CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212520Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	215520
Supporting document/s:	0001
Applicant's letter date:	09-16-2019
CDER stamp date:	09-16-2019
60-Day Filling date:	11-15-2019
74-Day Letter date:	11-29-2019
Review type:	Standard
PDUFA date:	07-16-2020
Product:	Oxymetazoline HCl Ophthalmic Solution, 0.1% (RVL-1201)
Indication:	Treatment of acquired blepharoptosis
Applicant:	RevitaLid, Inc.
Review Division:	DPT-ORPURM-OSM in support of the DO (Division of Ophthalmology)
Reviewer:	Muriel Saulnier, DVM, PhD, DABT, DPT-ORPURM-OSM
Supervisor/Team Leader:	Lori Kotch, PhD, DABT, DPT-ORPURM-OSM
Division Director:	Mukesh Summan, PhD, DPT-ORPURM-OSM
Project Manager:	Jacquelyn Smith

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 215520 are owned by RevitaLid, Inc. or are data for which RevitaLid, Inc. has obtained a written right of reference.

Any information or data necessary for approval of NDA 215520 that RevitaLid, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 215520.

TABLE OF CONTENTS

APPEARS THIS WAY ON ORIGINAL

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

RevitaLid, Inc. has developed RVL-1201 (oxymetazoline hydrochloride ophthalmic solution, 0.1%) for the treatment of acquired blepharoptosis (ptosis). The proposed clinical dose for RVL-1201 is 1 drop of 35 µL per eye once daily, equivalent to approximately 35 µg/eye/day or 70 µg/2 eyes/day (i.e. 1.16 µg/kg/day equivalent to 43 µg/M^2/day for a 60 kg subject, Maximum Recommended Human Dose or MRHD).

RevitaLid is pursuing the 505(b)(2) approval pathway proposing the Listed Drug (LD) RHOFADE™ (oxymetazoline HCl) Cream reviewed in NDA 208552[1] as the reference compound.

The Applicant indicated that oxymetazoline is a "direct-acting α2A-adrenergic agonist" whereas RHOFADE™[1] is classified as an α1A-adrenoceptor agonist.

The Applicant's hypothesis for the pharmacological effect in ptosis is by stimulation of α2A-adrenergic receptors in the Müller's muscle of the upper eyelid where they are the predominant sub-type in humans.

To fulfill the regulatory requirements RevitaLid has submitted 2 original toxicity studies in the rabbit, published literature for the pharmacology and toxicology of oxymetazoline, and a comparative bioavailability study in humans that bridges RVL-1201 to RHOFADE™ (Study RVL-1201-PKP01).

Study RVL-1201-PKP01 was a single dose, 2-treatment, 2-period, 2-sequence-study in 24 healthy volunteers who were administered either 1 drop of RVL-1201 to each eye (35 µg/eye) in Treatment A, or 0.3g (MRHD) RHOFADE™ applied to the entire face in Treatment B (7 days between Treatments A and B). Oxymetazoline C_{max} and AUC_{inf} values following RVL-1201 were 30.5 pg/mL and 468 pg•h/mL for RVL-1201 compared to 47.6 pg/mL and 950 pg•h/mL for RHOFADE™.

Thus, the systemic exposure to oxymetazoline at the proposed clinical dose of RVL-1201 was less than the exposure at the MRHD for RHOFADE™, thereby establishing a bridge between the 2 products.

---

[1] https://www.accessdata.fda.gov/scripts/cder/ob/index.cfm

1.2 Brief Discussion of Nonclinical Findings

Pharmacology
In vitro functional and binding studies with human cells indicated that oxymetazoline has affinity and potency for several subtypes of alpha adrenoreceptors including α1A- and α2A- adrenoreceptors. These receptors are widely distributed in different tissues in all animal species, including in the eye. There is species specificity in the amount and location of the different adrenoreceptor subtypes.

In the human eye, α2 adrenergic receptors are the predominant subtype (especially α2A) in the Mueller's muscle of the upper eyelid, and both agonist binding of the α1 and α2 adrenergic receptors at this location can mediate muscle contraction. The α1 adrenergic receptor was also found to play a role in canine and murine models of ptosis.
At clinically relevant dosages, oxymetazoline was found to produce mydriasis (rabbit) and decrease intraocular pressure (IOP) (rabbit and monkey). Mydriasis and decrease IOP have not been observed with topical ocular administration of RVL-1201 in clinical studies so far.

In safety pharmacology studies in rats, a single intravenous (IV) clinically relevant dose of oxymetazoline significantly increased blood pressure (BP) and lowered heart rate (HR) shortly after the injection. No significant changes in BP or HR have been observed with topical ocular administration of RVL-1201 in clinical studies so far. Further, this effect is easily monitorable in patients.

Pharmacokinetics/ADME
After topical binocular administration of 12.5 µg/eye in New Zealand White rabbits, oxymetazoline distributed mostly to the external ocular tissues reaching a peak after 1 hour, penetrating poorly the cornea. Twenty-three per cent of the dose was excreted by the kidney including 33% in an unchanged form. Among rat, rabbit and human, the rabbit metabolized oxymetazoline the most extensively. In all species, the metabolism has the potential to generate 2 reactive intermediate species. No unique human metabolite was found.

Toxicology
The toxicological profile of chronic RVL-1201 exposure was assessed in a GLP-compliant 26-week ocular toxicity study in New Zealand White rabbits with topical administration 2 times *per* day (BID) and 3 times *per* day (TID) and a 4-week post-treatment recovery period. This study included IOP measurement, ophthalmic examination of the anterior and posterior segments by slit-lamp biomicroscopy and indirect ophthalmoscopy, respectively, and analysis of the electrophysiological function of the retina by dark-adapted electroretinography (ERG).

Under the conditions of this study, a NOAEL of 105 µg/eye/day provided a local safety margin of 3X based on a MRHD of 35 µg/eye/day or 70 µg/2 eyes/day. In term of systemic toxicity, the safety margin was 32.4 based on a MRHD of 1.16 µg/kg/day for a 60 kg subject. At the NOAEL at Day 182, systemic oxymetazoline exposures achieved were 1516 and 1984 pg/mL for C_{max} and 10484 and 14889 pg.h/mL for AUC_{0-24} in male and female rabbits, respectively. Incidences of findings of fibrin in the anterior chamber of the eye were related to drug treatment- and procedure (ERG). They were reversible.

Oxymetazoline was not found to be genotoxic or carcinogenic.

Oxymetazoline was a reproductive and developmental toxicant in the rat and a developmental toxicant in the rabbit. Male and female fertility in the rat was affected at doses as low as 0.03, and 0.01 mg/kg/day subcutaneously (Human Equivalent Dose or HED of 4.9 and 1.6 µg/kg/day, based on Body Surface Area or BSA conversion) in males and females, respectively. Developmental toxicity was observed in the rabbit and the rat fetuses in the presence of maternal toxicity. However, in the rat, the structural abnormality findings in fetuses from dams treated with 0.1 mg/kg/day subcutaneously (HED of 16 µg/kg/day, based on BSA conversion) could not be solely attributed to maternal toxicity. The no-effect level of oxymetazoline for post-natal pup growth and development in the rat was 0.05 mg/kg/day subcutaneously (HED of 8.1 µg/kg/day, based on BSA conversion). Oxymetazoline was excreted into the milk of lactating rats.

1.3 Recommendations

1.3.1 Approvability

Reliance on NDA 212520 - RHOFADE™ provides nonclinical safety support for RVL-1201 at the proposed clinical dose and indication. Additionally, there is extensive clinical experience with oxymetazoline. This reviewer considers RVL- 1201 to be approvable from a toxicology perspective.

1.3.2 Additional Non-Clinical Recommendations

None.

1.3.3 Labeling

The preclinical information proposed by Applicant was consistent with that from RHOFADE™ PI 2017, except for the pharmacological classification indicated in “12 Clinical Pharmacology 12.1 Mechanism of action”. This part has been modified by Applicant.

APPEARS THIS WAY ON ORIGINAL

Sponsor's proposed text with Reviewer's recommendations in red	Reviewer's additional comments

APPEARS THIS WAY ON ORIGINAL

8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Risk Summary [Rhofade PI 2017] There are no available data on PROPRIETARY NAME ~~RHOFADE~~ use in pregnant women to inform a drug-associated risk for major birth defects and miscarriage. **A literature article describing intranasal decongestant use in pregnant women identified a potential association between second-trimester exposure to oxymetazoline (with no decongestant exposure in the first trimester) and renal collecting system anomalies [*see Data*].** In animal reproduction studies, there were no adverse developmental effects observed after oral administration of oxymetazoline hydrochloride in pregnant rats and rabbits at systemic exposures up to 7 and 278 times the MRHOD (maximum recommended human ophthalmic dose), respectively, based on dose comparison. [*see Data*]. The estimated background risks of major birth defects and miscarriage for the indicated population are unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively. **<u>Clinical Considerations</u>** **[Rhofade PI 2017, Kuang 2018]** ***Fetal/Neonatal Adverse Reactions*** **Following repeated use of oxymetazoline hydrochloride solution nasal spray for the treatment of nasal congestion at a dose 5 times higher than recommended, one case of fetal distress was reported in a 41-week pregnant patient. The fetal distress resolved hours later, prior to the delivery of the healthy infant.** **<u>Data</u>** **[Rhofade PI 2017]** ***Human Data*** **No adequate and well-controlled trials of PROPRIETARY NAME ~~RHOFADE~~ have been conducted in pregnant women. Across all clinical trials of PROPRIETARY NAME ~~RHOFADE~~, two pregnancies were reported. One**	PT defers to the clinical team regarding the adequacy of the clinical data (bolded text) for inclusion in the labeling. Applies to all bolded sections below. <u>Clinical Considerations:</u> PT defers to the clinical team regarding the adequacy of the data and inclusion of this section in the label. If text is retained, exposure margins in last sentence will need to be recalculated, based on dose (if that can be derived from label data). *Human Data* We defer to clinical team regarding the adequacy of the data and inclusion of this section in the label. If retained trade name should be inserted.

pregnancy resulted in the delivery of a healthy child. One pregnancy resulted in a spontaneous abortion, which was considered to be unrelated to the trial medication. A literature article summarizing the results of exploratory analyses of intranasal decongestant use during pregnancy identified a potential association between second-trimester exposure to oxymetazoline hydrochloride solution (with no decongestant exposure in the first trimester) and renal collecting system anomalies.

Animal Data
Effects on embryo-fetal development were evaluated in rats and rabbits following oral administration of oxymetazoline hydrochloride during the period of organogenesis. Oxymetazoline hydrochloride did not cause adverse effects to the fetus at oral doses up to 0.2 mg/kg/day in pregnant rats during the period of organogenesis (28 times the MRHOD, on a dose comparison basis). Oxymetazoline hydrochloride did not cause adverse effects to the fetus at oral doses up to 1 mg/kg/day in pregnant rabbits during the period of organogenesis (278 times the MRHOD, on a dose comparison basis). Maternal toxicity, including decreased maternal body weight, was produced at the high dose of 1 mg/kg/day in pregnant rabbits and was associated with findings of delayed skeletal ossification.
In a rat prenatal and postnatal development study, oxymetazoline hydrochloride was orally administered to pregnant rats once daily from gestation day 6 through lactation day 20. Maternal toxicity was produced at the high dose of 0.2 mg/kg/day (28 times the MRHOD, on a dose comparison basis) in pregnant rats and was associated with an increase in pup mortality and reduced pup body weights. Delayed sexual maturation was noted at 0.1 ~~and 0.2~~ mg/kg/day (14 times the MRHOD on a dose comparison basis).
Oxymetazoline hydrochloride did not produce adverse effects on fetal development at a dose of 0.05 mg/kg/day (7 times the MRHOD, on a dose comparison basis).

