OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA:	200890
Submission Date(s):	December 22, 2009; May 7, 2010
Brand Name	ISOPTO® Carpine
Generic Name	Pilocarpine hydrochloride
Primary Reviewer	Yongheng Zhang, Ph.D.
Team Leader	Charles Bonapace, Pharm.D.
OCP Division	DCP4
OND Division	DAIOP
Applicant	Alcon Inc.
Relevant IND(s)	Not applicable
Submission Type; Code	505(b)(2) ; Priority Review
Formulation; Strength(s)	Pilocarpine hydrochloride ophthalmic solution 1%, 2% , and 4%
Indication	The reduction of IOP in patients with open angle glaucoma or ocular hypertension (b) (4) acute angle-closure glaucoma; the prevention of (b) (4) postoperative elevated IOP associated with (b) (4) laser surgery and (b) (4) miotic

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LIST OF ABBREVIATIONS:

AUC	area under the plasma concentration-time curve
AUC0-∞	area under the concentration vs. time curve from time 0 extrapolated to infinity
AUClast	area under the concentration vs. time curve from time 0 to last measurable concentration
BQL	below quantifiable limit
C	Celsius
C_{max}	maximum plasma concentration
CYP450	cytochrome P450
CV%	percent coefficient of variation
ECG	electrocardiogram
EDTA	ethylenediaminetetra-acetic acid
h	hour(s)
HPLC	high performance liquid chromatography
IOP	intraocular pressure
i.v.	intravenous
kg	kilogram(s)
L	liter
LC-MS	liquid chromatographic-mass spectrometry
LC-MS/MS	liquid chromatographic-triple quadrupole mass spectrometry
LOQ	limit of quantification
LLOQ	lower limit of quantification
mg	milligram(s)
µg	microgram(s)
mL	milliliter(s)
µM	micromole
min	minute(s)
N	number
NA	not applicable
NC	not calculated
NR	not reported
P-gp	P-glycoprotein
PD	pharmacodynamic
PK	pharmacokinetic
QID	four times daily
SD	standard deviation
t1/2	elimination half-life
t_{max}	time to maximum plasma concentration
TID	three times daily

1. EXECUTIVE SUMMARY

Pilocarpine is a pre-1938 drug that has been marketed for many years. It is perhaps the most commonly used and most extensively studied intraocular pressure (IOP)-lowering miotic agent. Currently the product is one of many on the FDA compliance list of Medically Necessary Unapproved Marketed Drugs.

The Applicant submitted this 505(b)(2) application for ISOPTO® Carpine (pilocarpine hydrochloride ophthalmic solution) 1%, 2% and 4% in the reduction of IOP in patients with open angle glaucoma or ocular hypertension (b) (4) acute angle-closure glaucoma, and the prevention of (b) (4) postoperative elevated IOP associated with (b) (4) laser surgery and (b) (4) miotic.

In support of this NDA, the applicant conducted one ocular PK study (C-92-56) to assess systemic exposure of pilocarpine following ocular topical administration of ISOPTO® Carpine 4%. The applicant also conducted four clinical efficacy studies and one comfort/acceptability study with an ISOPTO® Carpine 2% treatment.

1.1. Recommendations

The Clinical Pharmacology information provided by the applicant in the NDA submission is acceptable.

The reviewer's proposed label changes in Appendix 4.1 should be forwarded to the sponsor.

1.2. Phase IV Commitments

None.

1.3. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

One human PK study (C-92-56) with ISOPTO® Carpine 4% (pilocarpine hydrochloride ophthalmic solution) was performed by Alcon. Pilocarpine concentrations following topical ocular administration were found to be low relative to the reported pilocarpine concentrations following oral dosing. The total daily dose for ISOPTO® Carpine 4% administered as 2 drops to each eye QID was 27.52 mg (43µl × 2 drop/eye × 2 eyes × 4 time/day × 40 mg/mL). Mean C_{max} value at steady-state following topical ocular dosing was approximately 3.7 ng/mL on both Days 5 and 8. As reported in the SALAGEN® label, the plasma C_{max} values following 5 mg and 10 mg SALAGEN® Tablets are 15 ng/mL and 41 ng/mL, respectively. Thus, mean C_{max} value following topical ocular dosing was approximately 4% of the C_{max} value following oral administration of an equivalent dose. Mean peak plasma exposure following topical ocular administration of 2 drops of ISOPTO® Carpine 4% QID is 4 to 11 times lower than that observed with SALAGEN® 5 mg and 10 mg Tablets, respectively. A larger margin is expected with 1 drop QID and at lower concentrations (i.e., 1% and 2%) of ISOPTO® Carpine.

No dose adjustment for ISOPTO® Carpine is warranted based on intrinsic factors including age and organ dysfunction (i.e. renal or hepatic impairment).

