pyridostigmine; E, edrophonium) and interference (I, CH_2Cl_2 ; Pi = picrate). Relationship between capacity ratios (k') and acetonitrile content of mobile phase. Column: 5- μ m Ultrasphere Octyl (15 \times 0.46 cm); eluent: 0.01 M $C_7H_7SO_3^-Na^+$, 0.01 M NaH_2PO_4 and 0.0025 M TMA^+Cl^- in acetonitrile-water adjusted to pH 3.0.

2. Standard curve linearity/lower limit of detection

Linearity was observed over the range of 0-400 ng/mL for neostigmine, 0-1000 ng/mL for pyridostigmine and 0-1500 ng/mL for edrophonium. The lowest points on the calibration graphs were 10 ng/mL for neostigmine, 30 ng/mL for pyridostigmine, and, 10

ng/mL for edrophonium. Quantitation down to 5 ng/mL (signal-to-noise ratio > 4) was achievable for all analytes.

3. Within-day precision

Within-day precision of the assay measured by coefficient of variation (C.V.) was 1.5% (n=10, 100 ng/mL) for neostigmine, 1.7% (n=10, 80 ng/mL) for pyridostigmine and 1.5% (n=10, 100 ng/mL) for edrophonium.

4. An example of the procedure in PK assessment – no useful information from the presented plot

The procedure was employed to examine the pharmacokinetics of neostigmine (0.05 mg/kg) and edrophonium (0.5 mg/kg). Examples of concentration versus time curves for neostigmine and edrophonium was provided (Fig. 7). Serum concentrations could be followed up to 4 h after administration for neostigmine and beyond for edrophonium. The procedure also may be used to detect these drugs in urine.



Fig. 7. Concentration versus time curves for neostigmine (N), and edrophonium (E) after intravenous administration. Infusion, 2 min; 0.5 mg/kg for edrophonium and 0.05 mg/kg for neostigmine.

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The Neuromuscular Pharmacology of Neostigmine in Infants and Children

Dennis M. Fisher, M.D., Roy Cronnelly, M.D., PH.D., Ronald D. Miller, M.D., Manohar Sharma, PH.D. § Department of Anesthesia, University of California, San Francisco, California. *Anesthesiology* 59:220-225, 1983

Notes:

1. No within paper analytical information is given, although the authors stated that they used the analytical method described by De Ruyter, 1980.
2. No patient information is presented, except the range of age in each group (infants: 2-10 months; children: 1-6 years; and adults: 29-48 years)
3. No kg body weight information was presented, yet the dose was administered based on $\mu\text{g}/\text{kg}$.
4. Mean PK parameters were presented; no individual PK parameters were presented. However, by looking at the presented neostigmine profiles, there may be a reasonable assurance that the presented PK parameters are acceptable.
5. This paper may have been a good source of PK information in children; however, there are not enough information presented in the paper, e.g., analytical, patient dosing information, etc., to contribute to the overall neostigmine clinical pharmacology information.

Summary:

Authors determined the dose-response relationship and time course of action of neostigmine in infants and children during N_2O -halothane anesthesia. d-tubocurarine (*dTc*) was administered by continuous iv infusion to maintain twitch tension of the adductor pollicis at constant 90% depression. Then 12 infants (3-48 weeks) and 15 children (1-8 yr) were given neostigmine (6.25, 12.5, or 25 $\mu\text{g}/\text{kg}$) with atropine while the *dTc* infusion was continued. The ED₅₀ (dose of neostigmine, which produces 50% antagonism of *dTc*-induced neuromuscular depression) was 13.1 $\mu\text{g}/\text{kg}$ in infants and 15.5 $\mu\text{g}/\text{kg}$ in children. The ED₅₀ was 22.9 $\mu\text{g}/\text{kg}$ in adults studied under similar anesthetic conditions. The time to 30%, 50%, and 70% of peak antagonism was similar for infants, children, and adults. The course of antagonism was followed past its peak effect in 15 subjects. In these subjects, duration of antagonism was similar to values in adults.

In addition, authors determined the pharmacokinetics of neostigmine. Three groups of five patients (infants, 2-10 months; children, 1-6 years; and adults, 29-48 years) were administered neostigmine as a 2-min intravenous infusion. Infants' dose was 100 $\mu\text{g}/\text{kg}$; children and adults doses were 70 $\mu\text{g}/\text{kg}$. Atropine dose was 30 $\mu\text{g}/\text{kg}$. Blood samples were obtained intermittently for 4 h, and concentrations of neostigmine were determined using a high-pressure liquid chromatographic technique. These data were fitted to a three-compartment pharmacokinetic model. Distribution half-lives and distribution volumes were similar for infants, children, and adults. Elimination half-life for infants, children and adults were 39 ± 5 min, 48 ± 16 min, and 67 ± 8 min (mean \pm SD), respectively.

The authors conclude that the time course of onset and duration of antagonism is similar for infants, children, and adults. The dose of neostigmine required to antagonize *dTc*-

induced neuromuscular blockade is lower in infants and children than in adults. Authors stated that the difference in dose requirement cannot be explained by age-related changes in volume of distribution. These findings are in contrast to the commonly held (but not documented) belief that infants and children require larger doses of neostigmine.

Design:

Serum samples, 0.5 ml each, were obtained before the infusion and at 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after drug administration. The samples were stored at -70°C until assayed for neostigmine.

Analytical method:

Authors used analytical method described by De Ruyter, et al, 1980. Authors stated that the high-pressure liquid chromatographic technique separates the parent compound from metabolites, were sensitive to 3.0 ng/ml, and, had a coefficient of variation of 5%.

Pharmacokinetic analysis:

The conc.-time curve was fitted using a least-squares nonlinear regression to two- or three- compartment pharmacokinetic models adjusted for the infusion. Values were weighted by the inverse-square of the serum concentration.

Using standard formulas, we determined the following variables: rapid and slow distribution half-lives ($t_{1/2T}$, $t_{1/2a}$); elimination half-life ($t_{1/2j}$); volume of the central compartment (V_I); steady-state volume of distribution (V_{dss}); and total plasma clearance (Cl).

Results:

1. A typical conc. vs. time curve from an infant:

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FIG. 3. Pharmacokinetic data from a 9-month-old patient. Neostigmine (100 $\mu\text{g}/\text{kg}$) was infused for 2 min. Circles represent measured concentrations of neostigmine; the solid line represents the fitted function as determined by nonlinear regression.

2. PK data

TABLE 2. Pharmacokinetic Data for Neostigmine (Mean \pm SD)
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3. Neostigmine conc. vs. time profiles for infants, children and adults