8.2 Lactation
Risk Summary
{Rhofade PI 2017}
No clinical data are available to assess the effects of oxymetazoline on the quantity or rate of breastmilk production, or to establish the level of oxymetazoline present in human breastmilk post-dose. Oxymetazoline was detected in the milk of lactating rats. The developmental and health benefits of breastfeeding should be considered

along with the mother's clinical need for PROPRIETARY NAME ~~RHOFADE~~ and any potential adverse effects on the breastfed child from PROPRIETARY NAME ~~RHOFADE~~ ~~or from the underlying maternal condition~~.	
12 CLINICAL PHARMACOLOGY **12.1 Mechanism of Action** [Module 2.7.3 Section 1] Oxymetazoline is an alpha adrenoceptor agonist targeting a subset of adrenoreceptors in Mueller's muscle of the eyelid. ~~Oxymetazoline acts as a vasoconstrictor.~~	*This reviewer suggests changing the wording to* ***"oxymetazoline is an alpha adrenoceptor agonist targeting a subset of adrenoreceptors in Mueller's muscle of the eyelid."*** *Rationale for change of MoA: It is this reviewer's opinion that not enough evidence has been collected to infer that the α2A-adrenergic receptors in Mueller's muscle of the eyelid are solely responsible for the pharmacological effect in the proposed indication.*
13 NONCLINICAL TOXICOLOGY [Rhofade PI 2017] **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** <u>Carcinogenesis</u> Oxymetazoline hydrochloride was not associated with an increased incidence of neoplastic or proliferative changes in transgenic mice given oral doses of 0.5, 1.0, or 2.5 mg/kg/day oxymetazoline hydrochloride for 6 months. <u>Mutagenesis</u> Oxymetazoline hydrochloride revealed no evidence of mutagenic or clastogenic potential based on the results of two *in vitro* ~~in vitro~~ genotoxicity tests (Ames assay and human lymphocyte chromosomal aberration assay) and one *in vivo* ~~in vivo~~ genotoxicity test (mouse micronucleus assay). <u>Impairment of Fertility</u> Effects on fertility and early embryonic development were evaluated in rats following oral administration of 0.05, 0.1, or 0.2 mg/kg/day oxymetazoline hydrochloride prior to and during mating and through early pregnancy. Decreased number of	*It is this reviewer's opinion that the final sentence in the fertility statement is only partially correct, and the*

corpora lutea and increased post-implantation losses were noted at 0.2 mg/kg/day oxymetazoline hydrochloride (28 times the MRHOD, on a dose comparison basis). However, no treatment related effects on ~~fertility or~~ mating parameters were noted at 0.2 mg/kg/day oxymetazoline hydrochloride	*strikethrough words should be deleted (despite being in the label for Rhofade™), as they are not supported by the submitted data.*

Systemic Exposure Margins to Support Label Calculations

Clinical Exposure Margins (Based on Dose)			
Species/Type of Study	NOAEL or LOAEL (mg/kg/day)	HED (mg/kg/day#)	Exposure Margins based on a MRHOD of 0.070 mg/day = 0.00116 mg/kg*
Rabbit EFD	NOAEL= 1 mg/kg/day	0.323	278X
Rat EFD	NOAEL= 0.2 mg/kg/day	0.032	28X
Rat Fertility	LOAEL= 0.2 mg/kg/day	0.032	28X
Rat PPND	LOAEL= 0.1 mg/kg/day	0.016	14X
	NOAEL = 0.05 mg/kg/day	0.008	7X

NOAEL = no-observed-adverse-effect level
LOAEL = low-observed-adverse-effect level
MRHOD = maximum recommended human ophthalmic dose
HED = human equivalent dose
* based on a 60 kg adult

2 Drug Information

2.1 Drug

Trade Name

(b) (4) were proposed in IND 116915 in May 2019 and are pending FDA recommendation.

Nomenclature
Oxymetazoline Hydrochloride.

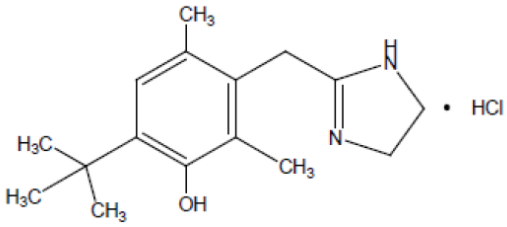
Code Name
RVL- 1201

Chemical Names
3- [4,5-Dihydro-1H-imidazol-2-yl) methyl] -6-(1,1- dimethylethyl)-2,4-dimethylphenol monohydrochloride;

Phenol,3-[(4,5-dihydro-1H-imidazol-2-yl) methyl]-6-(1,1- dimethylethyl)-2,4-dimethyl-, monohydrochloride;

6-tert-Butyl-3-(2-imidazolin-2-ylmethyl)-2,4- dimethylphenol monohydrochloride

Structure/Molecular Weight



MW 296.84

Molecular Formula
$C_{16}H_{24}N_2O \cdot HCl$

Pharmacologic Class
α-adrenergic agonist.

2.2 Relevant INDs, NDAs, and DMFs

IND 116915
NDA 208552[1]: LD
DMF # for the LD: (b) (4)

---

[1] https://www.accessdata.fda.gov/scripts/cder/ob/index.cfm

Product Details for NDA 208552

RHOFADE (OXYMETAZOLINE HYDROCHLORIDE)
1% Marketing Status: Prescription

Active Ingredient: OXYMETAZOLINE HYDROCHLORIDE
Proprietary Name: RHOFADE
Dosage Form; Route of Administration: CREAM; TOPICAL
Strength: 1%
Reference Listed Drug: Yes
Reference Standard: Yes
TE Code:
Application Number: N208552
Product Number: 001
Approval Date: Jan 18, 2017
Applicant Holder Full Name: ACLARIS THERAPEUTICS INC
Marketing Status: Prescription
Patent and Exclusivity Information (patent_info.cfm?Product_No=001&Appl_No=208552&Appl_type=N)

2.3 Drug Formulation

The drug product, oxymetazoline HCl ophthalmic solution, 0.1% is a clear, colorless to slightly yellow, aseptically prepared, preservative-free, sterile solution filled into clear, unit dose, blow/fill/seal single-use containers. The osmolality range of the solution is (b) (4) mOsm/kg, and the pH is adjusted to 5.8-6.8. The composition of the formulation is indicated in Table 1.

Table 1: RVL- 1201 Formulation (copied from Applicant's report)

Component	Quality Standard	Quantity (mg/mL)	Quantity (mg per vial)	Function
Oxymetazoline Hydrochloride	USP	1.00	(b) (4)	Active pharmaceutical ingredient
Sodium Chloride	USP	(b) (4)		(b) (4)
Potassium Chloride	USP			
Calcium Chloride, (b) (4)	USP			
Magnesium Chloride (b) (4)	USP			
Sodium Acetate (b) (4)	USP			
Tri-Sodium Citrate, (b) (4)	USP			
Hypromellose (b) (4)	Premium			
Hydrochloric Acid	NF			
Water for Injection	USP			
(b) (4)	NF			

2.4 Comments on Novel Excipients

They are no novel excipients. All inactive ingredients are within the range found in the FDA Inactive Ingredient Database (IID). The Applicant stated that the same formulation was used for all non-clinical, clinical, and registration batches.

2.5 Comments on Impurities/Degradants of Concern

All impurities except (b) (4) do not exceed the ICH Q3B(R2)[2] qualification threshold and are less than the threshold of toxicological concern (TTC) as described in ICH M7(R1)[3] (Table 2).

Table 2: RVL-1201 Specified Drug Product Impurities (copied from Applicant's report)

Impurity	Specification	Comments
Oxymetazoline-related (b) (4)	NMT (b) (4)	Specification is above the ICH Q3B qualification threshold of 1%; qualified for safety in 26-week ocular toxicity study. ICH M7-compliant QSAR evaluation (Gad Evaluation for RCA) indicates that there are no structural alerts, or there is an alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity (ICH M7 Class 5); considered non-mutagenic.
Other individual known oxymetazoline-related impurities:		
(b) (4)	NMT (b) (4)	Specification does not exceed the ICH Q3B qualification threshold of 1%. ICH M7-compliant QSAR evaluation (Gad Evaluation for other impurities) indicates that there are no structural alerts, or there is an alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity (ICH M7 Class 5); considered non-mutagenic. The specification for these impurities is below the TTC of 2.5 1.5 µg/day at the proposed clinical dose of 70 µg/day.[a]
Individual unknown impurities	NMT (b) (4)	Specification does not exceed the ICH Q3B reporting threshold.

NMT = not more than; QSAR = quantitative structure-activity relationship; TTC = threshold of toxicological concern as defined in ICH M7(R1)

[a] Clinical RVL-1201 dose based on one 35 µL drop of 0.1% oxymetazoline HCl per eye once daily.

---

[2]ICH Q3B(R2): Impurities in New Drug Products, July 2006.

[3]ICHM7(R1): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, February 2018.

Oxymetazoline-related [redacted] is a non-mutagenic impurity with a specification above the ICH Q3B qualification threshold of ≤1.0% for a maximum daily dose of less than [redacted] mg (the total daily clinical dose of oxymetazoline from RVL- 1201 is 70 µg for a 60 kg human).

The Applicant has proposed that [redacted] (b) (4) has been qualified in the 26-week ocular toxicity in the rabbit Study No. 74041B. The safety margins for oxymetazoline-related [redacted] (b) (4) showed that the local safety margin was less than [redacted] on a mg/eye/day basis, and the systemic safety margin was [redacted] based on doses normalized to body surface area (Table 3).

Table 3: Safety Margins for Oxymetazoline-Related [redacted] (b) (4) (copied from Applicant's report)

[redacted] Dose at NOAEL[a]		Clinical [redacted] Dose[bc]		Safety Margins[d]	
Local (µg/eye/day)	**Systemic (µg/m²/day)**	**Local (µg/eye/day)**	**Systemic (µg/m²/day)**	**Local**	**Systemic**
[redacted] (b) (4)					

[a] Based on the NOAEL from Study 74041B, a 26-week ocular toxicity study of RVL-1201, and an estimated level of [redacted] (b) (4) during the last 3 months of the study of at least [redacted] (b) (4) % (see below for further details).

[b] Based on the proposed clinical dose of one 35uL drop of RVL-1201 (oxymetazoline HCl, 0.1%) per eye once daily and the maximum proposed level of [redacted] (b) (4)

[c] Total daily dose of [redacted] (b) (4)

[d] Local and systemic animal dose at the NOAEL divided by the local and systemic clinical dose, respectively.

Nevertheless, RevitaLid has considered [redacted] (b) (4) qualified for safety at the proposed clinical dose of 1 drop *per* eye *per* day at a specification of ≤ [redacted] % and proposed the following impurity thresholds (Table 4).

Table 4: Proposed Impurity Thresholds (copied from Applicant's report)

						Test Article: RVL-1201
Batch No.	**Purity (%)**	**Specified Impurities (%)**			**Study Number**	**Type of Study**
PROPOSED SPECIFICATION:	[redacted] (b) (4)	[redacted] (b) (4)	Individual Known	Individual Unknown		
		NMT [redacted] (b) (4)	NMT [redacted] (b) (4)	NMT [redacted] (b) (4)		
R60701	[redacted] (b) (4)				74041B	26-week ocular toxicity in rabbits

NMT = not more than; ND = not detected

[a] Based on certificate of analysis included in the study report.

[b] Based on stability data generated at 9, 12, 14, and 18 months for drug product Batch R60701 samples for Related Compounds analyzed between 11/22/2017 and 7/18/2018 (rabbits treated from 12/18/2017 to 6/20/2018). Lowest and highest values during these test intervals are provided.

[c] For safety qualification purposes, [redacted] (b) (4) was estimated to be at least [redacted] % for the final 3 months of the 26-week rabbit study.

The following IR was sent to the Applicant by CMC in December 2019:

"Regarding the drug product specification, we note that acceptance criterion for Related (b) (4) is NMT (b) (4)% at stability, however the limit is above ICH Q3B qualification threshold of 1.0%. We acknowledge that you provided justification using 26-week ocular toxicity study to qualify the limit. However, according to the study, the local safety margin is only (b) (4), which is an issue. We recommend that you tighten the limit to NMT (b) (4)% to give a safety margin (b) (4) Please update the corresponding sections in NDA accordingly."

On 02-19-20, CMC informed the Division that Applicant has agreed to tighten (b) (4) (b) (4)specification to the recommended level.

2.6 Proposed Clinical Population and Dosing Regimen

The drug product is indicated for the treatment of acquired blepharoptosis (droopy eyelid) in adults and adolescents at the dose of 1 drop of 35 µL oxymetazoline hydrochloride ophthalmic solution, 0.1% into each eye daily.

2.7 Regulatory Background

Oxymetazoline was first approved by FDA in 1964 and commonly used as a vasoconstrictor in over the counter (OTC) eye drops at a concentration of 0.025% (Visine L.R.®; Johnson & Johnson Healthcare Products, Inc., Division of McNeil PPC, Inc.), and in nasal spray at 0.05% (Afrin, Nostrilla, other names; MSD Consumer Care Inc., Insight Pharmaceuticals, multiple other manufacturers). Oxymetazoline hydrochloride 1% cream (RHOFADE™, Allergan) was approved in 2017 for topical use to treat persistent facial erythema associated with rosacea.

RevitaLid is relying on FDA's prior findings of safety for the listed drug RHOFADE™ regarding the potential for RVL-1201 to induce genotoxicity, carcinogenicity, and reproductive and developmental toxicities (Prescribing Information (PI) dated 2017) but does not own a right of reference to NDA 208552 for RHOFADE™.

RevitaLid's reliance on the prior findings of safety for RHOFADE™ is scientifically justified based on the results of a clinical comparative bioavailability study (RVL-1201-PKP01) in which the systemic exposure to oxymetazoline from the proposed clinical dose of RVL-1201 was less than the exposure from a clinical dose of topically applied RHOFADE™

based on plasma C_{max} and AUC values. Thus, a bridge has been established between RVL-1201 and the listed drug in accordance with FDA suggestion at a Type C meeting with the Applicant (minutes dated May 2, 2018).