Pilocarpine is metabolized by esterase and CYP2A6. CYP2A6 can be inhibited by pilocarpine (Ki =1- 4 µM). Given the low systemic exposure following topical ocular administration of ISOPTO® Carpine, clinically relevant drug-drug interactions based on CYP450 interactions is not expected for ISOPTO® Carpine.

2. QUESTION BASED REVIEW

2.1. General Attributes of the Drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

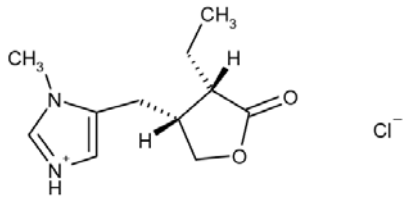
Pilocarpine is a white or almost white, crystalline powder or colorless crystals in which (b) (4) has been observed. Pilocarpine hydrochloride is freely soluble in water and alcohol and most stable at a pH range of 4 to 5.5.

Molecular Formula: $C_{11}H_{16}N_2O_2$ HCl

Molecular Weight: 244.72 Dalton

CAS Index Name: 6,11-dihydro-11-(1-methyl-4-piperidinylidene)-5*H*-imidazo[2,1-b] [3] benzazepine-3-carboxaldehyde

Chemical Structure:



Drug Product:
ISOPTO® Carpine has been marketed in the US and Europe for over 50 years. ISOPTO® Carpine (pilocarpine hydrochloride ophthalmic solution) 1%, 2% or 4% is a sterile, preserved ophthalmic solution containing 1%, 2% or 4% of pilocarpine hydrochloride formulated at a pH of approximately (b) It is a clear, colorless to pale yellow, slightly viscous solution with a target viscosity of approximately (b) (4) at room temperature and packaged in a multiple-dose container with a plastic dispensing plug and plastic closure. The composition of ISOPTO Carpine is given in **Table 2.1.1-1**.

Table 2.1.1-1: Composition of ISOPTO Carpine 1%, 2% and 4%

Component	Concentration (%w/v)			Function	Compendial Status
	Isopto Carpine 1%[a]	Isopto Carpine 2%[b]	Isopto Carpine 4%[c]		
Pilocarpine hydrochloride	1.0[d]	2.0[d]	4.0[d]	Active Ingredient	USP
Boric acid	(b) (4)				NF
Sodium chloride					USP
Sodium citrate, dihydrate					USP
Benzalkonium chloride	0.01	0.01	0.01		NF
Hypromellose 2910 (HPMC)					USP
Hydrochloric Acid and/or Sodium Hydroxide				pH Adjustment	NF NF
Purified Water					USP

[a] Formulation ID No. 10026; [b] Formulation ID No. 11631; [c] Formulation ID No. 99222;
[d] Up to (b) overage may be added (b) (4)

2.1.2. What is the proposed mechanism of drug action and therapeutic indication?

Pilocarpine is a direct acting cholinergic parasympathomimetic agent acting through direct stimulation of muscarinic receptors and smooth muscle such as iris and secretory glands. It contracts the cilliary muscle, causing increased tension on the scleral spur and opening of the trabecular meshwork spaces to facilitate outflow of aqueous humor, therefore reducing IOP. It also produces miosis through contracting of the iris sphincter muscle. The therapeutic indications include the reduction of IOP in patients with open angle glaucoma or ocular hypertension, the (b) (4) for acute angle-closure glaucoma, and the prevention of (b) (4) postoperative elevated IOP associated with (b) (4) laser surgery and (b) (4) miotic.

2.1.3. What are the proposed dosage(s) and route(s) of administration?

The recommended dosing for open-angle glaucoma and ocular hypertension is one drop of ISOPTO Carpine, 1%, 2% or 4% applied topically in the eye(s) up to four times daily. The frequency of instillation and concentration of ISPOTO Carpine are determined by the severity of the glaucoma and miotic response of the patient.

The recommended dosing for the initial treatment of acute angle-closure glaucoma is one drop of ISOPTO Carpine 1% or 2% applied topically in the eye(s) up to three times over a 30-minute period. (b) (4) . If laser iridoplasty is used to break the attack, one drop of ISOPTO Carpine 4% should be administered prior to the procedure. Following laser iridoplasty, one drop of ISOPTO Carpine 1% should be administered four times daily until laser iridotomy can be performed.

The recommended dosing for the prevention of (b) (4) postoperative elevated IOP associated with (b) (4) laser surgery and for induction of miosis is one drop of ISOPTO Carpine 1%, 2%, or 4% (or two drops administered five minutes apart) applied topically in the eye(s) 15 to 60 minutes prior to surgery.