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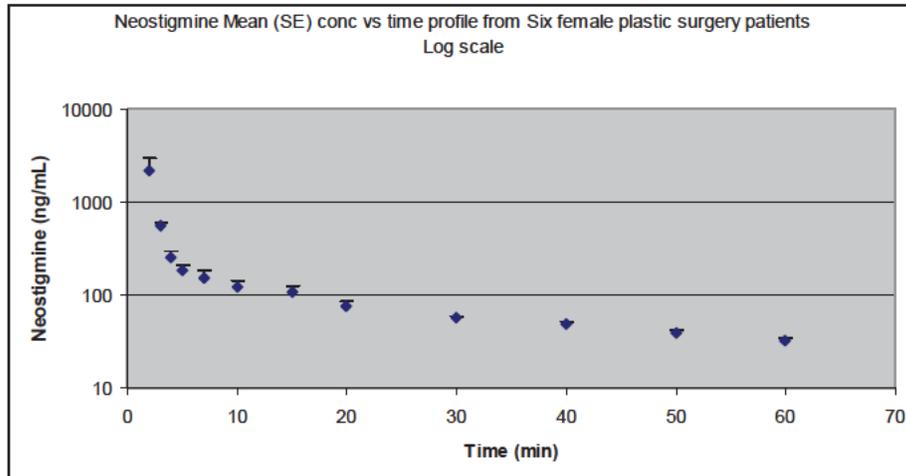
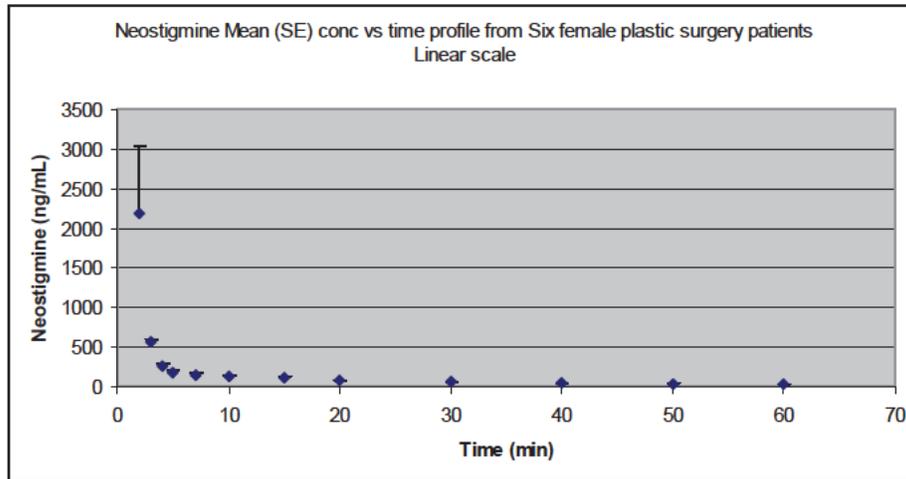
Pharmacokinetics and pharmacological effects of Neostigmine in man

T.N. Calvey¹, M. Wareing¹ & N.E. Williams¹, K. Chan²

¹Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX), ²School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF, British Journal of Clinical Pharmacology, Br. J. clin. Pharmac. (1979), 7, 149-155.

Notes:

1. This paper quotes Chan, et al., 1976, analytical paper. No within paper analytical information was presented;
2. Studies were carried out in n=6 female patients during plastic surgery; neostigmine methylsulphate, 5.0 mg, and atropine sulphate, 1.2 mg, were administered over a 15 s period;
3. No plasma conc. profiles were shown from the female subjects; however, mean plasma conc vs time table was provided: (Excel program drawn by this Reviewer)



Summary:

1. The pharmacokinetics of neostigmine was studied in six patients during the reversal of neuromuscular block induced by tubocurarine chloride. The effect of the drug on neuromuscular function was simultaneously assessed by electromyography.
2. Neostigmine plasma profile showed two slopes after intravenous administration. The fast disposition (distribution) half-life of the drug was invariably less than 1 min; the slow disposition (elimination) half-life ranged from 15.4-31.7 min.

3. Neostigmine usually increased the amplitude of the compound muscle action potential and diminished electromyographic decrement within 2 min of intravenous injection. The pharmacological effect of neostigmine was usually maximal between 7 and 15 min. There was an inverse relationship between the plasma concentration of the drug and the facilitation of neuromuscular transmission.

4. Red cell acetylcholinesterase activity was almost completely inhibited within 2-3 min of intravenous injection of neostigmine. Enzyme activity recovered to approximately 28% of control values by 30 min and to 55% by 60 min.

Introduction

This paper attempted to address the clearance of neostigmine from plasma during the antagonism of neuromuscular block induced by tubocurarine chloride. The effect of the quaternary amine on red cell acetylcholinesterase activity and voluntary muscle function was also studied.

Methods

Studies were carried out in six female patients, otherwise healthy, during plastic surgical procedures. The subjects were 22-62 years old (BW: range 48.5-72.6 kg). All the subjects were premedicated with nitrazepam (10 mg orally, given on the previous night) and diazepam (5-10 mg orally) on the day of operation. Anesthesia was induced by thiopentone sodium (200-400 mg), followed by tubocurarine chloride (45 mg); in one subject (patient 3) an additional dose of tubocurarine chloride (15 mg) was administered during the course of the operation. Anesthesia was maintained with nitrous oxide (60-70%) and halothane (0.5%) in oxygen, using intermittent positive pressure ventilation. At the end of the operation, neuromuscular block was reversed by standard techniques (neostigmine methylsulphate, 5.0 mg, and atropine sulphate, 1.2 mg, injected over a 15 s period). In the six patients studied, the dose of neostigmine methylsulphate ranged from 68.9-103.01g/kg.

Blood samples (approximately 8 ml) were taken at 2, 3, 4, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min. Neostigmine was extracted from plasma as an iodide complex, and its concentration was estimated by gas-liquid chromatography using a nitrogen detector (Chan, Williams, Baty & Calvey, 1976).

Results

1. Note: Neostigmine plasma conc vs time profile presented in the paper is from a 'man,' shown from 'a typical experiment.'

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Figure 1 Plasma concentration of neostigmine after intravenous injection in man. The figure shows the results of a typical experiment. ●, observed results; solid line, calculated curve from the biexponential function. The dashed lines correspond to the first and second exponential terms.

2. Mean neostigmine plasma conc. vs time

Table 1 Plasma concentration of neostigmine and red cell acetylcholinesterase activity (% of control) after intravenous injection of neostigmine methylsulphate (5.0 mg). Values correspond to mean \pm s.e. mean in the six experiments

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3. Individual pharmacokinetic parameters

Table 2 Pharmacokinetic parameters for intravenous neostigmine

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4. Neostigmine conc vs time fitted to a biexponential function and calculated amounts of neostigmine in central and peripheral compartments (according to 'standard methods' by Gibaldi & Perrier, 1975)

Table 3 Amount of neostigmine in the central and the peripheral compartment of a two compartment open model. Values correspond to mean \pm s.e. mean in the six experiments

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Pharmacokinetics of Edrophonium and Neostigmine When Antagonizing d-Tubocurarine Neuromuscular Blockade in Man

Robert B. Morris, M.D., Roy Cronnelly, M.D., Ph.D., Ronald D. Miller, M.D., Donald R. Stanski, M.D., and Mark R. Fahey, M.D.

Departments of Anesthesia, University of California, San Francisco 94143, and Stanford University, School of Medicine, Stanford, California 94305. *Anesthesiology* 54:399-402, 1981

Note:

1. Subjects are surgical patients
2. Assay mentioned in the paper is from De Ruyter et al., *J Chromatogr* 183: 193-201, 1980)
3. No within paper analytical information is presented in the paper
4. No neostigmine plasma profiles or data were presented in the paper
5. However, there is one table with neostigmine distribution and elimination half-lives, volume of distribution and clearance
6. The information is not useful

Summary:

The pharmacokinetics and effectiveness of edrophonium antagonism of d-tubocurarine neuromuscular blockade were compared with that of neostigmine in surgical patients anesthetized with halothane and nitrous oxide. After an intravenous (iv) injection of d-tubocurarine (0.3 mg/kg), the single twitch tension was allowed to return to five per cent of the control level. Edrophonium, 0.5 or 1.0 mg/kg (n = 12), or neostigmine, 0.07 mg/kg (n = 6), was then given intravenous in combination with atropine, 1.0 mg, as a 2-min controlled infusion. Train-of-four and single twitch tension were followed for 60 min in all patients. Twelve patients were monitored for 90 min, six patients for 120 min, four patients for 150 min, and two patients for 240 min. Blood was sampled intermittently for four hours and assayed for edrophonium or neostigmine using high-pressure liquid chromatography. Edrophonium was found to promptly antagonize the d-tubocurarine blockade. Twitch tension rapidly increased to a plateau (a rate of increase in twitch tension of less than 2 per cent of control per min) which was sustained in all cases. The mean time to plateau for edrophonium was 2.9 ± 0.21 (\pm SE) min as compared to 6.1 ± 0.75 min for neostigmine. Neuromuscular blockade did not reappear in any patient. The degree of antagonism of the neuromuscular blockade by neostigmine and edrophonium was not significantly different. Except for a longer distribution half-life, the pharmacokinetic variables for edrophonium did not differ significantly from those for neostigmine. The elimination half-lives of edrophonium and neostigmine were 110 ± 34 min (mean \pm SD) and 77 ± 47 min, respectively. The authors conclude that edrophonium, 0.5-1.0 mg/kg, have pharmacokinetic variables comparable to neostigmine and produces prompt, sustained, and effective antagonism of d-tubocurarine neuromuscular blockade.