Several prior "Pharmacology and Toxicology Correspondences" with the Division relevant to NDA 212520 submission took place, for which the minutes have been archived in DARRTS. These are:

- Document by Maria Rivera, PhD, dated 06-13-2014:
This was an "End of Phase 2" meeting requested by RevitaLid, Inc. (RevitaLid) to discuss the development program and regulatory requirements for approval of RVL- 1201 (Oxymetazoline Ophthalmic Solution 0.1%).

Dr. Rivera notified sponsor that "for NDA submission, a repeat-dose ocular toxicity study of 6 month duration was recommended to support a clinical treatment duration greater than 1 month […] as per ICH Guidance for Industry M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatory Information/Guidances/UCM073246.pdf)."

- Document by Maria Rivera, PhD dated 02-24-2017:
This was a "Type C" meeting to discuss the results of the Phase 3 clinical study, further development program, and regulatory requirements for submission and filing of an NDA for the (b) (4) of RVL-1201 Ophthalmic Solution in the treatment of acquired blepharoptosis.

Dr. Rivera notified sponsor that "as per M3(R2) ICH Guidance for Industry, a repeat-dose ocular toxicity study of 6-month duration was recommended to support a clinical treatment duration greater than 1 month and up to 3 months. Therefore, the current response included similar recommendations to those conveyed to the sponsor previously after review of the End of Phase 2 briefing document."

- Document by Maria Rivera, PhD dated 03-26-2018:
RevitaLid requested a Type C meeting to reach agreement on the remaining portions of the development program to support submission of a 505(b)(2) NDA for the use of RVL-1201 in the treatment of acquired blepharoptosis.

RevitaLid was seeking FDA agreement that the ongoing 26-week ocular toxicity study in rabbits, along with the previously submitted 28-day rabbit toxicity study and published literature for pharmacology/toxicology are adequate to support a 505(b)(2) NDA for RVL-1201.

Dr. Rivera noted that the sponsor "will need to be able to bridge (e.g. via comparative PK or dose) any published nonclinical safety data that […]" the sponsor "plans to rely on to fulfill nonclinical requirements."

- Document by Maria Rivera, PhD dated 05-24-2019:
RevitaLid requested a Pre-NDA meeting to discuss the structure and content of the NDA application to ensure that it meets the Division's expectations and is accepted for filing.

At the meeting Dr. Rivera reminded sponsor that "all nonclinical elements should be provided, either directly (original studies or published literature) or by relying on the FDA's findings of safety and effectiveness for a listed drug. If literature […]" was "being relied upon to support the NDA, to include a summary of all published nonclinical literature being relied upon and a copy of all publications cited. The nonclinical summary is typically organized to address each of the nonclinical elements (e.g. pharmacology, pharmacokinetics, ocular toxicity, systemic toxicity, genotoxicity, reproductive toxicity, carcinogenicity, etc.). See Guidance for Industry M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals for further information regarding required nonclinical elements. (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation /Guidances/UCM073246.pdf)"

3 Studies Submitted

The following original toxicity studies were conducted by Applicant and submitted to the Agency to support the approval of RVL-1201:

Type of Study	Route	Species/ Strain	Duration of Dosing	Compounds Administered	GLP	Study Number
Repeat dose toxicity	Ocular, topical	NZW rabbits	28 days	RVL-1201	Yes	12C145Q2R3G25
	Ocular, topical	NZW rabbits	26 weeks	RVL-1201	Yes	74041B

Preclinical data for genotoxicity, carcinogenicity, and reproductive and developmental toxicities were extracted from the label for RHOFADE™ PI 2017.

Applicant has also submitted summaries accompanied by copies of complete literature articles in support of the pharmacology, safety pharmacology, PK/ADME, and toxicology

sections of the pre-clinical development of their product as indicated for a 505(b)(2) application.

3.1 Studies Reviewed

Study 74041B: RVL-1201: A 26-Week Topical Ocular Toxicity Study of Oxymetazoline Hydrochloride Ophthalmic Solution, 0.1% with a 4-Week Recovery Period in New Zealand White Rabbits.

3.2 Studies Not Reviewed

Study 12C145Q2R3G25.

3.3 Previous Reviews Referenced

Study 12C145Q2R3G25 was reviewed by Maria Rivera, PhD and report filed in DARRTS on 01-27-2013.

4 Pharmacology

No original pharmacology studies were submitted. The Applicant has relied for this section on the published literature and the product label information for RHOFADE™. The Applicant's methodology for the literature search appeared to be adequate.

4.1 Primary Pharmacology

The Applicant' s hypothesis is that the ability of oxymetazoline to enhance adrenergic activity in the Mueller's muscle *via* stimulation of both α2A- and α1A-adrenoreceptors is the most likely explanation for its efficacy in treating ptosis.

4.1.1 Role of α1 and α2 adrenoreceptors in ptosis

- Esmaeli-Gutstein, 1999
The Mueller's muscle in the upper eyelid plays a modest role in elevating the eyelid, and a malfunction in this muscle can lead to blepharoptosis. Both α- and β-adrenoceptors have been identified in Mueller's muscle from human patients using immunohistochemical staining in the following descending order of prevalence: α2 (specifically α2A) >> β1 > α1. The authors concluded that the interactions between the α1 and the β2 receptors in the upper eyelid retractor muscles may contribute to the development of dysthyroid eyelid retraction.

-Garibaldi, 2006

The predominant influence of the α2-adrenoceptor on controlling Mueller's muscle was demonstrated in a small number of patients who showed dramatic resolution of ptosis after ocular instillation of apraclonidine, a selective α2-receptor agonist with weak α1 activity.

4.1.2 *In vitro*

- Haenisch, 2010

Oxymetazoline was shown to have high capacity to displace the binding of selective α-adrenoceptor antagonists from α-adrenoceptor subtypes transiently expressed in human embryonic kidney 293 (HEK293) cells. Oxymetazoline showed the highest affinity for the α_{1A}, α_{2C}, α_{1B}, and α_{2A} adrenoceptor subtypes with corresponding IC_{50} (concentration at 50% of maximum inhibition) values of 0.02, 0.13, 0.25, and 0.58 μM, respectively (Table 5).

Table 5: IC_{50} Values for Oxymetazoline in the Binding Displacement of α−Adrenoceptor Antagonists (copied from Applicant's report)

IC_{50} (μM)					
α-adrenoceptor subtype					
α_{1A}	**α_{1B}**	**α_{1D}**	**α_{2A}**	**α_{2B}**	**α_{2C}**
0.02	0.25	4.1	0.58	5.8	0.13

[^{3}H]prazosin was the α_1-adrenoceptor antagonist

[^{3}H]RX821002 was the α_2-adrenoceptor antagonist

In functional assays, oxymetazoline appeared to be a highly potent full agonist at α2B-adrenoceptors and a partial agonist at α1A-adrenoceptors with a potency at the latter receptor much lower than its affinity.

- Sudgen, 1996

In an early study with rat pineal gland membranes, oxymetazoline was shown to competitively inhibit radioligand binding at α1-adrenoceptors, suggesting oxymetazoline may bind these receptors.

- Yano, 2010

In an isolated canine model of upper eyelid preparation, selective α1A-adrenoceptor stimulation was shown to induce a sustained contraction of Mueller's muscle.

4.1.3 *In vivo*

Deniard, 1983

In this early study, the relative potency and selectivity of several predominantly α2 or α1-adrenoceptor agonists were evaluated for their capacity to reverse reserpine-induced

ptosis in male mice. All agonists produced dose-related reversal of ptosis that was inhibited in the presence of α-adrenoceptor antagonists such as yohimbine and prazosin.

Although oxymetazoline was not explicitly investigated in this study, the results of this study suggested that α1 adrenoreceptors played a predominant role in this mouse model of ptosis.

4.2 Secondary Pharmacology

4.2.1 Effects in the eye

4.2.1.1 *In vitro*

- Ishikawa, 1996

Alpha-adrenoceptors are widely distributed in the eye, especially in the smooth muscle cells of the iris, the blood vessels of the conjunctiva as well as those of the ciliary processes and the aqueous outflow tract where α2-adrenergic receptors may play a role in the mediation of ocular vascular and muscular tones.

In this article, the authors compared post-junctional α-adrenoreceptors in iris dilator muscles of humans, and albino and pigmented rabbits.

Oxymetazoline induced contractile responses in albino and pigmented rabbits, and in human iris dilator muscles in a concentration-dependent manner with maximum % contraction (E_{max}) values of 72%, 80%, and 48%, respectively. The effects were reversed by an adrenergic antagonist such as prazosine. Oxymetazoline concentrations eliciting 50% of E_{max} (EC_{50}) were 142, 62, and 189 nM (or 42, 62, and 56 ng/mL, respectively) (Table 6).

The authors suggested that the post-junctional α-adrenoreceptors in iris dilator of humans may be like that in pigmented rabbit iris, i.e. characterized by low affinity to prazosine whereas the subtype of α-adrenoreceptor in the albino rabbit iris dilator appeared to be of high affinity to prazosin.

Table 6: Oxymetazoline-Induced Contractile Responses in the Iris Dilator Muscle (extracted from the article by Ishikawa, 1996)

EC_{50} (nmol/l) and Emax (% maximum contraction) values with 95% confidence limits of iris dilator after directly acting sympathomimetic drugs in the human and rabbit iris dilator. The average maximum tension induced by 100 μmol/l PE was 96±11 mg, 197±11 mg and 45±5 mg, in albino rabbit, pigmented rabbit and human iris dilator, respectively. *Emax*, the responses to PE (100 μmol/l) in the beginning of the experiment was considered as 100%

		Albino rabbit iris dilator		Pigmented rabbit iris dilator		Human iris dilator	
Phenylephrine control	EC_{50}	7900	(5800–11 100)	3570	(2440–5210)	1220	(970–1540)
	Emax	100%	(n=10)	100%	(n=11)	100%	(n=27)
Phenylephrine[a] with uptake blockers	EC_{50}	710	(480–1050)	710	(580–880)	1220	(860–1730)
	Emax	88%	(74–101, n=20)	81%	(69–103, n=16)	121%	(95–147, n=12)
Norepinephrine control	EC_{50}	9050	(5000–16 100)	3820	(1570–9290)	1310	(980–1760)
	Emax	125%	(78–171, n=7)	92%	(74–110, n=3)	99%	(87–111, n=4)
Norepinephrine[a] with uptake blockers	EC_{50}	99	(70–137)	121	(27–533)	195	(93–405)
	Emax	131%	(85–176, n=5)	93%	(68–119, n=3)	98%	(85–110, n=4)
Oxymetazoline	EC_{50}	142	(98–205)	62	(36–105)	189	(87–407)
	Emax	72%	(56–87, n=7)	80%	(67–89, n=6)	48%	(23–72, n=6)
P-aminoclonidine	EC_{50}	247	(189–323)	115	(65–202)	189	(80–441)
	Emax	51%	(29–72, n=5)	57%	(46–68, n=8)	36%	(16–62, n=7)
Naphazoline	EC_{50}	261	(117–578)	440	(180–1090)	1500	
	Emax	66%	(57–75, n=6)	80%	(74–84, n=3)	58%	(n=1)
Methoxamine	EC_{50}	2330	(2140–2540)	2750	(1160–6500)	740	
	Emax	90%	(67–112, n=5)	94%	(86–105, n=3)	100%	(n=1)

[a] pretreatment with cocaine, hydrocortisone and U-0521. Refer to the text for details

- Patil and Ishikawa, 2004

At 10 or 100 μM, oxymetazoline competitively blocked the myotic activity of carbachol on the isolated human iris sphincter or ciliary muscles. The IC_{50} for oxymetazoline was 61 μM while the dissociation constant (K_b) for both ciliary and iris was ~5.2 μM.

Based on these results, the investigators concluded that oxymetazoline is a weak antimuscarinic compound with potent α-adrenoceptor–mediated vasoconstrictive activity

and that the therapeutic benefit in ocular decongestion recovery may be partly related to its weak anticholinergic activity.

4.2.1.2 *In vivo*
Several of these models are further described in the section “Toxicology”

- Murray, 1985
Oxymetazoline was administered topically to the eyes of normal and sympathectomized (surgical excision of the superior cervical sympathetic ganglion) albino rabbits.

At 0.003, 0.03, 0.1, 0.3, and 3 mM (or 0.89, 8.9, 27, 89, and 890 µg/mL, respectively) administered to normal rabbits (6 per group) oxymetazoline caused an initial dose-related rapid IOP increase of up to 4.5 mm Hg at 0.3 mM (lasting for 15-30 minutes) but then IOP decreased from baseline over 8 hours.