2.2. General Clinical Pharmacology

2.2.1. What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The applicant conducted one ocular PK study (C-92-56) to assess systemic exposure of pilocarpine following ocular topical administration of ISOPTO® Carpine 4%. The applicant also conducted four clinical efficacy studies and one comfort/acceptability study with an ISOPTO® Carpine 2% treatment (**Table 2.2.1-1**).

2.2.2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics [PD]) and how are they measured in clinical pharmacology and clinical studies?

IOP is accepted as a surrogate endpoint for assessing the efficacy of treatments for open-angle glaucoma and ocular hypertension. The IOP endpoint has served as the basis for approval for all IOP-lowering agents for open-angle glaucoma or ocular hypertension.

2.2.3. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes, the sponsor used a validated gas chromatography with electron capture detection to quantitate concentrations of pilocarpine in human plasma (*Refer to Section 2.6*).

2.2.4. Exposure-response

2.2.4.1. What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

Neither dose-response nor concentration-response studies were performed by Alcon. ISOPTO® Carpine is available in three strengths: 1%, 2% and 4%, allowing for dose titration based on the indication and patient response.

2.2.4.2. *What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.*

Neither dose-response nor concentration-response studies were performed by Alcon.

2.2.4.3. Does this drug prolong the QT or QTc interval?

Cardiovascular safety and tolerability was not evaluated following topical administration of ISOPTO® Carpine.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

There is no unresolved dosing or administration issues.

Table 2.2.1-1: Completed Clinical Studies for ISOPTO® Carpine (pilocarpine hydrochloride ophthalmic solution) 2%

Protocol Type (No.)	Study Design	Subject/Patient Population	Treatment Groups	Dosing Regimen	Dosing Duration	Total No. Randomized: Total No. Exposed to ISOPTO Carpine 2%, 4%
Safety/Efficacy C-90-42	Randomized, double-masked, parallel group	Adults, primary open-angle glaucoma or ocular hypertension	ISOPTO Carpine 2% Betaxolol Susp. 0.25% Betaxolol Susp. 0.25%/Pilocarpine 1% Betaxolol Susp. 0.25%/Pilocarpine 2%	1 drop BID 1 drop BID 1 drop BID 1 drop BID	90 days	76[a] total: 18 ISOPTO Carpine 2%
Safety/Efficacy C-90-105	Randomized, double-masked, parallel group	Adults, primary open-angle glaucoma	ISOPTO Carpine 2% Betaxolol Sol. 0.5% Timolol Sol. 0.5%	1 drop QID 1 drop BID 1 drop BID	24 months	69 total: 14 ISOPTO Carpine 2%
Safety/Efficacy C-91-47	Randomized, double-masked, parallel group	Adults, primary open-angle glaucoma or ocular hypertension	ISOPTO Carpine 2% Betaxolol Susp. 0.25% Betaxolol Susp. 0.25% /Pilocarpine 1.75%	1 drop TID 1 drop TID 1 drop TID	90 days	182 total: 61 ISOPTO Carpine 2%
Safety/Efficacy C-91-54	Randomized, double-masked, parallel group	Adults, primary open-angle glaucoma or ocular hypertension	ISOPTO Carpine 2% Betaxolol Susp. 0.25% Betaxolol Susp. 0.25% /Pilocarpine 1.75%	1 drop TID 1 drop TID 1 drop TID	90 days	186 total 64 ISOPTO Carpine 2%
Comfort C-95-17	Randomized, double-masked, parallel group	Healthy adults	ISOPTO Carpine 2% Betaxolol Susp. 0.25% Betaxolol Susp. 0.25% /Pilocarpine 1.75%	1 drop BID[b] 1 drop BID[b] 1 drop BID[b]	7 days	79 total: 26 ISOPTO Carpine 2%

2.2.5. What are the PK characteristics of the drug and its major metabolite?

The plasma PK of pilocarpine was studied in healthy adult volunteers following multiple topical dosing (i.e., QID for 8 days) of ISOPTO® Carpine 4%. Pilocarpine metabolites such as pilocarpic acid and 3-hydroxypilocarpine were not monitored and analyzed.

2.2.5.1. What are the single dose and multiple dose PK parameters?

Table 2.2.5.1-1: Plasma Pharmacokinetic Parameters Following Multiple Topical Ocular Administration of 4% Pilocarpine QID, two drops in each eye.

Parameters	Day 5 (mean ± SD)	Day 8 (mean ± SD)
C_{max} (ng/mL)	3.69 ± 2.31	3.71 ± 3.15
$T_{1/2}$ (hr)	2.3 ± 0.8	2.5 ± 1.5
$AUC_{0\text{-last}}$ (ng × hr/mL)	7.41 ± 8.65	7.70 ± 8.42

$T_{1/2}$ = terminal half-life; SD= Standard deviation

2.2.5.2. How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

The plasma PK of pilocarpine was only studied in healthy adult volunteers following multiple topical administrations (i.e., QID for 8 days) of ISOPTO® Carpine 4%.