Edrophonium, 0.5 mg/kg (n = 6), 1.0 mg/kg (n = 6), or neostigmine, 0.07 mg/kg (n = 6), was infused over 2 min in combination with atropine, 1.0 mg. The length of the operative procedures permitted monitoring of all cases for at least 60 min following antagonist infusion. Twelve cases were monitored for 90 min, six cases for 120 min, four cases for 150 min, and two cases for 240 min.

Venous blood was sampled from the contralateral arm at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after beginning the antagonist infusion, and stored at -300 C until assayed.

Samples were assayed for edrophonium or neostigmine using a high-pressure liquid chromatography technique which separates parent compounds from metabolites, and is sensitive to 1 ng/ml. (De Ruyter MGM, Cronnelly R, Castagnoli N: Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds. Determination of pyridostigmine, neostigmine, and edrophonium in biological fluids. *J Chromatogr* 183: 193-201, 1980)

The data, appropriately corrected for infusion period, were analyzed by nonlinear least squares regression analysis and fitted to a two-compartment open pharmacokinetic model. Computed pharmacokinetic variables were distribution half-life, elimination half-life, volume of central compartment (V_I), volume of distribution of steady state (V_{Dss}), and clearance (Cl).

TABLE I. Pharmacokinetic Variables (Means \pm SD) in Normal Anesthetized Patients

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Bioavailability of Intranasal Neostigmine: Comparison with Intravenous Route

M. Brogгинi¹, C. Benvenuti², V. Botta¹, A. Fossati³ and M. Valenti³ 1991

¹Dept. of Medicine, F. Del Ponte Hospital, Varese, Italy; ²Medical Dept., Formenti, Milano, Italy; ³Pharmacological Dept. and Pharmaceutical Development, Formenti, Milano, Italy, *Meth Find Exp Clin Pharmacol* **1991**, 13(3): 193-198

Note:

1. The doses used were 0.5 mg intravenous and 21.6 mg intranasal neostigmine
2. Authors used their HPLC method to analyze neostigmine; the paper described analytical setup, sample preparation, and standard curve conc.; however, it does not provide any QC, reproducibility, stability information
3. Subjects' neostigmine profiles are presented
4. Subjects' PK parameters are presented
5. t_{1/2} is much larger than previously quoted in other papers submitted in this application: 1.88 h vs (1.28 h vs. 1.12 h)

Summary:

The pharmacokinetics of intranasal (i.n.) neostigmine was compared with the intravenous (i. v.) route in 6 healthy volunteers with a mean age of 25.5 years and a mean weight of 65.8 kg in a crossover design. The doses used were 0.5 mg i.v. and 21.6 mg i.n. Neostigmine was determined by HPLC. The plasmatic profiles of the two routes were similar. An early peak of plasmatic concentration after i.n. administration was observed. The absolute bioequivalence of the i.n. route was ten-fold greater than the oral one.

Subjects:

Six healthy volunteers, 3 male and 3 female subjects, had a mean age of 25.5 years (median 25.5, range 23-28) with a mean weight of $65.8 \text{ kg} \pm 7$ (SEM) and mean height of $175.3 \text{ cm} \pm 4.8$.

Experimental design

The experimental design carried out was a Latin square crossover, with randomized sequences. The neostigmine formulations tested were 6% nasal solution (*Neostigmine Spray*, Formenti) and 0.5 mg vials for intravenous use (*Prostigmina*, Roche).

Before utilization, the nasal spray device was primed by activating the pump with some puffs in the open air to assure a homogeneous and constant spread of the solution (6). Each puff dispensed 5.4 mg. The subjects received in an upright position, in random sequence, single doses of neostigmine, 0.5 mg administered intravenously and 21.6 mg intranasally (2 puffs per nostril), at a 7-day interval. After fasting overnight, the basal blood sample was drawn at 8.00 a.m. (time 0 or "blank") and immediately afterward the subjects were given the scheduled single dose. Breakfast was eaten 1.5 h after dosing.

Blood samples (5 ml) were then taken at 0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2 and 4 h, centrifuged, and the plasma stored frozen at 20°C until the chemical analysis.

Analytical method

Neostigmine was determined in plasma samples using the HPLC method and UV detector at a wavelength of 200 nm. Edrophonium chloride (Sigma E-2356 – lot 12/F0407) was used as internal standard (IS).

Experimental conditions

A Perkin Elmer high pressure liquid chromatographic unit was used. It consisted of a four solvent pump Mod. SE 410, a UV LC-95 detector having variable wavelength, an automatic sampler ISS 100, and an LCI 100 integrator. A Perkin Elmer HS C-8 column (12.5 x 0.46 cm 5 μ sphere) was used. The mobile phase was prepared using a mixture (15/85) of acetonitrile and buffer, NaH_2PO_4 0.01 M adjusted with concentrated H_3PO_4 to reach a pH of 3, and with the addition of 1 g/L of sodium salt of heptasulphonic acid. The flow was 1.5 mL/min, at room pressure and at a pressure of 1200 psi. At the conditions selected for the experiment, neostigmine and IS showed good resolution with retention times of around 12.6 and 7.0 minutes (Fig. 1).

Preparation of the calibration curve and samples

Standard neostigmine (provided by the Formenti Company) was dissolved in water and diluted down to the concentration of 5 µg/mL, and at this dilution the points for the plasma curve at concentrations of 10, 30, 100, 300, 1000 ng/ml were prepared. Forty µL of an aqueous solution of internal standard prepared at concentrations of 100 µg/mL and 100 µ of perchloric acid (70%) diluted in water to 1/3 were added to 1 mL samples of blank plasma. The samples were vigorously stirred for 30 sec and then centrifuged at 10,000 rpm for 10 min. Fifty µL of the aqueous supernatant were injected into an HPLC column. The ratios between height of the neostigmine peaks and the IS peaks were used to trace two points of the calibration curve. The reproducibility of the method was verified by repeating the dosage for each point of the calibration curve 10 times.

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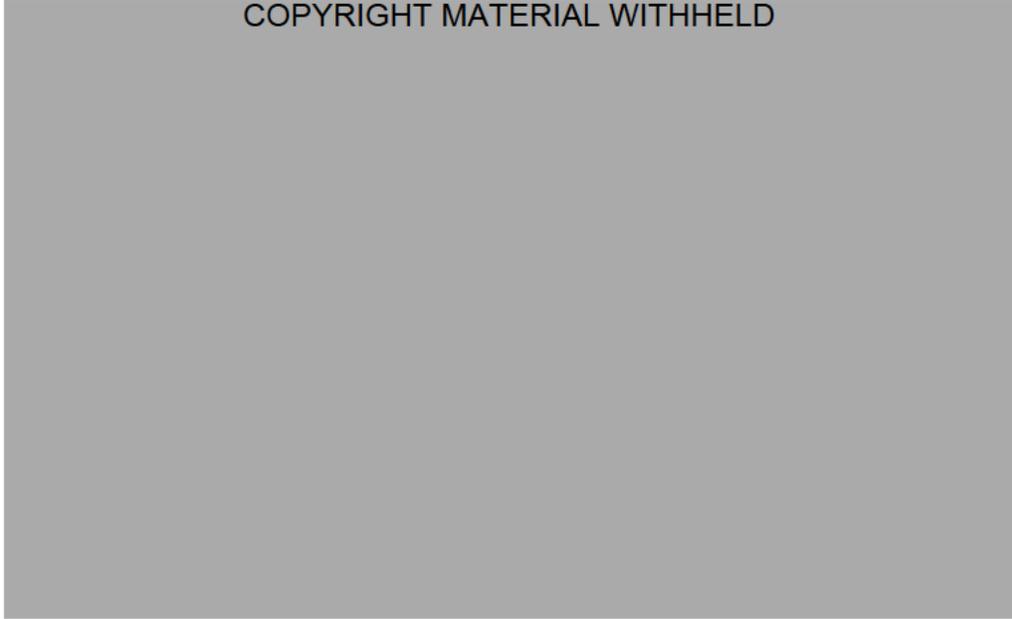


FIG. 1. Example of chromatogram.