Oxymetazoline also caused a dose-related increase in pupil diameter of up to 71% at 0.3 mM (the peak effect was attenuated at the highest dose of 3 mM). It is thought that these 2 actions are mediated by different α-adrenoreceptors.

The investigators postulated that the initial IOP rise may be due to stimulation of post-synaptic α-adrenergic receptors in the superficial episcleral and aqueous veins resulting in their constriction but was transient because drug was eliminated rapidly from these tissues. The subsequent decrease in IOP was thought to be due to reduction of the blood supply to the intra-scleral venous plexus.

In the sympathectomized rabbit model, 4 animals received 0.3 mM oxymetazoline in the sympathectomized eye and 4 received oxymetazoline in the eye contralateral to the sympathectomy. The response in sympathectomized eyes did not differ from that seen in normal eyes, but the early IOP rise in the treated eyes contralateral to the sympathectomy was absent or attenuated. These findings substantiated the hypothesis that postsynaptic α-adrenergic receptors were involved.

- Campbell ,1994; Campbell, 1995
The administration of oxymetazoline intracerebroventricularly (ICV) at 0.9 µg, 20 µL to 5 normotensive New Zealand White (NZW) rabbits resulted in bilateral ocular hypotension (maximum > 7.0 mm Hg) that peaked at 2 hours but had no effect on pupil diameter.

Unilateral topical ocular administration of oxymetazoline at 500 µg, 50 µL to 5 normotensive NZW rabbits resulted in bilateral ocular hypotension (maximum > 12 mm

Hg) that persisted more than 12 hours. Topical administration at this dose induced mydriasis of up to 3.3 mm with a duration of 60 minutes.

In a follow-up study, a single dose of oxymetazoline was administered to 5 normotensive NZW rabbits per group using the following doses, volumes and routes: ICV at 0.3, 0.9, 3.0 µg/20 µL to the lateral ventricle; 0.9 µg/20 µL to the third ventricle; 0.9 µg/1.0 µL to the rostral ventrolateral medulla (RVLM); topical ocular at 50 µg/50 µL.

Unilateral topical ocular application of oxymetazoline produced maximal, bilateral hypotensive responses from baseline of 6.4 (± 0.88) mm Hg ($P < 0.001$) and 8.7 (± 1.6) mm Hg ($P < 0.001$), at 2 hours in the contralateral and ipsilateral eyes, respectively. Intracerebroventricular and RVLM administration of oxymetazoline in this study also resulted in significant lowering of IOP when applied *via* each of those routes.

The oxymetazoline-induced ocular hypotension was alleviated by centrally-administered rauwolscine, an α2- adrenoceptor antagonist and efaroxan, an antagonist of the imidazoline receptor. These *in vivo* studies demonstrated that oxymetazoline could lower IOP through its agonistic activity on ocular and central adreno- and imidazole receptors.

- Chu, 1996
In a series of experiments with the NZW rabbit, Chu repeated Campbell's experiments and showed a dose-dependent decrease in IOP at topical doses of 7.5, 75, and 750 µg/eye oxymetazoline. Further, the authors noted that "oxymetazoline's ocular hypotensive effect was not totally dependent on intact sympathetic nerves and a major component resulted from activating post-junctional α2A- adrenoceptors of the ciliary epithelium".

- Wang, 1993
The authors demonstrated that in 8 glaucomatous monkeys (glaucoma induced by repeated argon laser photocoagulation of the mid-trabecular meshwork), 1 topical ocular application of oxymetazoline at 0.125%, 0.5%, 1.5% (or 62.5, 250, and 750 µg/eye), in a volume of 50µL significantly reduced IOP up to 6.0 mm Hg for up to 5 hours at the 2 higher concentrations ($p < 0.05$). The maximum effect on IOP was observed after application of 0.125% to 0.5% (50µL) test compound x2 *per* day for 5 days which resulted in a reduction of up to 7.0 (± 0.8) mm Hg for up to 16 hours. In this study (as in the rabbit studies above) it was shown that aqueous humor flow was concomitantly decreased.

- Nasal, 1995

In the Wistar rat, oxymetazoline at a single IV dose of 3000 µg/kg resulted in a weak mydriasis indicating that the "receptors determining mydriasis were not pure α2-adrenoreceptors".

- Xuan, 1997
"Short-term" and "long-term" conjunctivitis were induced in the NZW rabbit with histamine, and arachidonic acid, respectively. Animals with histamine-induced conjunctivitis received 0, 0.001%, 0.004%, and 0.016% (or 0, 0.25, 1, and 4 µg/eye, respectively) in a volume of 25 µL oxymetazoline topically in the eye, whereas arachidonic acid-induced conjunctivitis was treated with 0, 0.004%, 0.008%, and 0.016% (or 0, 1, 2, and 4 µg/eye, respectively) oxymetazoline.

In both models, all concentrations effectively decreased the inflammation in a dose-dependent fashion. The authors concluded that "oxymetazoline is a potent long acting decongestant (vasoconstrictive agent) for conjunctival red eye syndrome."

4.2.2 Effects on vascular smooth muscles
4.2.2.1 In vitro
- Wikberg-Matsson, 2001
In studies in isolated arteries from the intraocular part of the porcine ciliary artery, the α2-adrenergic agonist oxymetazoline was shown to be a potent vasoconstrictor with an EC_{50} of 5.26 nM (or 1.56 ng/mL) compared to noradrenaline (EC_{50} of 247 nM). The results suggested that it was an α2A-adrenoceptor subtype mediated action.

- Ruffolo, 1982
In rats and rabbits, oxymetazoline induced contraction in the aorta with a high degree of tissue selectivity, showing a higher potency relative to noradrenaline with the rabbit (relative potency 6.6; EC_{50} 11 nM or 3.27 ng/mL) than with the rat (relative potency 0.08; EC_{50} 220 nM or 65.3 ng/mL) aorta.

4.2.2.2 *In vivo*
- Malta, 1979
Oxymetazoline was shown in cats to cause an increase in hindlimb vascular resistance (demonstrating pre-junctional α-adrenoceptor activity), as well as reversal of partial tubocurarine blockade of contractions of the tibialis anterior muscles (demonstrating post-junctional activity). The effective intra-arterial doses for these effects were 1.9 and 21 µg, respectively. These potencies were ~17x lower than that of noradrenaline.

4.2.3 Effects in the gastro-intestinal tract

4.2.3.1 *In vitro*

- Liu, 1996

Oxymetazoline caused concentration-dependent inhibition of peristaltic contractions of isolated rat ileum with an IC_{50} of 4.23 nM or 1.26 ng/mL.

- Shujaa, 2011

Oxymetazoline inhibited electrically evoked contractions of the gastric fundus strip of the mouse with an EC_{50} of 4 nM or 1.19 ng/mL and this effect was reversed by an α2-adrenoceptor antagonist.

4.2.3.2 *In vivo*

- Fulop, 2005; Zadori, 2007

In the rat, oxymetazoline inhibited in a dose-dependent fashion, basal and stimulated gastric emptying after a SC or an IV dose of 0.17 to 6.8 µM/kg through activation of pre- and post-synaptic α2-adrenoceptors.

- Hikasa, 1992

Oxymetazoline induced dose-dependent emesis in dogs with an ED_{50} (dose at 50% of maximum effect) of 0.039 mg/kg IV; the effect was reversed by yohimbine, an α2-adrenoceptor antagonist, but not by prazosin, an α1-adrenoceptor antagonist.

4.2.3 Effects on the nasal mucosa and bronchopulmonary apparatus

- Hybinette, 1982

The intra-arterial administration of 0.001-10 µg/kg oxymetazoline to anesthetized white rabbits delayed mucociliary wave frequencies as measured with photoelectric oscilloscopy. In the dose-range 0.001-1 µg/kg (17 rabbits) the decrease in wave frequency was dose-dependent ($p < 0.01$), was observed within 30-60 seconds after injection, and the effect lasted for 5-15 minutes. It is thought that this may be due to a reduction in blood flow to the ciliated epithelium.

- Akerlund, 1993

The effect of intra nasal administration of oxymetazoline to NZW rabbits on nasal (n = 7) and sinus (n = 6) mucosal blood flow was measured with laser-doppler flow cytometry. Saline or oxymetazoline (0,0001 to 1 mg/mL) were administered cumulatively at the rate of 0.1 mL at a time increasing the next administration by a factor of 10. Oxymetazoline induced a dose-dependent decrease of nasal and sinus mucosa blood flow with 50% reduction observed at 0.1 mg/mL (equivalent to commercial nasal spray) compared to saline. Blood pressure and heart rate were unchanged. The authors hypothesized that it was due to a vasoconstrictive effect of oxymetazoline on the arteries penetrating the maxillary sinus ostium.

- Carrillo, 1969
The nasal and bronchopulmonary effects of oxymetazoline in comparison with epinephrine were evaluated in anesthetized mongrel dogs following IV (n = 15) and intracarotid (IC) (n = 7) administration. IC (0.2 and 0.4 µg/kg) and IV (0.5, 1.0 and 2.0 µg/kg) administration of oxymetazoline reduced nasal pressure dose-dependently up to 8.0 mm Hg for 7 minutes and 13.7 mm Hg for 9 minutes, respectively. The effect was prolonged compared with epinephrine. The pulmonary resistance decreased after oxymetazoline IV due to vasoconstriction in the bronchial mucosa.

4.2.4. Effects in the autonomic nervous system
- Briand, 1990
A single 2 µg/kg dose of oxymetazoline administered IV modulated responses of noradrenaline and neuropeptide-Y concentrations in portal venous blood in hemorrhage-induced dogs.

- Halpin, 1996
Net transport of an organic anion across isolated rat renal tubule was shown to increase with 10 µM oxymetazoline, suggesting a role of α-adrenergic stimulation in renal organic anion secretion.

4.2.5 Effects in inflammation, cellular defense and related activities
- Beck-Speier 2006
Oxymetazoline inhibited pro-inflammatory reactions in cell-free systems and canine alveolar macrophages (AMs) *in vitro.*

In cell-free systems, oxymetazoline (0.4 – 1 mM) (or 74 - 297 µg/mL) inhibited 5-lipooxygenase (LO) but not 15-LO activity and did not alter ultrafine carbon particle-induced oxidation of methionine.

At 0.1 mM (or 18.6 µg/mL) ($p < 0.05$) in canine AMs, oxymetazoline suppressed proinflammatory reactions including 5-LO activity, LTB4 formation, and respiratory burst and prevented particle-induced oxidative stress, whereas PLA2 activity and synthesis of immune-modulating PGE2 and 15-HETE were not affected.

- Kruszewska 2002
Over 160 non-antibiotic drugs were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa.* Oxymetazoline showed activity against *S. aureus* and *E. coli*; minimum inhibitory concentrations were 0.005 and 0.025 mg/mL, respectively.

- Isaacson 2005

Oxymetazoline concentrations of 0.025%, 0.05%, 0.1%, 0.2%, 0.4%, and 0.8% were studied to determine whether they had ability to inhibit the following middle ear pathogens: *Hemophilus influenza*, *S. aureus*, *P. aeruginosa*, *Moraxella catarrhalis*, and penicillin-resistant, penicillin intermediate, and penicillin-sensitive *Streptococcus pneumoniae*. Oxymetazoline HCl nasal spray (0.05%) and eye drops (0.025%) had activity against all species tested except *H. influenzae* and *P. aeruginosa*. The USP (United States Pharmacopeia) stated that this activity was very limited at the commercial preparations of 0.025% and 0.05%.

- Westerveld 1995
Antioxidant actions of oxymetazoline were evaluated in liver preparations from Wistar rats *in vitro*. Oxymetazoline was found to be a potent inhibitor of lipid peroxidation at concentrations of 5 µM (or 148.5 µg/mL) and higher, and effective as a hydroxyl radical scavenger at a concentration as low as 0.25 µM (or 7.4 µg/mL).

- Nickenig 1994
The effect of oxymetazoline on DNA synthesis in vascular smooth muscle cells from rat aorta was investigated with phase-contrast microscopy and a protein assay. Oxymetazoline was shown to induce moderate dose-dependent increase of [^{3}H] thymidine incorporation into the cell DNA.

4.3 Safety Pharmacology

4.3.1 Effects on the cardiovascular system

- Rhofade PI 2017
Alpha-adrenergic agonists as a class may affect blood pressure. In the label for the listed drug RHOFADE™, there is cautionary language for patients with cardiovascular disease, orthostatic hypotension, and/or uncontrolled or hypotension.

- Armstrong, 1982
The effect of oxymetazoline on cardiovascular activity has been shown in rats where a 4 µg/kg intra-arterial dose of oxymetazoline induced marked bradycardia in either methylatropine-pretreated conscious or pentobarbital-anesthetized, vagotomized rats. This effect appeared to be mediated through stimulation of peripheral and/or central neuronal α2-adrenoceptors.