2.2.5.3. What are the characteristics of drug absorption?

Following topical administration of ISOPTO® Carpine 4%, T_{max} for pilocarpine plasma concentration was 0.5-1 hr post dose. Pilocarpine concentrations following topical ocular administration were found to be low relative to the reported pilocarpine concentrations following oral dosing. The total daily dose for ISOPTO® Carpine 4% administered as 2 drops to each eye QID was approximately 27.52 mg (43µl × 2 drop/eye × 2 eyes × 4 time/day × 40 mg/mL). Mean C_{max} values at steady-state following topical ocular dosing were approximately 3.7 ng/mL on Days 5 and 8. Plasma C_{max} values following 5 mg and 10 mg SALAGEN® Tablets were 15 ng/mL and 41 ng/mL, respectively. Thus, the mean C_{max} value following topical ocular dosing was approximately 4% of the C_{max} value following oral administration of an equivalent dose. Mean peak plasma concentration following topical ocular administration of 2 drops of ISOPTO® Carpine 4% QID is 4 to 11 times lower than that observed with SALAGEN® 5 mg and 10 mg Tablets, respectively.

2.2.5.4. What are the characteristics of drug distribution?

Pilocarpine does not bind to human plasma proteins over a concentration range of 5 to 25,000 ng/mL.

2.2.5.5. Does the mass balance study suggest renal or hepatic as the major route of elimination? (This may include table with results of mass balance study.)

No mass balance study was performed.

2.2.5.6. *What are the characteristics of drug metabolism?*

The sponsor did not provide any *in vitro* or *in vivo* metabolism data on pilocarpine. From the reviewer's literature review (*See Appendix 4.2.2*), pilocarpine is metabolized to pilocarpic acid by a cation-dependent esterase in serum, aqueous humor, and other organs. Pilocarpine can also be metabolized to 3-hydroxypilocarpine by CYP2A6. Pilocarpine is both a substrate (K_m of 1.5 μM determined using human liver microsomes) and an inhibitor (K_i of 1-4 μM) of CYP2A6. Given the low systemic exposure (C_{max} = 3.7 ng/mL, equivalent to 0.01 μM) following topical administration, the projected [I]/ K_i for pilocarpine would be ≤ 0.01 and the inhibition of CYP2A6 *in vivo* by pilocarpine is not expected.

2.2.5.7. What are the characteristics of drug excretion?

Limited information is available about the elimination of pilocarpine in human. Pilocarpine and its metabolites, including pilocarpic acid and 3-hydroxypilocarpine, are excreted in the urine following pilocarpine oral administration.

2.2.5.8. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Not evaluated because PK parameters were derived from a PK study at one dose level, i.e., ISOPTO® Carpine 4%.

2.2.5.9. How do the PK parameters change with time following chronic dosing?

As shown in **Table 2.2.5.1-1**, pilocarpine plasma concentrations reached steady-state by Day 5 following multiple topical QID dosing. PK parameters including AUC, C_{max}, and $T_{1/2}$ were similar between Days 5 and 8.

2.2.5.10. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The CV% for C_{max} and $AUC_{0\text{-last}}$ values for pilocarpine were > 60% among healthy subjects (**Table 2.2.5.1-1**). This level of inter-subject variability in systemic exposure is not considered unexpected following topical ocular administration. Pilocarpine PK was not studied in patients.

2.3. Intrinsic Factors

2.3.1. What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

In the ocular PK study (C-92-56), a limited number of healthy subjects (n=14) were studied. The effect of the commonly known intrinsic factors including race, gender and age on the PK of pilocarpine following topical administration of ISOPTO® Carpine 4% has not been thoroughly studied. Given the low systemic exposure following topical administration, however, dose adjustment is not warranted in patients based on the commonly known intrinsic factors.

2.4. Extrinsic Factors

2.4.1. What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?
Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

The impact of the commonly known extrinsic factors on pilocarpine dose-exposure and/or – response has not been evaluated. Because of the systemic exposure is low, the impact, if any, would not be clinically significant.

2.4.2. Drug-drug interactions

2.4.2.1. Is there an in vitro basis to suspect in vivo drug-drug interactions?

No. Pilocarpine is metabolized to pilocarpic acid by a cation-dependent esterase in serum, aqueous humor, and other organs, and to 3-hydroxypilocarpine by CYP2A6 (with a K_m of 1.5 μM in human liver microsomes). With an *in vitro* K_i value of 1-4 μM for CYP2A6 and the low systemic exposure following topical administration, the projected [I]/ K_i for pilocarpine would be ≤ 0.01 and the inhibition of CYP2A6 *in vivo* by pilocarpine is not expected.