Pharmacokinetic parameters

The following pharmacokinetic parameters were evaluated: AUC_{inf}, t_{1/2}, total clearance (Cl_{tot}/F), V_d/F, C_{max}, T_{max}, MRT and absolute bioavailability.

RESULTS

The dose referred to body weight was 0.35 mg/kg (range 0.25-0.44) for i.n. administration and 0.008 mg/kg (0.006-0.01) for i.v. The plasma curves for each subject are given in Figure 2 and the individual and mean pharmacokinetic parameters and the statistical comparison are reported in Table 1.

TABLE 1. Pharmacokinetic parameters correlated with the actual dose administered of intranasal and intravenous neostigmine

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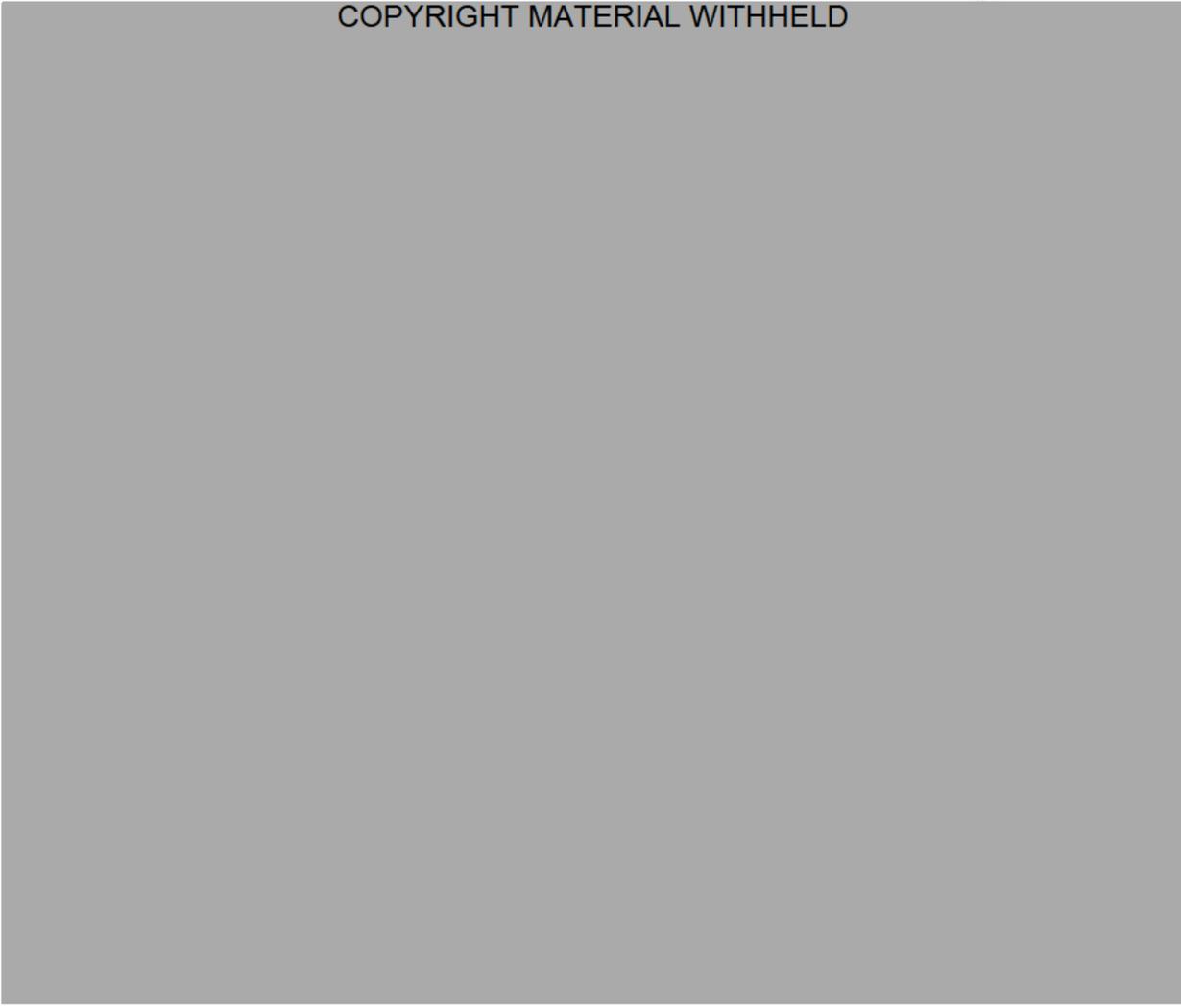




FIG. 2. Individual plasma curves after a single dose of neostigmine 0.5 mg i.v. or 10.8 mg per nostril (■—■ i.v.; ♦—♦ i.n.).

The values indicate that intranasal administration gave a higher absorption than intravenous at the doses tested: mean C_{max} 281.4 vs. 83.4 ng/mL and mean AUC 512.2 vs. 126.8 h.ng/mL. The maximum plasma concentration and the area under the concentration curve were constantly superior in each subject, although in subjects 4 and 6 the difference was less marked. The intranasal formulation was quickly absorbed as

shown by the early peak time. Half-life and mean residence time were similar for both administrations.

The absolute bioavailability varied between 3 and 21% (mean 11%) and these extreme values were obtained in subjects who received a similar dose/body weight of 0.01 and 0.44 mg/kg, respectively, for i.v. and i.n. administration.

Renal Function and the Pharmacokinetics of Neostigmine in Anesthetized Man

Roy Cronnelly, M.D., Ph.D., Donald R. Stanski, M.D., Ronald D. Miller, M.D., Lewis B. Sheiner, M.D., Yung J. Sohn, M.D.

Departments of Anesthesia and Pharmacology and the Division of Clinical Pharmacology. Department of Medicine. University of California. San Francisco. California. *Anesthesiology* 51:222-226, 1979.

Note:

1. Neostigmine dose was 0.07 mg/kg
2. Authors refer to Chan et al, 1976; however, there is no analytical information presented
3. neostigmine conc. vs time profiles were presented
4. neostigmine PK parameters in normal, newly transplanted and anephric patients are presented;
5. reported $t_{1/2}$ for normal subjects is in the similar range as of those other papers, 1.33 h

Summary:

The pharmacokinetics of neostigmine in patients with normal renal function ($n = 8$) were determined and compared with those of patients undergoing renal transplantation ($n = 6$) or bilateral nephrectomy ($n = 4$). All patients were anesthetized with nitrous oxide and halothane. d-Tubocurarine was infused at a rate sufficient to maintain 90 per cent depression of twitch tension. Ten to 15 minutes prior to the end of operation and anesthesia, the d-tubocurarine infusion was terminated and neostigmine, 0.07 mg/kg, and atropine, 0.03 mg/kg, were given by infusion over a 2-min period. Concentrations of neostigmine in blood drawn periodically during the following four hours were determined by gas-liquid chromatography and the data fitted to a two-compartment pharmacokinetic model. In anephric patients elimination half-life (181 ± 54 min, mean \pm SD) was significantly prolonged when compared with comparable values for patients with normal renal function (80 ± 48 min). Total serum clearance was significantly decreased from 16.7 ± 5.4 mL/kg/min in patients with normal renal function to 7.8 ± 2.6 mL/kg/min in anephric patients. Neostigmine pharmacokinetics following renal transplantation were not different from those in patients with normal renal function. It is concluded that renal excretion accounts for 50 percent of neostigmine clearance and, in

the absence of renal function, the serum half-life of neostigmine is prolonged, similar to that of d-tubocurarine.