- Timmermans, 1978
In this study, oxymetazoline (10 to 60 µg/kg IV) caused a dose-dependent decrease in heart rate in normotensive rats without decrease in mean arterial blood pressure whereas

it was reported to result in hypotension and bradycardia when administered intracisternally to dogs and vagotomized cats.

- Boudier, 1974
Oxymetazoline administered IV to rats (no dose given) caused an immediate, dose-dependent brief increase in blood pressure, but when injected into the anterior hypothalamus at 1, 10, or 100 nmol, did not have significant hypotensive or cardio-depressor effects. The authors concluded that the hypothalamic α-adrenergic receptors differ from the peripheral α-adrenergic receptors, and that perhaps only imidazolines with a 2-amino substitution (such as clonidine and tramazoline) show affinity for these central hypotensive α-adrenergic receptors.

- Doxey, 1981
Oxymetazoline administered IV at the doses of 1, 10, and 100 µg/kg in pentobarbitone-anesthetized rats caused a transient increased in mean arterial blood pressure and a reduction in heart rate without secondary drop in blood pressure. When administered intracerebroventricular (ICV), oxymetazoline at the doses of 3, 10, and 30 µg resulted in either a slight rise in blood pressure or no change and only slightly reduced heart rate.

The authors concluded that the lack of hypotensive activity of oxymetazoline may be due to poor access into the central nervous system (CNS), or a peripherally mediated vasoconstriction that counteracted any centrally mediated hypotension.

The bradycardia may be either central in origin, or due to peripheral presynaptic α-adrenergic receptors on the cardiac sympathetic nerves and that with ICV administration, poor lipophilicity may have limited the penetration to sites within the CNS.

- Bayorh, 1997
Oxymetazoline administered IV at 1, 5, and 10 µg/kg significantly increased blood pressure (BP) and decreased heart rate (HR) in normal conscious rats (n = 6). The changes of + 50, + 75, + 60 mm Hg (BP) and – 50, - 75, - 40 beats/min (HR) from baseline were measured at 1, 5, and 10 µg/kg, respectively at 1 minute after the injection.

In rats with bilateral vagotomy (pithed), basal blood pressure average was 50 mm Hg lower than in conscious normal rats. In pithed rats the blood pressures increased more than in conscious rats after oxymetazoline injections, but the heart rates were not significantly affected. In a third experiment, pithed rats were stimulated at 0.1, 0.3, and 1 Hz before and after oxymetazoline administered at 10 µg/kg IV. Sympathetic stimulation caused frequency-dependent increases in blood pressure that were not significantly affected by pretreatment with oxymetazoline. On the contrary frequency dependent

elevations in heart rates were produced by sympathetic stimulation and were significantly reduced by pre-treatment with oxymetazoline at all frequencies tested.

The investigators advanced the theory that oxymetazoline, although more selective for imidazole I1 receptors than α2-adrenoceptors in the brainstem, may activate peripheral presynaptic I1 receptors, and to some extent α2-adrenoceptors, which may result in sympathetic inhibition that was preferentially greater in cardiac tissue than in the peripheral vasculature. Thus, the result will be a reduction in heart rate without significant drop in blood pressure.

- Kobinger, 1983
Intracisternal injection of 30 µg/kg of oxymetazoline to anaesthetized cats lowered blood pressure, heart rate, and rates of electric discharge in the sympathetic splanchnic nerve.

When blood pressure and heart were lowered with pre-treatment (reserpine and α-methyl-p-tyrosine) oxymetazoline did not lower them further but did decrease splanchnic nerve discharge rates. The investigators theorized that central stimulation with oxymetazoline decreased sympathetic nerve activity independently of endogenous catecholamines.

4.3.2 Effects on the respiratory system
Suh, 1995; Min, 1996
Changes in the histology of the nasal respiratory mucosa were observed following long-term intranasal administration of oxymetazoline to rabbits. Two puffs of 0.1 mL each of 0.05% oxymetazoline hydrochloride solution were administered BID for periods of 1, 2, or 4 weeks (10 rabbits *per* group).

Ciliary loss, epithelial ulceration, inflammatory cell infiltration and subepithelial edema were noted, and the changes were more pronounced with increasing administration duration of the decongestant.

Dilatation or vacuolization of mitochondria and endoplasmic reticula and vesicles in the cytoplasm were observed in the 2- and 4-week oxymetazoline groups. The results of this study suggested that the administration of decongestants may cause ciliary loss with subsequent inflammatory changes in the nasal respiratory mucosa.

4.3.3 Effects on the Central Nervous System
- Loomis, 1992

Oxymetazoline produced antinociception in rats (ED_{50} values of 53.7 nmol (16 ng/mL) and 93.3 nmol (28 ng/mL) after intrathecal administration in the tail-flick and paw pressure withdrawal tests, respectively) with no detectable spinal neurotoxicity.

- Sherman 1987
A single intrathecal (IT) injection of oxymetazoline at 50, 100, 200, 250 and 300 nmol to Sprague Dawley rats was evaluated to determine the dose-response relationship and duration of antinociception and whether it was mediated by spinal α-adrenoceptors. Dose-dependent antinociception was observed as measured by the tail flick and paw pressure tests, with significant antinociception at all doses except 50 nmol in the paw pressure test. The ED_{50} in the tail flick and paw pressure tests were 120 nmol (36 ng/mL) and 148 nmol (44 ng/mL), respectively.

Oxymetazoline produced long lasting antinociception in the rat following (IT) injection (8 hours at 100 nmol), and this effect was thought to be mediated through α2-adrenoceptors in the spinal cord.

5 Pharmacokinetics/ADME/Toxicokinetics

For pre-clinical development, no original PK/ADME studies were submitted. The Applicant has relied for this section on the published literature and on a repeat dose toxicology study conducted according to Good Laboratory Practice (GLP) regulations. This study evaluated the toxicity and toxicokinetic of oxymetazoline after 26 weeks of ocular administration in the New Zealand White rabbit (Study No. 74041B). The Applicant's methodology for the literature search appears to be adequate.

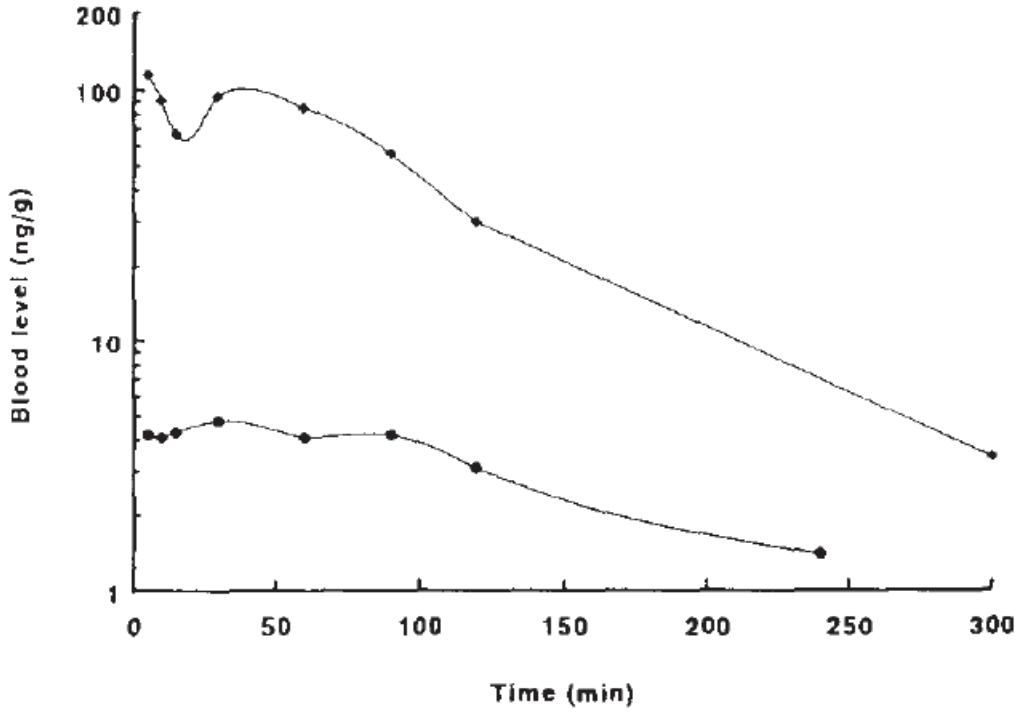
5.1 PK/ADME

5.1.1 Absorption and Distribution

5.1.1.1 Single dose
5.1.1.1.1. In the rat
- Hayes, 1995
In this study, the authors developed an HPLC assay in whole blood to estimate the PK of a single dose of 40 µg oxymetazoline in Sprague-Dawley rats (4/group, 200-300g) after IV and intranasal (IN) routes of administration using 300 µL samples. Pharmacokinetic parameter values were not reported however, authors showed typical blood PK profiles of oxymetazoline in rats generated form IV and IN dosing of the drug in saline (Figure 1). The results indicated that oxymetazoline was not extensively absorbed into the systemic

circulation after IN exposure leading to a bioavailability of 6.8% calculated from the $AUC_{0-90min}$.

Figure 1: Pharmacokinetic Profiles of Oxymetazoline Generated from 40 µg IV (upper trace) and IN (lower trace) Solutions Administered to Rats (n = 4) (copied from the publication by Hayes, 1995)



- Dowty, 1997

The oral absorption and *in vitro* steady-state intestinal permeability coefficients were determined for oxymetazoline. Male Sprague-Dawley rats (5/group) were fasted overnight and for 6 hours post-dose. Rats were administered either an oral or IV dose of 91.2 µg/kg [^{14}C]-oxymetazoline (10 µCi/kg). The fraction of orally absorbed oxymetazoline was calculated from the ratio of the mean % radioactivity recovered in urine after oral administration to that after IV administration. The fraction of orally absorbed oxymetazoline was 43% (90% confidence interval of 38-48%).

5.1.1.1.2. In the rabbit

- Duzman, 1983

Method

Twelve NZW rabbits received binocular topical applications of 50 µL of 0.025% (12.5 µg/eye) [^{14}C]-oxymetazoline (specific activity, 6.3 mCi/mM) in an isotonic formulation containing polyvinyl alcohol and polyvinyl pyrrolidone at pH 5.3. The measurements of

the exposure to the radioactivity in the different compartments of the eye were performed at 30 minutes, 1, 3, and 6 hours post administration.

Results
Oxymetazoline was poorly absorbed into the cornea (0.405% of the dose was recovered at 30 minutes). Only 0.006% of the radioactive dose was present in the aqueous humor 30 minutes after drug administration, which increased to approximately 0.02% of the dose 3 and 6 hours after drug administration. Radioactivity was also low in other ocular tissues including the choroid/retina, ciliary body, iris, lens and vitreous humor (Table 7).

The total amount of radioactivity found in all tissues was only 0.084% of the dose at 30 minutes, 0.091% at 1 hour, 0.127% at 3 hours and 0.107% at 6 hours. The highest concentrations were found in the external ocular tissues such as the cornea, conjunctiva, sclera and nictitating membrane. The radioactivity gradually decreased in all ocular tissues after 1 hour (Table 8).

This reviewer's comment: This is an albino species. It is unknown if the compound will be retained in pigmented tissues. The results of this study likely underpredict human exposure, given that the concentration/dose tested is less than clinical dose.

Table 7: Radioactivity in Rabbit Eye Tissues Following Topical Ocular Application of 0.025% Oxymetazoline (percentage of original dose) (copied from the publication by Duzman, 1983)

	Oxymetazoline, % of Original Dose							
	30 min		1 hr		3 hr		6 hr	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aqueous humor	0.006	0.001	0.014	0.008	0.020	0.018	0.018	0.005
Choroid/retina	0.058	0.032	0.045†	0.030	0.046	0.021	0.043†	0.018
Ciliary body	0.007	0.003	0.016	0.013	0.030	0.017	0.018	0.005
Cornea	0.405	0.183	0.458‡	0.085	0.247†	0.149	0.100	0.023
Iris	0.005	0.001	0.008	0.005	0.012†	0.007	0.012	0.003
Lens	0.006	0.002	0.004	0.001	0.012	0.011	0.013	0.005
Vitreous humor	0.002	0.001	0.004	0.003	0.002	0.001	0.003†	0.001
Total	**0.489**	...	**0.549**	...	**0.369**	...	**0.207**	...

*Six eyes tested unless otherwise noted. Oxymetazoline hydrochloride applied.
†Five eyes tested.
‡Four eyes tested.