2.4.2.2. Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Pilocarpine was identified to be a CYP 2A6 substrate. Therefore, pilocarpine metabolism can be influenced by CYP2A6 polymorphism. However, due to the low systemic exposure, the intersubject variability on pilocarpine plasma exposure as the result of CYP2A6 polymorphism is unlikely to be clinically relevant following ocular topical dosing.

2.4.2.3. Is the drug an inhibitor and/or an inducer of CYP enzymes?

Pilocarpine can inhibit CYP2A6 *in vitro* with K_i of 1-4 μM (*Refer to Section 2.4.2.1*).

2.4.2.4. Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

It is unknown if pilocarpine is a substrate and/or an inhibitor of P-glycoprotein or not.

2.4.3. What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

No issues related to dose, dosing regimens, or administration are unresolved.

2.5. General Biopharmaceutics

Not applicable to the current application because pilocarpine is formulated as ophthalmic solution for topical administration.

2.6. Analytical Section

2.6.1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

The sponsor used a validated gas chromatography with electron capture detection to quantitate concentrations of pilocarpine in human plasma. (b) (4) was used as the internal standard.

2.6.2. Which metabolites have been selected for analysis and why?

No metabolites were selected for analysis.

2.6.3. For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Pilocarpine does not bind to human plasma protein over a concentration range of 5 to 25,000 ng/mL. Thus, free concentrations were measured.

2.6.4. What bioanalytical methods are used to assess concentrations?

Refer to *Section 2.6.1.* for further information.

2.6.4.1. What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

The standard curve for plasma samples ranged from 1 ng/mL to 40 ng/mL for pilocarpine. The observed plasma concentrations of pilocarpine in clinical studies did not exceed the upper limit of quantitation. The linear regression of the curves for peak area ratios *versus* concentration was weighted $1/x^2$ for the standard curves.

2.6.4.2. What are the lower and upper limits of quantification (LLOQ/ULOQ)?

The lower and upper limits of quantitation were 1 ng/mL and 40 ng/mL for pilocarpine, respectively.

2.6.4.3. What are the accuracy, precision, and selectivity at these limits?

The accuracy (%RE) and precision (%CV) ranges for pilocarpine were -4.3% to 6.0% and 3.5% to 10.4%, respectively.

Selectivity was demonstrated by the lack of interference by potential endogenous interfering substances in blank human plasma with or without internal standard and plasma samples collected pre-dosing. No interference from either isopilocarpine (the optical diasteroisomer of pilocarpine) or pilosin was detected.

2.6.4.4. What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

Pilocarpine was shown to be stable in EDTA-stabilized plasma at room temperature up to 2 days and at -20°C for more than 16 months, in extracted samples for 4 days at room temperature, and following three freeze/thaw cycles.

2.6.4.5. What is the QC sample plan?

The concentrations of the QC samples consisted of 2.0, 8.0, and 20 ng/mL. Between-run and within-run accuracy and precision were evaluated using replicates (n=3) from each of the three concentrations.

3. LABELING RECOMMENDATIONS

See Appendix 4.1. for detail.

4. APPENDICES

4.1. Proposed Package Insert (Original and Annotated; Dec, 2009)

5 Page(s) of Draft Labeling has been Withheld in Full immediately following this page as B4 (CCI/TS)

4.2. Individual Study Review

4.2.1. PK Study Following Ocular Topical Administration

Study Number: C-92-56
Plasma Concentrations of Pilocarpine Following Pilocarpine 4% Solution QID or Pilocarpine 1.75% TID in Combination with Betaxolol 0.25% in Healthy, Adult Subjects

Dates: October 21, 1992 to November 8, 1992
Clinical investigator: Robert A. Laibovitz, MD, Austin, TA, USA
Analytical site: (b) (4)

OBJECTIVES:

The objectives of this study were to determine the steady-state plasma concentration-time profile of pilocarpine in healthy subjects topically dosed with either pilocarpine 4% solution QID or Pilocarpine 1.75% TID in combination with Betaxolol 0.25%.

FORMULATION & ADMINISTRATION

Pilocarpine hydrochloride ophthalmic solution (Isoptocarpine®) 4% (Lot 2RDAE) was dosed two drops in each eye QID.

Betaxolol 0.25%/Pilocarpine 1.75% combination was prepared from sterile pilocarpine hydrochloride 8.75% solution and Betaxolol 0.313% suspension. The resulting solution was dosed two drops in each eye TID.

STUDY DESIGN:

This was a prospective, single-center, open-label, cross-over study. Total 16 normal, healthy subjects (with a mean age of 60.1 years, age matched to glaucoma patients) were enrolled and administered QID in both eyes for 8 days.