Eight patients (23-52 years old) had normal renal function as evidenced by normal values for blood urea nitrogen (BUN) and creatinine. Four patients (38-45 years old) were undergoing bilateral or transplant nephrectomy, and six patients (29-44 years old) were undergoing renal transplantation. All patients were premedicated with diazepam, 10 mg, orally. Anesthesia was induced with thiopental, 2-4 mg/kg, and maintained with nitrous oxide, 60 per cent, and halothane, 0.4 to 0.5 per cent, end-tidal.

Ten to 15 min prior to the end of operation and anesthesia, the d-tubocurarine infusion was terminated and neuromuscular blockade antagonized by neostigmine, 0.07 mg/kg, combined with atropine, 0.03 mg/kg, administered intravenously over a 2-min period by use of an infusion pump.

Blood sampling:

Venous blood was withdrawn from the contralateral arm 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min following neostigmine administration. Serum was then stored at -35 C until assayed for neostigmine. Neostigmine was extracted from serum and concentrations determined by the technique of Chan *et al.* Serum containing neostigmine in concentrations ranging from 15 to 300 ng/mL was prepared with pyridostigmine as the internal standard.

Serum concentration data were analyzed with weighted nonlinear least-squares regression analysis. Data appropriately corrected for the infusion period were fitted to a two-compartment mamillary (or open) model:

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FIG. 1. Diagrammatic representation of the two-compartment pharmacokinetic model used for data analysis of neostigmine. V_1 and V_2 represent apparent volumes of the central and peripheral compartments. k_{12} and k_{21} are first-order rate constants of drug transfer between compartments. k_e is the first-order rate constant for drug elimination from the central compartment. Also shown is the biexponential equation that characterized the model.

Pharmacokinetic parameters calculated were $T_{a1/2}$, $T_{b1/2}$, V_d , V_{dss} , CL_t .

Results:

1. A typical plasma conc. vs time curves for normal, immediate renal transplantation and anephric subjects

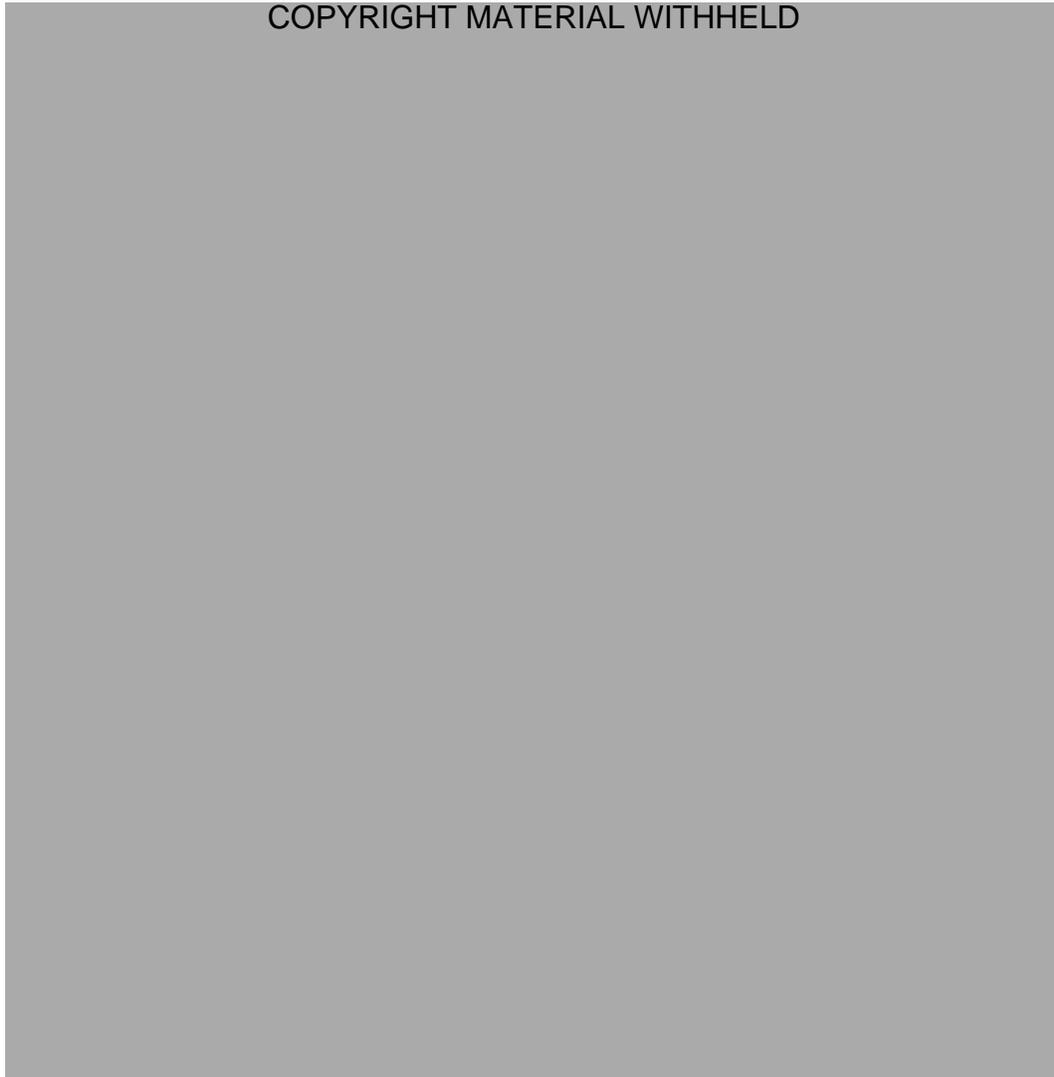


FIG. 2. Examples of the quality characterization of individual patient data by the pharmacokinetic model. Each figure represents data from one patient. In the upper left figure, the data are from a patient with normal renal function. In the upper right figure, data are from a patient with a newly transplanted kidney. In the lower figure, the data are from an anephric patient. Solid lines represent the fitted function.

2. Neostigmine conc. vs time profiles in normal subjects

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FIG. 3. Disappearance of neostigmine (0.07 mg/kg) from serum following 2-min intravenous infusion in eight patients with normal renal function. Solid lines represent fitted functions. The lengths of the individual lines indicate the times at which serum levels of neostigmine were no longer detectable.

3. Neostigmine conc. vs time profiles in patients with immediate renal transplantation

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Fig 4. Disappearance of neostigmine (0.07 mg/kg) from serum following 2-min intravenous infusion in six patients immediately following renal transplantation. Solid lines represent the fitted functions. The lengths of solid lines represent times at which serum levels of neostigmine were no longer detectable.