Table 8: Radioactivity in Rabbit Eye Tissues Following Topical Ocular Application of 0.025% Oxymetazoline (ng/g of Tissue) (copied from the publication by Duzman, 1983)

	Oxymetazoline, ng/g of Tissue							
	30 min		1 hr		3 hr		6 hr	
Tissues	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aqueous humor	2.92	0.73	6.34	4.02	8.81†	8.02	8.06	2.05
Choroid/retina	53.87	32.78	46.21†	30.19	42.51	20.50	34.54†	17.64
Ciliary body	19.66	8.32	43.15	32.09	76.56	46.11	48.87	9.61
Conjunctiva	781.84	346.87	739.35	348.72	270.40	105.78	162.00	110.41
Cornea	739.25	311.72	782.66‡	137.89	396.07†	240.54	152.17	32.99
Iris	37.08	12.53	55.50	32.46	96.59†	54.39	93.93	19.60
Lens	1.92	0.73	1.27	0.40	3.61	3.16	4.20	1.33
Nictitating membrane	1907.42	836.61	1457.95	639.89	650.47	259.15	202.18‡	43.47
Optic nerve	35.67	22.51	22.12	7.66	15.97	3.61	12.14†	6.33
Sclera	479.58	213.19	531.32	331.35	353.56	108.85	187.05	79.59
Vitreous humor	0.19	0.05	0.29	0.23	0.15	0.07	0.21†	0.07

*Six eyes tested unless otherwise noted. Oxymetazoline hydrochloride applied.
†Five eyes tested.
‡Four eyes tested.

5.1.1.2 Multiple doses
See TK

5.1.3 Metabolism and Excretion

5.1.3.1 *In vivo*
- Duzman, 1983
In their paper, the authors have also studied the effect of the route of administration on the metabolism and excretion of oxymetazoline in NZW female rabbits.

Method
A solution of 0.025% (50 µL) of [^{14}C]-oxymetazoline (specific activity, 6.3 mCi/mM) was administered to 9 (3/group) female NZW rabbits by the nasal, ocular, and IV routes of administration (Table 9). The urine was collected for a period of 48-hours after drug administration.

Table 9: Study Design (copied from the Applicant's report)

Route of Administration	% Oxymetazoline Hydrochloride	Dose, μL	Radioactivity, ×10⁶ (DPM)
Nasal	0.25	20.0*	2.35
Topical ocular	0.05	100.0†	2.35
Intravenous	0.01	1.0 mL	4.7

*Twenty microliters into each nostril.
†Fifty microliters into each eye.

DPM = Disintegration *per* Minute

Results:
During the first 24-hour post-dose period, the mean (±SD) recovery of the administered radioactivity in urine was 44 ± 11.0%, 20.0 ± 3.4 % and 18.6 ± 6.1% after IV, topical ocular and nasal administration, respectively. During the next 24-hours, amounts excreted after each route of administration were similar. Radioactivity excreted in the urine during the first 48 hours was approximately 48% of the IV dose and 23% of the IN and ocular doses (Table 10).

HPLC analysis indicated that 29.7 ± 2.0%, 29.6 ± 22.0% and 33.3 ± 10.0% of the 24-hour urine radioactivity comprised of unchanged oxymetazoline, after IV, topical ocular and nasal administration, respectively. The metabolite peak had the same retention time in all the urine samples. The ratio of metabolite to unchanged drug was independent of the route of administration (Table 11).

Table 10: Per Cent of Administered Radioactivity Excreted in Urine (copied from Applicant's report)

	Radioactivity Excreted in Urine, %	
Route of Administration	0-24 hr	24-48 hr
Nasal	18.6 ± 6.1	3.8 ± 2.4
Topical ocular	20.0 ± 3.4	2.9 ± 1.0
Intravenous	44.7 ± 11.0	3.3 ± 4.3

*Three rabbits were used for each administration route. Values are means ± SD.

Table 11: Per Cent of Radioactivity as Metabolite or Parent Drug in 24-Hour Urine Samples (copied from Applicant's report)

	Radioactivity, %	
Route of Administration	Unchanged Oxymetazoline	Metabolite
Nasal	33.3 ± 10.0	47.7 ± 16.5
Topical ocular	29.6 ± 22.0	52.7 ± 15.5
Intravenous	29.7 ± 2.0	56.0 ± 7.8

* Three rabbits were used for each administration route. Values are means ± SD.

5.1.3.2 *In vitro*

- Mahajan, 2011a

A study was conducted to identify and characterize the glucuronide metabolite of oxymetazoline, identify the specific UGT isoforms involved in the *in vitro* glucuronidation of oxymetazoline and determine the kinetics of oxymetazoline glucuronidation in pooled human liver microsomes (HLM) using expressed human uridine glucuronosyltransferases (UGTs).

The glucuronide metabolite of oxymetazoline was identified as the β-O-glucuronide and UGT1A9 isoform was identified as the only UGT catalyzing the β-O-glucuronidation pathway.

The kinetic studies indicated that oxymetazoline is not glucuronidated at the nanomolar intranasal therapeutic dose and thus is eliminated unchanged. UGT1A9 would only contribute to its elimination at toxic plasma concentrations.

The authors concluded that the low incidence of adverse events observed in the clinic with the therapeutic use of oxymetazoline intranasally cannot therefore be attributed solely to its elimination through the glucuronide detoxification pathway and that other toxic metabolites must be formed.

- Mahajan, 2011b

Pooled human, Sprague-Dawley rat, or NZW rabbit liver S9 fractions and microsomes were analyzed after incubation with 10 μL containing 50 μM oxymetazoline.

Results

Four major metabolites (M1, M2, M6 and M7) and seven minor metabolites (M3. M4, M5, M8, M9, M10, and M11) of oxymetazoline were detected (Table 12).

Table 12: Oxymetazoline Metabolites Identified in S9 Fractions in Rat, Rabbit and Human (copied from Applicant's report)

Metabolite	Retention Time (min)	Identification	Human Liver S9	Rat Liver S9	Rabbit Liver S9
Parent	12.2	Oxymetazoline	√	√	√
M1	4.95	Monohydroxylation of the t-butyl group	√	√	√
M2	12.36	Oxidative dehydrogenation of the imidazoline to an imidazole moiety	√	√	√
M3	5.64	Monohydroxylation of M2	√	√	√
M4	2.54	Dihydroxylation of oxymetazoline		√	√
M5	2.77	Dihydroxylation of M2		√	√
M6	4.22	Glutathione conjugate of oxymetazoline	√	√	√
M7	4.65	Glutathione conjugate of M2	√	√	√
M8	3.17	Minor metabolite	√		√
M9	3.48	Minor metabolite			√
M10	3.86	Minor metabolite		√	√
M11	6.34	Minor metabolite			√

Source: Mahajan2011b

√: metabolite present

Unchanged oxymetazoline was the largest component recovered from the different species. The metabolites of oxymetazoline identified included M1 (mono-hydroxylation of the t-butyl group), M2 (oxidative dehydrogenation of the imidazoline to an imidazole moiety), M3 (mono-hydroxylation of M2), M4 (di-hydroxylation of oxymetazoline), and M5 (di-hydroxylation of M2). Glutathione conjugates of oxymetazoline (M6) and M2 (M7) were also identified in the liver S9 fractions.

Oxymetazoline was more extensively metabolized by rabbit liver S9 fractions (65% of the original dose) than by rat (20% of the original dose) or human (10% of the original dose). The rabbit S9 liver fractions exhibited a markedly different metabolite profile compared to the other species producing, in addition to the 4 major and 7 minor metabolites observed in the HPLC-UV chromatogram, other second-generation trace metabolites. Analysis of the LC-UV peak areas for rabbit liver S9 fractions indicated that the glutathione conjugates (M6 and M7) accounted for approximately 10 and 9% of the original dose of oxymetazoline, respectively, after 60-min incubation.

The human and rat liver microsomes produced fewer oxidative metabolites than their respective liver S9 fractions; in contrast, rabbit liver microsomes generated the same number of oxidative metabolites (M1–M5) as the S9 fractions.

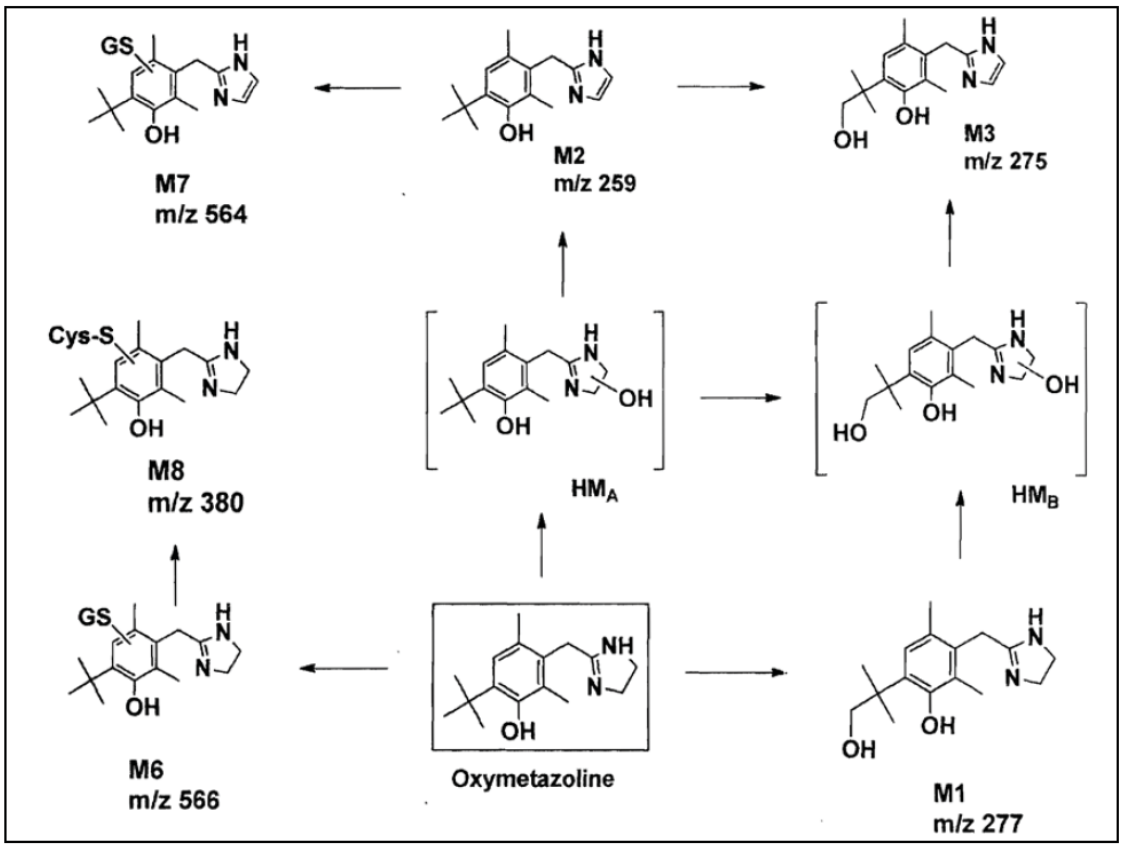
Screening of 9 human-expressed cytochrome P450s identified CYP2C19 as the single isoform catalyzing the formation of M1, M2, and M3. Detection of M6, M7 indicated the capability of oxymetazoline to undergo bioactivation to reactive intermediate species. M6 was identified as the glutathione conjugate of a p-quinone methide.

The 50 µM substrate concentration used in this screening study to predict the relative contribution of each isoform to the total oxymetazoline metabolism was at least 130-fold greater than the usual therapeutic intranasal dose of 400 nM. At this concentration of 50 µM oxymetazoline, no detectable oxidative metabolites were produced by the other P450s, suggesting that the other enzymes were unlikely to participate in a clinically relevant oxidation. This study also confirmed that CYP2C19 can catalyze the bioactivation of oxymetazoline to a reactive intermediate. Considering that p-quinone methide is abundant in liver S9 incubations of oxymetazoline in the three animal species used, the toxicological significance of the formation of this reactive intermediate *in vivo* cannot be overlooked.

The overall conclusion of the paper was that the bioactivation pathway of oxymetazoline was unlikely to be a safety concern with nasal products due to the briefness of exposure and the low doses used.

The proposed metabolic pathway of oxymetazoline after incubation in human liver S9 fractions is depicted in Figure 2.

Figure 2: Proposed Metabolic Pathway of Oxymetazoline in Human Liver S9 Fractions (copied from Applicant's report)



Source: Mahajan 2011b

5.2 Toxicokinetics

See Study No. 74041B in "General Toxicology".

6 General Toxicology

The toxicological properties of RVL-1201 have been evaluated in a 26-week ocular toxicity and toxicokinetic (TK) study in rabbits. Prior to the initiation of that study, a 28-day ocular toxicity study was conducted. These studies, along with FDA's prior findings of safety for the listed drug RHOFADE™, comprise the nonclinical safety assessment of RVL-1201.

The FDA agreed that a 26-week ocular toxicity study in rabbits, in addition to a previously completed 28-day rabbit study, would be enough to support a 505(b)(2) NDA for RVL-1201 (Type C meeting minutes dated May 2, 2018) (Table 13), pending review.