In the part 1, pilocarpine hydrochloride ophthalmic solution (Isoptocarpine®) 4% was administered QID for eight days followed by a two-day washout. Subsequently, subjects were dosed TID for 8 days with the Betaxolol 0.25%/Pilocarpine 1.75% combination in the part 2 of the study.

Series plasma samples were taken from 14 subjects (two subjects dropped out prior to plasma sampling, due to pilocarpine-related adverse events) on Days 5 and 8 at pre-dose, and 0.5, 1, 2, 3, 4, 5 and 6 hours post-dose.

Note: This review is focused on the part 1 of the study, i.e., pilocarpine plasma exposure following the administration of pilocarpine 4% solution QID for eight days.

DATA ANALYSIS

Non-compartmental analysis was used to estimate key pharmacokinetic parameters including C_{max}, T_{max}, AUC, and the elimination $T_{1/2}$ for those subjects (n=13) with plasma concentration values above the lower limits of quantification (i.e., 1.0 ng/mL).

RESULTS:

For the 13 subjects among all the subjects (n=14) with blood samples, pilocarpine concentrations were detectable at ≥ 4 time points. For one subject, pilocarpine plasma concentrations were below the LLOQ at all the time points analyzed.

Pharmacokinetics
Following topical administration of ISOPTO® Carpine 4%, T_{max} for pilocarpine plasma concentration was 0.5-1 hr post dose. The total daily dose for ISOPTO® Carpine 4% administered as 2 drops to each eye QID was approximately 27.52 mg (43µl × 2 drop/eye × 2 eyes × 4 time/day × 40 mg/mL). The mean C_{max} at steady-state following topical ocular dosing was approximately 3.7 ng/mL on both Days 5 and 8. Individual Pilocarpine C_{max} values and $AUC_{0\text{-last}}$ values ranged from 1.63 to 11.3 ng/mL and from 1.4 to 26.6 ng×hour/mL, respectively.

As expected, pilocarpine systemic exposure was lower in subjects when receiving Pilocarpine 1.75% in combination with Betaxolol 0.25% TID compared to that when receiving ISOPTO® Carpine 4% QID. (*Note: the exposure data from the combination treatment was not thoroughly reviewed by the reviewer*)

Table 2: Plasma Pharmacokinetic Parameters Following Multiple Topical Ocular Administration of 4% Pilocarpine QID, two drops in each eye.

Parameters	Day 5 (mean ± SD)	Day 8 (mean ± SD)
C_{max} (ng/mL)	3.69 ± 2.31	3.71 ± 3.15
C_{max} range (ng/mL)	1.63 – 8.07	1.88 – 11.3
$T_{1/2}$ (hr)	2.3 ± 0.8	2.5 ± 1.5
$AUC_{0\text{-last}}$ (ng × hr/mL)	7.41 ± 8.65	7.70 ± 8.42

$T_{1/2}$ = terminal half-life; SD= Standard deviation

Safety
Ten of sixteen subjects receiving pilocarpine 4% experience adverse events and two of them discontinued from the study due to nonserious events including hemorrhage vitreous, hyperemia eye, blurred vision and headache related to pilocarpine 4%. All the adverse events were considered nonserious, generally mild to moderate in intensity.

COMPARISON WITH ORAL PHARMACOKINETICS

Following ocular topical administration of ISOPTO® Carpine 4%, pilocarpine concentrations were found to be low relative to the reported pilocarpine concentrations following oral dosing. The total daily dose for ISOPTO® Carpine 4% administered as 2 drops to each eye QID was approximately 27.52 mg (43µl × 2 drop/eye × 2 eyes × 4 time/day × 40 mg/mL). Mean C_{max} values at steady-state following topical ocular dosing were approximately 3.7 ng/mL on Days 5 and 8. As reported in the SALAGEN® label, the plasma C_{max} values following 5 mg and 10 mg Tablets are 15 ng/mL and 41 ng/mL, respectively. Thus, mean C_{max} following topical ocular

dosing was approximately 4% of the C_{max} following oral administration of an equivalent dose. Mean peak plasma concentrations following topical ocular administration of 2 drops of ISOPTO® Carpine 4% QID is 4 to 11 times lower than that observed with SALAGEN® 5 mg and 10 mg Tablets, respectively.

REVIEWER'S ASSESSMENT & RECOMMENDATION:

Results from Study C-92-56 adequately described the pharmacokinetics of pilocarpine in plasma following repeated daily ocular topical administration of pilocarpine 4% ophthalmic solution in healthy, adult subjects.