4. Neostigmine conc. vs time profiles in anephric patients

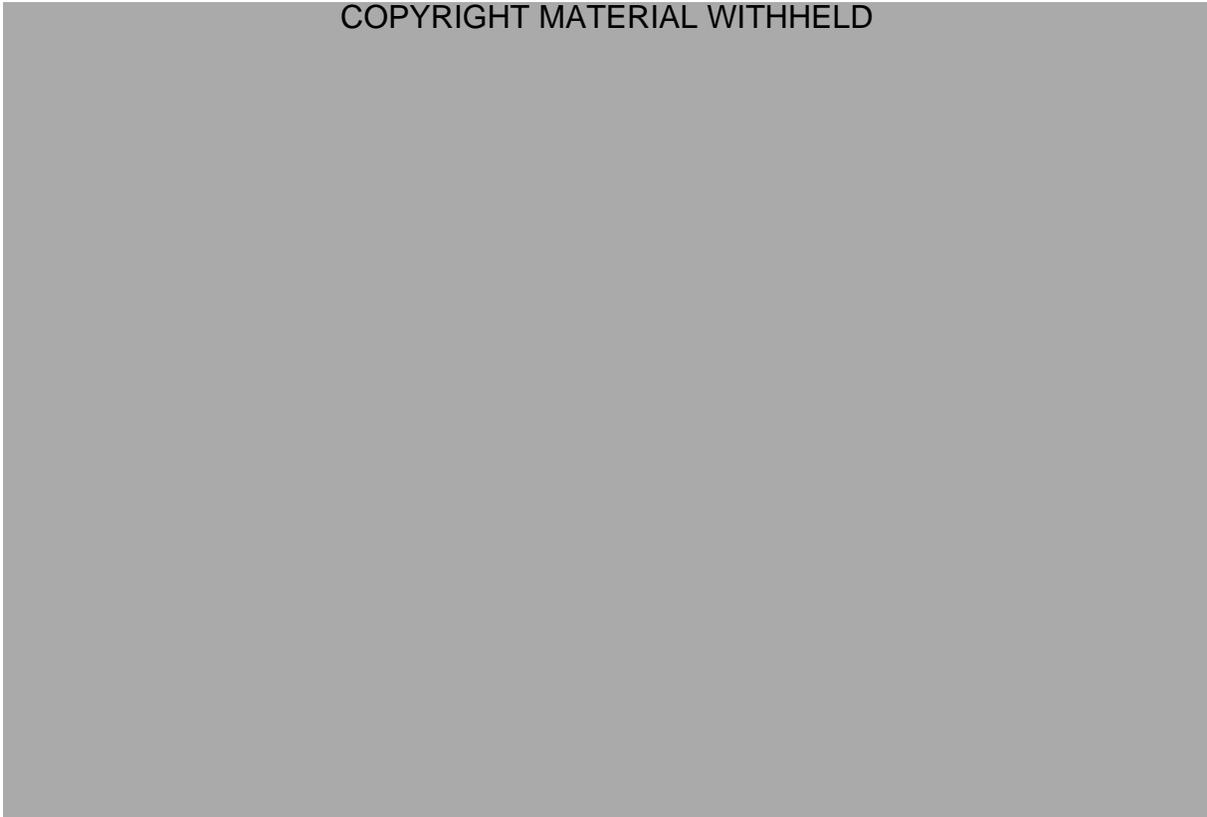


FIG. 5. Disappearance of neostigmine (0.07 mg/kg) from serum following 2-min intravenous infusion in anephric patients (n = 4). Solid lines represent fitted functions. The lengths of solid lines represent times at which serum levels of neostigmine were no longer detectable.

5. Pharmacokinetic parameters for normal, immediate renal transplantation, and anephric subjects

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Neostigmine pharmacokinetics were not significantly different in patients with normal renal function and those undergoing renal transplantation. In contrast, anephric patients had a significantly prolonged elimination half-life and decreased total serum clearance of neostigmine when compared with patients with normal renal function or those undergoing renal transplantation.

=====
4.3 Consult Review (including Pharmacometric Reviews)

Not Applicable.

4.4 Cover Sheet and OCPB Filing/Review Form

Office of Clinical Pharmacology New Drug Application Filing and Review Form			
General Information About the Submission			
	Information		Information
NDA/BLA Number	203629	Brand Name	Neostigmine Methylsulfate Injection, USP
OCP Division (I, II, III, IV, V)	II	Generic Name	
Medical Division	DAAAP	Drug Class	Non-depolarizing neuromuscular blocking agent
OCP Reviewer	David Lee, Ph.D.	Indication(s)	Reversal of non- depolarizing neuromuscular blocking agent
OCP Team Leader	Yun Xu, Ph.D.	Dosage Form	Injection 0.5 and 1 mg
Pharmacometrics Reviewer	-	Dosing Regimen	
Date of Submission	Dec 28, 2011	Route of Administration	Injection
Estimated Due Date of OCP Review	Sept 28, 2012	Sponsor	APP Pharmaceuticals

Medical Division Due Date	Sept 28, 2012	Priority Classification	Standard
PDUFA Due Date	Oct 28, 2012		
Clin. Pharm. and Biopharm. Information			
	“X” if included at filing	Number of studies submitted	Number of studies reviewed
STUDY TYPE			
Table of Contents present and sufficient to locate reports, tables, data, etc.			
Tabular Listing of All Human Studies			
HPK Summary			
Labeling	x		
Reference Bioanalytical and Analytical Methods			
I. Clinical Pharmacology			
Mass balance:			
Isozyme characterization:			
Blood/plasma ratio:			
Plasma protein binding:			
Pharmacokinetics (e.g., Phase I) -			
Healthy Volunteers-			
single dose:			
multiple dose:			
Patients-			
single dose:			
multiple dose:			
Dose proportionality -			
fasting / non-fasting single dose:			
fasting / non-fasting multiple dose:			
Drug-drug interaction studies -			
In-vivo effects on primary drug:			
In-vivo effects of primary drug:			
In-vitro:			
Subpopulation studies -			
ethnicity:			
gender:			
pediatrics:			
geriatrics:			
renal impairment:			
hepatic impairment:			
PD -			
Phase 2:			
Phase 3:			
PK/PD -			
Phase 1 and/or 2, proof of concept:			
Phase 3 clinical trial:			
Population Analyses -			
Data rich:			
Data sparse:			
II. Biopharmaceutics			
Absolute bioavailability			
Relative bioavailability -			
solution as reference:			
alternate formulation as reference:			
Bioequivalence studies -			
traditional design; single / multi dose:			
replicate design; single / multi dose:			
Food-drug interaction studies			
Bio-waiver request based on BCS			
BCS class			
Dissolution study to evaluate alcohol induced dose-dumping			
			Sponsor requests a biowaiver.

III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	X			
Total Number of Studies				

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	No clinical study was conducted with the proposed product.
2	Has the applicant provided metabolism and drug-drug interaction information?			x	
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?		x		Sponsor requests a biowaiver.
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?			x	
5	Has a rationale for dose selection been submitted?			x	
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?			x	
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?			x	
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?			x	
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?			x	
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?			x	
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product			x	

	(i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?				
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			x	
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			x	
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	The applicant submitted literature information
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?			x	
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?			x	
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? _____yes____

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

 Reviewing Clinical Pharmacologist Date

 Team Leader/Supervisor Date

APP Pharmaceuticals submitted a New Drug Application (NDA) for Neostigmine Methylsulfate Injection, USP, in accordance with Section 505(b)(2) of the Federal Food, Drugs, and Cosmetic Act. The Applicant seeks an indication of a reversal agent to the neuromuscular blocking effects of non-depolarizing muscle relaxants. The Applicant requests for approval of this NDA submission is based on the literature for both pediatrics and adult population. Neostigmine Methylsulfate Injection has a long history of clinical use in patients as a reversal agent to the neuromuscular blocking agents and has been marketed as an unapproved drug.

A pre-IND meeting was held with the Applicant on December 22nd, 2009, to discuss the appropriateness of literature information to support approval as well as a request for biowaiver. The Agency conveyed to the Applicant that an NDA submission can be based on the appropriate literature if the formulations used in the literature are appropriate for reference. In this submission the Applicant submitted literature information for the approval as well as a request for biowaiver. The referenced literature in the submission included studies with neostigmine intravenous injections. It is noted that the proposed Neostigmine Methylsulfate Injection formulation contains two inactive ingredients namely, liquefied phenol, USP, and sodium acetate, USP. (b) (4) acetic acid, USP, and sodium hydroxide, NF, are used to adjust pH of the injection solution.

From a clinical pharmacology perspective, the adequacy of the literature information in the application for the product labeling purpose will be a review issue. The application is recommended for filing, and, there are no comments/information requests to be conveyed to the Applicant at this time.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DAVID J LEE
08/24/2012

YUN XU
08/24/2012

**BIOPHARMACEUTICS
GENERAL APPLICATION REVIEW
Office of New Drug Quality Assessment**

Application No.:	203-629	Reviewer: Minerva Hughes, Ph.D.	
Submission Date:	28 Dec 2011		
Division:	Division of Anesthesia, Analgesia, and Addiction Products	Team Lead: Angelica Dorantes, Ph.D.	
Sponsor:	APP Pharmaceuticals	Supervisor: Richard Lostritto, Ph.D.	
Trade Name:	Neostigmine Methylsulfate Injection, USP	Date Assigned:	11 Jan 2012
Generic Name:	Neostigmine Methylsulfate Injection	Date of Review:	23 August 2012
Indication:	Reversal of non-depolarizing neuromuscular blocking agents.	Type of Submission: Original NDA – Marketed Unapproved Drug Biowaiver Request	
Formulation/strengths	Injection/ 0.5 mg and 1.0 mg		
Route of Administration	Injection		

SUBMISSION: NDA 203-629 was submitted in accordance with section 505(b)(2) of the FDC Act for the use of neostigmine methylsulfate as a reversal agent to the neuromuscular blocking effects of nondepolarizing muscle relaxants. The active moiety, neostigmine, is an anticholinesterase agent, which inhibits the hydrolysis of acetylcholine by competing with acetylcholine for binding to acetylcholinesterase at sites of cholinergic transmission. The proposed drug product, Neostigmine Methylsulfate Injection, is a marketed, unapproved drug with a long history of medical use for the proposed indication.