Table 13: Nonclinical Studies Conducted to Support Approval of RVL-1201 (copied from Applicant)

Type of Study	Route	Species/ Strain	Duration of Dosing	Compounds Administered	GLP	Study Number
Repeat dose toxicity	Ocular, topical	NZW rabbits	28 days	RVL-1201	Yes	12C145Q2R3G25
	Ocular, topical	NZW rabbits	26 weeks	RVL-1201	Yes	74041B

6.1 Single-Dose Toxicity

No single-dose toxicity studies were conducted with RVL-1201. The acute ocular effects of oxymetazoline on intraocular pressure and mydriasis have been described in the literature.

- Murray, 1985

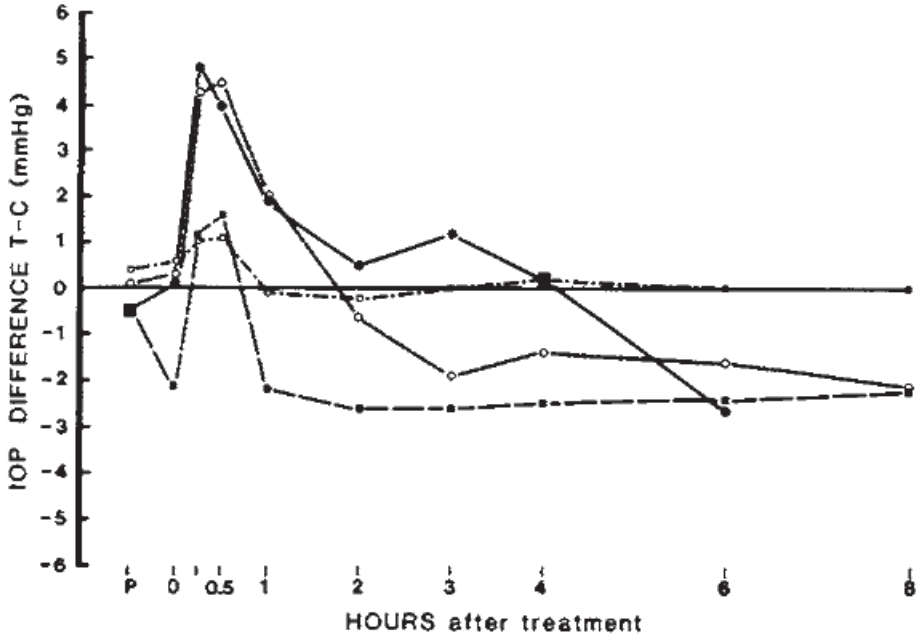
Method

Fifty microliters of oxymetazoline hydrochloride solutions of 0.003, 0.03, 0.1, 0.3, and 3 mM (0.04, 0.4,1.3, 4.5, and 44.5 µg/treated eye, respectively) prepared in distilled water were administered topically into the eyes of normal female albino rabbits (6 per group). Intraocular pressure (IOP) was measured in unrestrained animals 30 and 15 minutes prior to, immediately before and 0.25, 0.5, 1, 2, 3, 4, 6 and 8 hours after drug treatment. The effects on pupil diameter was determined on photography taken before and after treatments.

Results

Oxymetazoline caused dose-related rapid increases in IOP in treated eyes of up to 4.5 mm Hg at 0.3 mM with a peak response occurring within 15-30 minutes after treatment. The duration of the effect increased with dose, up to 4 hours at the highest dose of 3nM. At this dose, statistically significant “rebound” reductions in IOP at 6 hours post-treatment was also observed ($p<0.001$) (Figure 3).

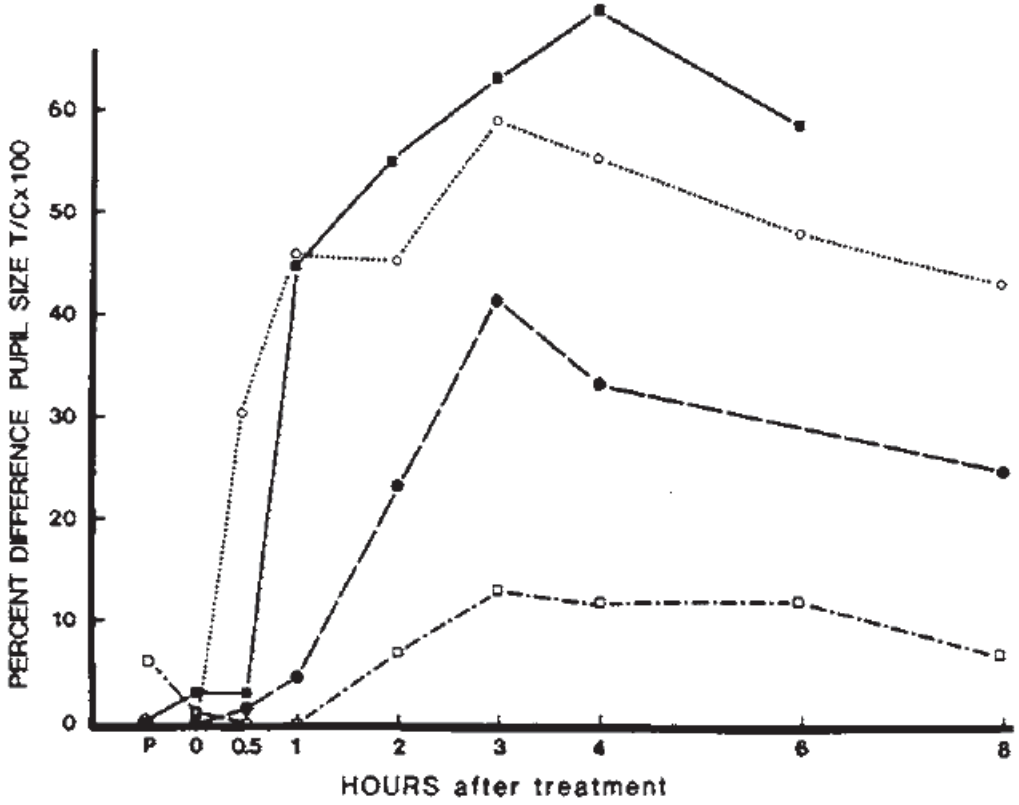
Figure 3: Differential IOP Dose Responses to Oxymetazoline Drops in Normal Rabbit Eyes (copied from the publication by Murray, 1985)



(●) 3 mM; (○) 0.3 mM;
(■) 0.03 mM; (□) 0.003
mM; N=6.

Oxymetazoline produced dose-dependent increases in pupil diameter of up to 71% at 3 mM. This effect appeared within 30 minutes after administration of the test compound and lasted at least for 8 hours (Figure 4).

Figure 4: Mydriatic Dose Responses to Oxymetazoline Drops in Normal Rabbit Eyes (copied from the publication by Murray, 1985)



(□) 0.03
mM; (●) 0.1 mM; (○) 0.3
mM; (■) 3 mM. N=6.

- Campbell, 1994
In this study, unilateral topical ocular administration of oxymetazoline hydrochloride at 500µg, 50µL to 5 normotensive rabbits resulted in bilateral ocular hypotension (maximum > 12 mm Hg) that persisted for more than 12 hours. Topical administration at this dose induced mydriasis of up to 3.3 mm with a duration of 60 minutes.

This reviewer's comment: The results confirm the findings of Murray described above.

- Wang, 1993
Method

Single and multiple dose testing with oxymetazoline was performed with 8 glaucomatous monkey eyes (4 animals with bilateral glaucoma).

In the single dose protocol, each eye received topically 1 drop of oxymetazoline at 0, 0.125%, 0.5%, 1.5% concentration/50 µL in normal saline with 2-week washout periods between each application.

In the multiple dose protocol, 1 drop 0.5% oxymetazoline/50 µL was applied to each eye twice daily for 5 consecutive days. In addition, 8 normotensive monkeys were used to measure the uveoscleral outflow prior to, and repeatedly, beginning 1 hour after application of 1 drop 0.5% or 1% oxymetazoline in 1 eye/50 µL.

Results

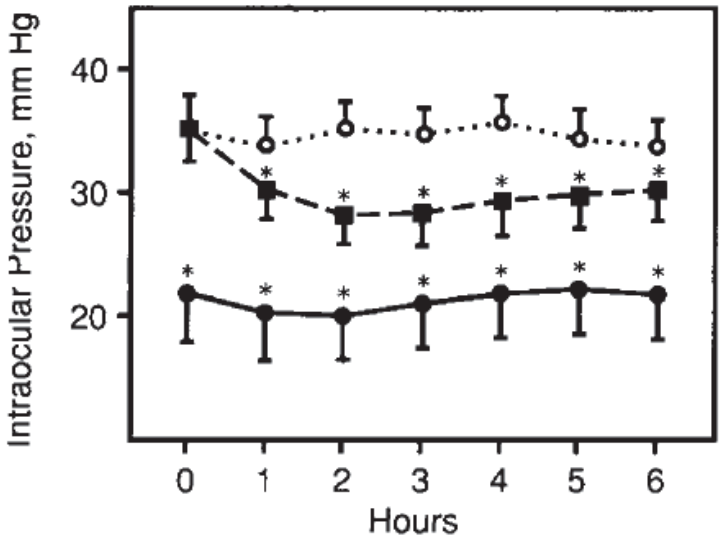
One single topical ocular application of oxymetazoline at 0.125%, 0.5%, 1.5%/50 µL significantly reduced IOP up to 6.0 mm Hg in glaucomatous monkeys and for up to 5 hours at the 2 highest doses tested ($p < 0.05$). A small dose-dependent increase in magnitude and duration of IOP reduction was observed (Table 14). In the multiple dose protocol, IOP was reduced up to 7.0 (± 0.8) mm Hg, with an effect duration of up to 16 hours after the second daily dose (Figure 5). The results with normotensive monkeys indicated decreased aqueous humor flow rates and increased uveoscleral outflows (Tables 15 and 16).

Table 14: Effects of a Single Dose at 0.125%, 0.5%, or 1.5% Oxymetazoline on IOP in Eight Glaucomatous Monkey Eyes (copied from the publication by Wang, 1993)

Oxymetazoline Concentration	Mean (±SEM) IOP, mm Hg						
	Baseline	1 h	2 h	3 h	4 h	5 h	6 h
0.125%	0.1±1.3	−4.0±1.4*	−5.0±1.3*	−4.1±1.4*	−4.5±1.2*	−3.1±1.8	−3.4±1.8
0.05%	0.8±0.9	−3.9±0.8*	−5.5±0.8*	−6.0±0.9*	−6.1±1.2*	−5.0±0.9*	−2.3±1.5
1.5%	1.0±1.1	−5.0±0.6*	−4.5±0.8*	−4.5±1.0*	−3.6±0.9*	−3.5±1.1*	−2.3±1.3

*Differences in IOP between drug-treated and vehicle-treated eyes were significant at $P<.05$ using the two-tailed paired t test.

Figure 5: Effects of Twice Daily Administration 0.5% Oxymetazoline on IOP in Eight Glaucomatous Monkey Eyes (copied from the publication by Wang, 1993)



Effect of twice-daily administration of 0.5% oxymetazoline to eight glaucomatous monkey eyes for 5 days. Points represent means at baseline (open circles), day 1 (squares), and day 5 (closed circles) of treatment and the limits (±SEMs). Asterisk indicates statistically significant differences in intraocular pressures between drug-treated and vehicle-treated eyes ($P<.05$ using two-tailed paired t test).

Table 15: Effect of 0.5% Oxymetazoline on Outflow Facility and IOP in Eight Normal Monkeys (copied from the publication by Wang, 1993)

Treatment Received	Mean (±SEM) IOP, mm Hg	Mean (±SEM) Outflow Facility, µL/min
Oxymetazoline		
Treated	14.4±0.6*	0.72±0.07
Baseline	15.3±0.6	0.64±0.06
Vehicle		
Treated	15.0±0.7	0.68±0.06
Baseline	15.4±0.4	0.66±0.06

*Differences in IOP or outflow facility in the oxymetazoline-treated eyes compared with either baseline values or vehicle-treated eyes were significant at $P<.01$.

Table 16: Effect of 1% Oxymetazoline on Aqueous Humor Flow in Eight Normal Monkeys (copied from the publication by Wang, 1993)

Treatment Received	Mean (±SEM) Aqueous Humor Flow, µL/min	
	Baseline	Treated
Oxymetazoline	1.67±0.09	1.02±0.05*
Vehicle	1.70±0.14	1.62±0.19

*Differences in aqueous humor flow rates in the oxymetazoline-treated eyes compared with either baseline values or vehicle-treated eyes were significant at $P<.01$.

Lethal dose

In rats, the lowest lethal doses of oxymetazoline administered orally or subcutaneously were 83- (oral) and 183- (SC) fold higher than the MRHD of 1 µg/kg (Table 17).