- The mean C_{max} at steady-state following topical ocular dosing was approximately 3.7 ng/mL on both Days 5 and 8.
- Individual pilocarpine C_{max} values and AUC $_{0\text{-last}}$ values ranged from 1.63 to 11.3 ng/mL and from 1.4 to 26.6 ng×hour/mL, respectively. T_{max} values ranged from 0.5 to 1 hour.
- Mean peak plasma exposure following topical ocular administration of 2 drops of ISOPTO® Carpine 4% QID is 4 to 11 times lower than that observed with SALAGEN® Tablets. A larger margin is expected with 1 drop QID and at lower concentrations (i.e., 1% and 2%) of ISOPTO® Carpine.

4.2.2. Literature Review on Pilocarpine Metabolism In Vitro and In Vivo

Title: Involvement of CYP2A6 in the formation of a novel metabolite, 3-hydroxypilocarpine, from pilocarpine in human liver microsomes.
Authors: Takuro Endo, Masaaki Ban, Kazuma Hirata, Akitoshi Yamamoto, Yoshiki Hara, and Yasunori Momosel.
Journal: Drug Metabolism & Disposition, 35:476-483 (2007)

Objectives:
(1) To confirm and identify a previously reported pilocarpine metabolite (M1) other than pilocarpic acid in human urine after the oral administration of pilocarpine.
(2) To identify the human CYP450 responsible for the formation of M1.

Method:

Metabolite identification from human urine samples
A single oral dose of pilocarpine hydrochloride (10 mg) was administered to six male volunteers. Urine samples were collected at 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h after the dose. Three milliliters of the 0- to 4-h urine sample from each volunteer was pooled and used for the isolation of the metabolites. The pooled human urine was analyzed using an LC-MS/MS system to confirm the retention time of pilocarpic acid, pilocarpine and M1. The isolated M1 was characterized and identified by NMR spectroscopy. Based on the mass spectral and NMR analyses, the proposed structure for M1 possessed a hydroxyl group on carbon-3 with (R)-stereochemistry. To confirm this structure, an authentic sample was synthesized. The synthesized (R)- and (S)-diastereomers, namely, 3-hydroxypilocarpine and 3-hydroxyisopilocarpine, were separated on a C18 column. The configuration of the authentic sample was confirmed from its single crystal X-ray diffraction pattern.

In vitro metabolism
Pilocarpine *in vitro* metabolism was investigated in human liver microsomes using ^{14}C-labeled pilocarpine. To identify the human CYP450 responsible for the metabolite formation, *in vitro* experiments using CYP450 isoform-selective inhibitors, cDNA expressed human CYP450s (Supersomes; CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4), and liver microsomes from different donors were conducted.

Results:

A metabolite of pilocarpine (M1) was isolated by HPLC and identified to be 3-hydroxypilocarpine be by LC-MS/MS and NMR analyses.

The generation of 3-hydroxypilocarpine was observed in human liver microsomes with an NADPH-generating system, and this fact suggested that P450 is involved in the generation. Pilocarpic acid was not detected in human liver microsomes regardless of the presence or absence of an NADPH-generating system.

Figure 1 shows an Eadie-Hofstee plot for the 3-hydroxylation of pilocarpine in pooled human liver microsomes. The plot was almost monophasic and indicated that a single enzyme was responsible for the formation of 3-hydroxypilocarpine from pilocarpine. The apparent Km and Vmax values were 1.5 μM and 8.3 pmol/min/mg, respectively.

Table 1: Demographics of the subjects

Variable		N = 16	PERCENT
Age (Yrs)	Mean	60.1	-
	STD	7.25	-
	Range	45-71	-
Sex	Male	6	37.5
	Female	10	62.5
Race	Caucasian	14	87.5
	Black	1	6.2
	Other	1	6.2
Iris Color	Brown	8	50.0
	Blue	7	43.7
	Hazel	1	6.2

ASSAY METHODOLOGY:

A validated gas chromatography with electron capture detection was used to quantitate concentrations of pilocarpine in EDTA-stabilized human plasma. (b) (4) was used as the internal standard. The plasma samples were extracted by methylene chloride at pH 8.5. Following evaporation, the extracts were derivatized by heptafluorobutyric anhydride. The derivatized analytes were then back extracted into acid and washed with hexane. Finally, the derivatized analytes were extracted into benzene at pH 9.25. Extracts were analyzed by capillary gas chromatography with electron capture detection.