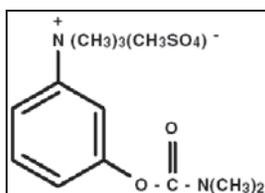
The Applicant referenced the literature for all efficacy, safety, and clinical pharmacology information. At the 22 December 2010 pre-IND meeting for the referenced IND 106574, FDA requested that the Applicant include in the NDA a formal waiver request for conducting bioavailability studies using the drug product that is the subject of this NDA.

This Biopharmaceutics review evaluates the acceptability of the biowaiver request in accordance with the applicable FDA regulations and guidance.

BIOPHARMACEUTICS REVIEW:

Drug Substance Information

The Applicant referenced DMF (b) (4) for the drug substance, neostigmine methylsulfate, chemistry, manufacturing, and controls information. Neostigmine methylsulfate has no known polymorphs and is water soluble. The chemical structure and molecular formula are illustrated below.



Neostigmine methylsulfate chemical structure, MW = 334.39 g/mol and MF = C₁₃H₂₂N₂O₆S

Drug Product Information

The proposed drug product is formulated as an injectable dosage form, available in 0.5 mg/mL and 1.0 mg/mL strengths. The formulation will be packaged in Type 1 glass vials, which are then (b) (4). The following table provides the quantitative drug product formulation information.

Packaging Configuration	10 mL fill in a 10-cc vial			
Vial	10 cc, Type 1, (b) (4) amber glass vial			
Stopper	(b) (4) amber glass vial			
Seal	20 mm (b) (4) light green			
Ingredient	0.5 mg/mL Unit	1.0 mg/mL Unit	Function	Quality Standard
Neostigmine methylsulfate, USP	0.5 mg	0.5 mg	Active Ingredient	USP
Phenol (as liquid phone, USP)	4.5 mg	4.5 mg	Preservative	USP
Sodium acetate, USP (trihydrate)	0.2 mg	0.2 mg	(b) (4)	USP
Water for injection	qs to 1 mL	qs to 1 mL		USP
(b) (4) acetic acid, USP	As needed	As needed	pH Adjuster	USP
Sodium hydroxide, NF	As needed	As needed	pH Adjuster	NF

Bioavailability Data

As per 21 CFR 320.21(1) and §320.21(2), all NDA applicants are required to include in the NDA either evidence measuring the in vivo bioavailability of the drug product that is the subject of the NDA or information to permit FDA to waive the submission of evidence measuring in vivo bioavailability.

The Applicant did not conduct any bioavailability studies with the proposed drug product under this NDA. However, as requested by FDA, a formal biowaiver request was included in the NDA. To satisfy the CFR’s bioavailability evidence requirement, the Applicant included pharmacokinetic literature data, in lieu of the drug product specific bioavailability studies.

FDA’s acceptance of the provided literature data as evidence of satisfying the bioavailability requirement is contingent on the appropriateness of the scientific bridge between the formulation and routes of administration used in the literature studies and the NDA’s proposed formulation and route of administration.

A summary of the referenced literature formulations and routes of administration is provided in the following table.

Author/Journal	Study Objective/Design	No. of Subjects	Neostigmine Formulation	Route
Williams, 1978/ British Journal of Anesthesia	Neostigmine PK parameters after neuromuscular block	5	Neostigmine methylsulfate (excipients not specified)	IV
Chan, 1976/ Journal of Chromatography	Neostigmine bioassay and measure neostigmine in human plasma after neuromuscular block	1	Neostigmine bromide in water, no other excipients	IV
De Ruyter, 1980/ Journal of Chromatography	PK of neostigmine in infants, children and adults after neuromuscular block with	Not specified	Salt form not specified (used in water only)	IV

Fisher, 1983/ Anesthesiology	PK of neostigmine in infants, children and adults after neuromuscular block with	15	Neostigmine salt form not specified	IV
Calvey, 1979/ British Journal of Clinical Pharmacology	PK of neostigmine during reversal of neuro-muscular block with Tubocurarine	6	Neostigmine methylsulfate (Prostigmin)*	IV
Morris, 1981/ Anesthesiology	PK of neostigmine during reversal of neuromuscular block with d-tubocurarine	6	Neostigmine salt not described	IV
Broggini, 1991/ Meth Exp Clin Pharmacology	Single dose PK of neostigmine when administered intranasal and IV	6	6% nasal spray (Neostigmine Spray) 0.5 mg/mL (Prostigmina**, Roche)	intranasal IV

Reviewer's Notes:

* External research by this reviewer regarding the trade name Prostigmin found that the formulation includes: 1 mg/mL neostigmine methylsulfate, 4 mg/mL phenol, and sodium hydroxide for pH adjustments.

**External research by this reviewer regarding the registered trade name Prostigmina found that the drug substance was neostigmine methylsulfate.

Reviewer's Assessment: Satisfactory

Neostigmine methylsulfate is a salt of the active moiety neostigmine, a quaternary ammonium ion, and sulfate ion. The drug substance is water soluble and is intended for intravenous (IV) administration, which affords direct delivery to the systemic circulation without any absorption step. There are no rate-release controlling excipients in the formulation. Phenol is used as a preservative and is not known to interfere with the drug's pharmacology. Thus, for this IV product, bioavailability can be considered self-evident. It is important to note, however, that there are no approved neostigmine products. As such, bioequivalence studies or a waiver of bioequivalence studies is not applicable for this product.

The submitted literature includes data on different neostigmine formulations, including one formulation with phenol as the preservative. From a scientific perspective, the counter ion is not believed to play a role in drug distribution and metabolism. Thus, the pharmacokinetic (PK) data from the different neostigmine salts administered by IV are expected to be similar to the Applicant's product, with the exception of the intranasal route of administration. Thus, the scientific link to the literature IV data is reasonable and the bioavailability data from the literature can be considered representative of the drug product that is the subject of this NDA.

Data from the intranasal route is not scientifically appropriate to support the Applicant's NDA. Intranasal administration involves an absorption step prior to systemic exposure, which varies depending on mucosal health, the nasal cavity size, and the physicochemical properties of the drug substance. The Applicant, however, is seeking approval for only the intravenous route of administration, which is the preponderance of literature PK data submitted.

CONCLUSIONS:

The scientific bridge to the literature bioavailability data has been appropriately established for only the intravenous literature studies. As there are no listed drugs for reference, an additional waiver of bioequivalence studies does not apply for this product.

The submitted literature, if acceptable, can be used to satisfy the “evidence” of bioavailability requirement as per §320.24(b)(6). Therefore, from the perspective of Biopharmaceutics, additional studies are not necessary to meet the bioavailability data submission requirement. However, it should be noted that the adequacy of the submitted PK data to support bioavailability, clinical, and labeling decisions are two different issues. The adequacy of the literature PK (bioavailability) data is under the purview of the Office of Clinical Pharmacology, assigned primary reviewer Dr. David Lee. Additional PK studies may be requested, if deemed appropriate by the Office of Clinical Pharmacology, irrespective of the literature data submitted.

APPROVAL RECOMMENDATION:

Approval from the perspective of Biopharmaceutics.

Minerva Hughes, Ph.D.

Biopharmaceutics Reviewer
Office of New Drug Quality Assessment
cc: see DARRTs list

Angelica Dorantes, Ph.D.

Biopharmaceutics Team Leader
Office of New Drug Quality Assessment

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/s/

MINERVA HUGHES
08/23/2012

ANGELICA DORANTES
08/23/2012

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Office of Clinical Pharmacology New Drug Application Filing and Review Form				
General Information About the Submission				
	Information		Information	
NDA/BLA Number	203629	Brand Name	Neostigmine Methylsulfate Injection, USP	
OCP Division (I, II, III, IV, V)	II	Generic Name		
Medical Division	DAAAP	Drug Class	Non-depolarizing neuromuscular blocking agent	
OCP Reviewer	David Lee, Ph.D.	Indication(s)	Reversal of non-depolarizing neuromuscular blocking agent	
OCP Team Leader	Yun Xu, Ph.D.	Dosage Form	Injection 0.5 and 1 mg	
Pharmacometrics Reviewer	-	Dosing Regimen		
Date of Submission	Dec 28, 2011	Route of Administration	Injection	
Estimated Due Date of OCP Review	Sept 28, 2012	Sponsor	APP Pharmaceuticals	
Medical Division Due Date	Sept 28, 2012	Priority Classification	Standard	
PDUFA Due Date	Oct 28, 2012			
Clin. Pharm. and Biopharm. Information				
	“X” if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.				
Tabular Listing of All Human Studies				
HPK Summary				
Labeling	x			
Reference Bioanalytical and Analytical Methods				
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD -				

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				Sponsor requests a biowaiver.
replicate design; single / multi dose:				
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	X			
Total Number of Studies				

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	No clinical study was conducted with the proposed product.
2	Has the applicant provided metabolism and drug-drug interaction information?			x	
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?		x		Sponsor requests a biowaiver.
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?			x	
5	Has a rationale for dose selection been submitted?			x	
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?			x	
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can			x	

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

	begin?				
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?			x	
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?			x	
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?			x	
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			x	
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			x	
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			x	
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	The applicant submitted literature information
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?			x	
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?			x	
19	Was the translation (of study reports or other study information) from another language needed and provided in this			x	

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

submission?				
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IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

_____yes_____

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Clinical Pharmacologist

Date

Team Leader/Supervisor

Date

APP Pharmaceuticals submitted a New Drug Application (NDA) for Neostigmine Methylsulfate Injection, USP, in accordance with Section 505(b)(2) of the Federal Food, Drugs, and Cosmetic Act. The Applicant seeks an indication of a reversal agent to the neuromuscular blocking effects of non-depolarizing muscle relaxants. The Applicant requests for approval of this NDA submission is based on the literature for both pediatrics and adult population. Neostigmine Methylsulfate Injection has a long history of clinical use in patients as a reversal agent to the neuromuscular blocking agents and has been marketed as an unapproved drug.

A pre-IND meeting was held with the Applicant on December 22nd, 2009, to discuss the appropriateness of literature information to support approval as well as a request for biowaiver. The Agency conveyed to the Applicant that an NDA submission can be based on the appropriate literature if the formulations used in the literature are appropriate for reference. In this submission the Applicant submitted literature information for the approval as well as a request for biowaiver. The referenced literature in the submission included studies with neostigmine intravenous injections. It is noted that the proposed Neostigmine Methylsulfate Injection formulation contains two inactive ingredients namely, liquefied phenol, USP, and sodium acetate, USP. (b) (4) acetic acid, USP, and sodium hydroxide, NF, are used to adjust pH of the injection solution.

From a clinical pharmacology perspective, the adequacy of the literature information in the application for the product labeling purpose will be a review issue. The application is recommended for filing, and, there are no comments/information requests to be conveyed to the Applicant at this time.

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/s/

DAVID J LEE
02/22/2012

YUN XU
02/23/2012

**BIOPHARMACEUTICS
FILING REVIEW
Office of New Drug Quality Assessment**

Application No.:	203-629	Reviewer: Minerva Hughes, Ph.D.	
Submission Date:	28 Dec 2011		
Division:	Division of Anesthesia, Analgesia, and Addiction Products	Team Lead/Supervisor: Angelica Dorantes, Ph.D.	
Sponsor:	APP Pharmaceuticals		
Trade Name:	Neostigmine Methylsulfate Injection, USP	Date Assigned:	11 Jan 2012
Generic Name:	Neostigmine Methylsulfate Injection	Date of Review:	8 Feb 2012
Indication:	Reversal of non-depolarizing neuromuscular blocking agents.	Type of Submission: Original NDA – Marketed Unapproved Drug	
Formulation/strengths	Injection/ 0.5 mg and 1.0 mg	Biowaiver Request	
Route of Administration	Injection		

SUBMISSION: NDA 203-629 was submitted in accordance with section 505(b)(2) of the FDC Act for approval of neostigmine methylsulfate as a reversal agent to the neuromuscular blocking effects of nondepolarizing muscle relaxants. The drug product is formulated as a (b) (4) injectable solution of neostigmine methylsulfate (0.5 mg or 1.0 mg), phenol (4.5 mg, (b) (4)%) and sodium acetate (0.2 mg, (b) (4)%).

Neostigmine Methylsulfate Injection is a marketed, unapproved drug. The active ingredient, neostigmine, is an anticholinesterase agent, which inhibits the hydrolysis of acetylcholine by competing with acetylcholine for binding to acetylcholinesterase at sites of cholinergic transmission. There is a long history of clinical use of neostigmine for the proposed indication. The drug has been used clinically since the 1930s.

The applicant's request for approval is based solely on the literature. Literature evidence in the form of case reports, dose-response studies and clinical studies form the basis for submission. A biowaiver request was also submitted on the basis of literature data.

A pre-IND meeting was held with the Applicant on 22 December 2009, to discuss the suitability of literature information/data to support approval. The Agency stated that an NDA submission may be made on the basis of the literature, if scientifically appropriate. Additionally, literature may be used to support a biowaiver request, if the formulations used in the literature are appropriate for reference.

BIOPHARMACEUTICS INFORMATION: This NDA submission includes the Applicant request for a waiver of the CFR requirement to provide in vivo bioavailability or bioequivalence data to support the approval of their proposed Neostigmine Methylsulfate Injection product. The waiver request was explicitly stated in the cover letter; however, the language used in Section 1.12.15 Request for Waiver of In Vivo Bioavailability Studies was ambiguous.

“A teleconference was held between APP Pharmaceuticals, LLC (APP) and FDA’s Division of Anesthesia, Analgesia and Rheumatology on December 22, 2010, to discuss submission plans for a 505 (b)(2) NDA for Neostigmine Methylsulfate Injection, USP. Please see the enclosed PIND 106,574 MEETING MINUTES. As documented in the Agency meeting minutes, the Agency stated

that the formal review of submitted information in the NDA application will determine the adequacy of the literature to support a request to waive pharmacokinetic/bioavailability studies for the proposed adult and pediatric populations. APP Pharmaceuticals has submitted this evidence in this 505 (b) (2) application and it can be found in Module 2 (SECTION 2.5.2, SECTION 2.5.3, SECTION 2.7.1, SECTION 2.7.2).”

Information in the referenced NDA sections:

- Tabular summary of literature data, with active ingredients and doses specified. Referenced literature included studies with neostigmine methylsulfate IV.
- Proposed formulation components
 - *There are no novel excipients in the proposed formulation.*

Reviewer’s Comments:

1. *It should be noted that the date of the teleconference in the FDA’s Meeting Minutes and in the NDA’s text are different.*
2. *It is acknowledged that the NDA includes a request for a biowaiver for the proposed Neostigmine Methylsulfate Injection product.*
3. *The submitted literature will be reviewed as part of the NDA review process to determine if this information supports the biowaiver request and whether a biowaiver can be granted.*

FILING REVIEW RECOMMENDATION: The application is recommended for filing.

COMMENTS FOR DAY-74 LETTER: There are no comments for the Day 74 letter.

Minerva Hughes, Ph.D.

Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Angelica Dorantes, Ph.D.

Biopharmaceutics Team Leader/Supervisor
Office of New Drug Quality Assessment

cc: see DARRTs list

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/s/

MINERVA HUGHES
02/08/2012

ANGELICA DORANTES
02/08/2012