Criterion	Pilocarpine	Comments
Conc. range, ng/mL	1.0– 40	satisfactory
LLOQ, ng/mL	1.0	satisfactory
Linearity, r^2	> 0.99	satisfactory
Accuracy, % RE	-2.7 – 6 [a] -4.3 – 5.0 [b]	Satisfactory
Precision, % CV	7.8 – 10.4 [a] 3.5 – 7.6 [b]	Satisfactory
Recovery	Evaluated for the analyte (89.8%) and internal standard (62.9)% at three QC concentrations (low, medium and high)	Satisfactory
Specificity	Interference by endogenous compounds evaluated	Satisfactory
Stability	Stable in EDTA-stabilized plasma at room temperature up to 2 days and at -20°C for more than 16 months, extracted samples for 4 days at room temperature, and following three freeze/thaw cycles	Satisfactory

[a], QC samples including 2.0, 8.0 and 20 ng/mL
[b], Calibration standards including 1.0, 2.0, 3.0, 5.0, 10, 20 and 40 ng/mL

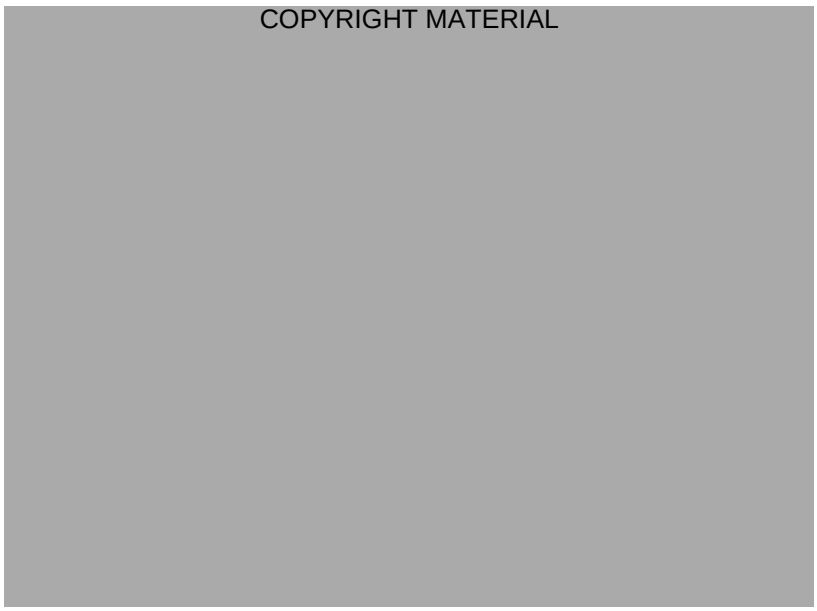


Figure 1: An Eadie-Hofstee plot for 3-hydroxypilocarpine formation from pilocarpine in human liver microsomes. Pooled human liver microsomes were incubated with 0.125 to 25 μM pilocarpine at 37°C for 60 min. Each data point represents the mean of duplicate determinations.

The formation of 3-hydroxypilocarpine in human liver microsomes was significantly inhibited (> 90%) by 200 μM coumarin. Other selective inhibitors of CYP1A2 (furafylline and α-naphthoflavone), CYP2C9 (sulfaphenazole), CYP2C19 [(S)-mephenytoin], CYP2E1 (4-methylpyrazole), CYP2D6 (quinidine), and CYP3A4 (troleandomycin) had a weak inhibitory effect (< 20%) on the formation. The highest formation activity was expressed by recombinant CYP2A6. The Km value for recombinant CYP2A6 was 3.1 μM, and this value is comparable with that of human liver microsomes (1.5 μM). The pilocarpine 3-hydroxylation activity was correlated with coumarin 7-hydroxylation activity in 16 human liver microsomes (r = 0.98). These data indicated that CYP2A6 is the main enzyme responsible for the 3-hydroxylation of pilocarpine.

It is established that pilocarpine is an inhibitor of CYP2A6 (i.e., coumarin 7-hydroxylase activity; Ki = 1–4 μM). Therefore, pilocarpine is not only a substrate of CYP2A6 but also an inhibitor for CYP2A6.

Summary of Pilocarpine Metabolism:

One pilocarpine metabolite, pilocarpic acid, is produced by the cleavage of the pilocarpine lactone ring. The enzyme responsible for the formation of pilocarpic acid has been characterized as a cation-dependent esterase present in serum and other organs. The authors in the article confirmed and identified another pilocarpine metabolite to be 3-hydroxypilocarpine in human urine after the oral administration of pilocarpine. Pilocarpine, pilocarpic acid, and 3-hydroxypilocarpine were excreted in the urine following pilocarpine oral administration.

CYP2A6 is the main enzyme responsible for the 3-hydroxylation of pilocarpine. Pilocarpine is an inhibitor of CYP2A6 (i.e., coumarin 7-hydroxylase activity; Ki = 1–4 μM) and a substrate of CYP2A6 with a Km value of 1.5 μM in human liver microsomes.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-200890	ORIG-1	ALCON INC	PILOCARPINE HYDROCHLORIDE OPHTHALMIC SOLUTION, 1%, 2% AND 4%

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/s/

YONGHENG ZHANG
05/21/2010

CHARLES R BONAPACE
05/21/2010