Table 17: Oxymetazoline Lethal Doses in the Rat (copied from Applicant's report)

Method of Administration	Approximate Lethal Dose (mg/kg)	Study
Oral	5 days (age): <1; 90 days: 0.8 (0.5-1.2)	Goldenthal, 1971
Subcutaneous	5 days: <1; 90 days: 1.1 (0.8-1.7)	Goldenthal, 1971
Intranasal (4-hr inhalation)	0.02 mg/L	Mucinex Nasal Spray-MSDS, 2009

6.2 Repeat-Dose Toxicity

6.2.1 Study title: RVL-1201: A 26-Week Topical Ocular Toxicity Study of Oxymetazoline Hydrochloride Ophthalmic Solution, 0.1% with a 4-Week Recovery Period in New Zealand White Rabbits

Study no.:	74041B
Study report location:	Edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6th, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RVL-1201, R60701, 98.6%

Key results

Under the conditions of this study, the no-observed adverse effect level (NOAEL) of RVL-1201 was 0.21 mg/animal/day (0.1% oxymetazoline, 1 drop of 35 µL three times daily to both eyes or 105 µg/eye/day). The local safety margin was therefore 3X based on a MRHD of 35 µg/eye/day. In terms of systemic toxicity, at the NOAEL of 0.117 mg/kg/day in a 1.8-kg rabbit (or 0.038 mg/kg HED based on BSA conversion), the safety margin was 32.4X based on a MRHD of 1.16 µg/kg/day for a 60 kg subject.

At the NOAEL, systemic exposures achieved at Day 182 were 1516, and 1984 pg/mL for C_{max} and 10484 and 14889 pg.h/mL for AUC_{0-24} in males and female rabbits, respectively. In the bioequivalence study (Study RVL-1201-PKP01), when healthy volunteers were administered 1 drop of RVL-1201 to each eye, C_{max} and AUC_{inf} values achieved 30.5 pg/mL and 468 pg•h/mL, respectively, which represented systemic toxicity safety margins of 57X and 27X for C_{max} and AUC, respectively, compared to combined male and female rabbit exposure values at the NOAEL of 105 µg/eye/day.

Methods

Doses:	0, 0.14, 0.21 mg/animal/day
Frequency of dosing:	Day
Route of administration:	Ocular Instillation
Dose volume:	35 µL
Formulation/Vehicle:	Vehicle for ophthalmic solution
Species/Strain:	New Zealand white rabbits
Number/Sex/Group:	10 main group including 5 for recovery
Age:	4 months
Weight:	2.5 to 4 Kg
Satellite groups:	No
Study design:	See below

The test and control/vehicle items were administered to groups of rabbits by ocular instillation of 1 drop onto the superior corneal surface of both eyes two or three times a day (6 hours ± 30 minutes apart) for 26 consecutive weeks:

Group Numbers	Group Designation	Doses/day	Dose Level (µg/eye/ dose)*	Dose Level (mg/animal/ day)*	Dose Concentration (% w/v)	Toxicology Animals			
						Main		Recovery	
						M	F	M	F
1	Control#	3	0	0	0	10	10	5	5
2	Low Dose	2	35	0.14	0.1	10	10	5	5
3	High Dose	3	35	0.21	0.1	10	10	5	5

M= Male, F= Female
# The control animals received the vehicle item only.
* One drop from an appropriate blow/fill/sealed (BFS) vial of RVL-1201 (hydrochloride ophthalmic solution, 0.1%) is equal to 35µL of placebo or test item, which is also equal to 35µg.

Observations and Results

Mortality

Mortality checks were performed once a day (in the afternoon) during all phases of the study.

- One Main Study Female (Animal No. 2503A) treated with 0.14 mg/animal/day was found dead on Day 183. The day prior, at the time of blood draw for TK analysis, the animal presented with signs of convulsion, lying on the cage floor, teeth grinding, dilated pupils, slight salivation and paresis of the right side of the body. The paresis persisted prior to the demise of this animal. There were no macroscopic or microscopic findings.

- One Recovery Male (Animal No. 2012D) treated with 0.14 mg/animal/day was found dead on Day 195. This animal had no macroscopic or microscopic findings.

Although the cause was unclear, the deaths of these animals were not considered by Applicant test-article related as no other animal in this group or at 0.21 mg/animal/day died or presented with macroscopic/microscopic changes at necropsy.

Clinical Signs

Cage-side clinical signs were recorded once daily during the acclimation period and once a day during the treatment, and recovery periods. Detailed clinical examination occurred for each rabbit at least once pre-treatment, weekly during the treatment and recovery periods and before necropsy.

There were no test article-related effects on clinical signs.

Body Weights

Body weights were recorded for all animals once prior to group assignment and approximately one week prior to initiation of treatment. Body weights were recorded for all animals once weekly thereafter during the treatment (before dosing on each occasion) and recovery periods, as well as terminally prior to necropsy (fasted).

There were no test article-related effects on body weights.

Feed Consumption

Individual daily food intake was recorded for all animals commencing on Day -7 and continuing throughout the treatment and recovery periods.

There were no test article-related effects on feed consumption.

Ophthalmoscopy

During the detailed ocular examination, the following order of conduct was followed:
1) Tonometry conducted at the same day/time as the ophthalmic examination when applicable; tonometry was not performed on the same days as electroretinography;
2) Slit-lamp bio-microscopy of the anterior segment with Hackett-McDonald scoring, followed by fluorescein dye application (1% or fluorescein ophthalmic strips) and final scoring assessment;
3) Dilation of pupils;
4) Lens and ocular fundus examination by indirect ophthalmoscopy.

Funduscopic and biomicroscopic examinations were performed for all animals as follow:
- Once during the pre-treatment period (between Day -8 and Day -6);
- Once at the end of treatment Week 13 (between Day 89 and Day 91);
- Once at the end of treatment Week 17 (between Day 117 and Day 119);
- Twice at the end of treatment Week 26 (Day 180);
- Once at the end of recovery period (Day 209).

All examinations were conducted by a Board-Certified Veterinary Ophthalmologist.

Since fibrin was observed at the ocular examination during the end of treatment Week 13 that could potentially be due to the ERG manipulation (which was performed prior to the ophthalmology), an additional ocular examination was performed 1 day after the ERG measurements at the end of treatment-Week 26 time-point. On this occasion, only the anterior segment of the eyes in all animals was examined for the presence of fibrin by slit-lamp biomicroscopy.

Results

Slit-lamp biomicroscopy and fundoscopy (Table 18)
The left eye of Animal No. 3514E (treated with 0.21 mg/day) at Week 13 had a negative pupillary light response which remained present until the end of the recovery period. Beginning at Week 17, it was associated with anterior cortex opacities of the lens, temporal and multifocal. In the opinion of this reviewer, this effect observed at the high dose was consistent with the pharmacological activity on alpha 1 adrenoreceptors in the iris (mydriasis). However, since it only happened in 1 eye out 120 (60 drug- treated animals on study) this finding could also be incidental.

Anterior chamber fibrin was noted during Weeks 13 and 26 in test-article treated animals when the ophthalmology was performed shortly after the ERG sessions. Fibrin was not observed in any of the control animals or at pre-treatment when the ERG was performed (Days-13 to -11) a few days before the ophthalmic examination (Days -8 to -6). This finding was reversible. Anterior chamber fibrin was not observed either during Week 17 when no ERG procedures were performed, during Week 26 when ophthalmology examinations were conducted before ERGs, or during Week 30 when ERG was performed 1 day after the ophthalmic examination.

A total of 6/30 rabbits treated with 0.21 mg/animal/day and 2/30 rabbits treated with 0.14 mg/animal/day had fibrin in the anterior chamber of the eye during Week 13. The fibrin was generally graded very slight and was unilateral in all animals (all in the right eyes,

except for Animal No. 2515E where it was found in the left eye). On this occasion, Animal No. 2514E treated with 0.14g/day had also very slight anterior chamber flare and a positive fluorescein staining. In all other animals with this finding, there was no obvious association between the presence of fibrin and overt inflammation.

During Week 26, a brief ocular exam was performed following the ERG procedure and a total of 4 eyes (4 rabbits including 2 at 0.14 mg/animal/day and 2 at 0.21 mg/animal/day) had minor anterior chamber fibrin. All were graded very slight except for one eye where it was graded slight. Since it was only found in treated animal and was dose-related the finding was therefore treatment-related. Since it was reversible, it was not considered adverse by this reviewer.

Other findings linked to eye manipulations were very slight to slight conjunctival congestion (hyperemia) noted in all groups with higher incidences in animals that had an ERG procedure shortly before the ophthalmic examination.

Throughout the study multiple eyes had “faint positive” fluorescein results observed in control and treated animals. There was no obvious association between treatment and positive findings.

Table 18: Treatment Related Slit-Lamp Bio-Microscopy and Fundoscopy Findings (this Reviewer’s table)

Animal # / Lesions	Week 13 (post-ERG	Week 17 (No ERG)	Week 26 (Pre-ERG)	Week 26 (Post-ERG)	Week 30 (Pre-ERG)
Anterior chamber aqueous fibrin	*2514E (OD) *2515E (OS) &3007C (OD) &3011D (OD) &3507C (OD) &3509C (OD) &3512D (OD) &3014E (OD)			*2007B (OD) *2506B (OD) &3501A (OS) &3010C (OD)	
Anterior chamber aqueous flare	*2514E (OD)				
Negative pupil light response	&3514E (OS)	&3514E (OS)	&3514E (OS)	&3514E (OS)	&3514E (OS)
Lens opacity cortex anterior multifocal		&3514E (OS)	&3514E (OS)	&3514E (OS)	&3514E (OS)

* 0.14 mg/animal/day; & 0.21 mg/animal/day, OD = Right Eye; OS = Left Eye

Tonometry
Intraocular pressure (IOP) was measured as follows:
- Once during the pretreatment period (between Day -8 and Day -6);
- On Day 1 after the first dose;
- Once at the end of treatment Week 4 (between Day 26 and Day 28) at 1-hour post first dose;
- Once at the end of treatment Week 13 (between Day 89 and Day 91);
- Once at the end of treatment Week 26 (Day 180);
- Once at the end of recovery period (Day 209).

IOP measurements were performed without the use of topical anesthetic with a rebound tonometer for veterinary use. Pre-study IOPs were measured at approximately the same time of day as the end of dosing period IOPs and the recovery period IOPs. The rabbits underwent measurements in the same order on each occasion.

IOP measurements were not performed on the same day as the ERG examinations. All examinations were conducted by competent technical staff.

Results
There were no changes in IOP that could be attributed to the test article during the treatment or recovery periods.

This reviewer's comment: The TonoVet® rebound tonometer that they have used in this study in not GLP compliant.

Electroretinography (ERG)

It was performed as follows:
- Once during the pre-treatment period (between Day -13 and Day -11)
- Once at the end of treatment Week 13 (between Day 89 and Day 91)
- Once at the end of treatment Week 26 (Day 181)
- Once at the end of recovery period (Day 210)

Per Applicant, all the examinations were conducted by a neurophysiologist specializing in animal electroretinography and performed under GLP conditions.

Animals were dark-adapted for at least 2 hours and sedated prior to ERGs.
The GLP compliant ERG apparatus setting was as follow:
Preamplifier gain of 1000,
Computer interface gain of +/-10.

Stimulus duration of 50 ms.
Pre-stimulus delay of 20 ms.
Inter-flash interval of 5 s.
Acquisition time of 500 ms.

Typical unprocessed, dark-adapted ERGs were obtained at up to 5 stimulus intensities increasing in 1 log unit increments from Intensity 5 (bottom, dimmest) up to Intensity 1 (top, brightest). Stimuli were presented in order of increasing intensity. The ERGs at Intensity 5 and Intensity 1 were designated as the 'standard' responses for quantitative analysis.

The ERGs were assessed qualitatively and quantitatively compared to historical controls.

Parameters obtained included a- and b-wave time-to-peak (A-/B-TTP), a- and b-wave Amplitude (A-/B-AMP), a- and b-wave slope (A-/B-SLP) and a- to b-wave ratio (A/B).

Results
There were no test item-related findings at the end of treatment or at recovery suggesting retinal degeneration or other physiological abnormality in any of the treated rabbit.

According to the ophthalmologist that performed the ERG, there was no evidence from previous ERG studies on any species suggesting a cause-and-effect relation between the ERG procedure and the presence of anterior chamber fibrin in this study. Therefore, the presence of fibrin in this study is unexplained.

ECG
NA

Clinical Pathology

Blood sampling occurred on all animals prior to the start of treatment, at the end of the treatment period (at Day 182) and at the end of the recovery period (at Day 210).

Hematology and Coagulation

Parameter evaluated were:

Red blood cell count	Mean corpuscular hemoglobin (calculated)
Hematocrit (calculated)	Mean corpuscular volume
Hemoglobin	Morphology of cells
White blood cell count	Platelet count
WBC differential (absolute)#	Reticulocyte (absolute and percentage#)
Mean corpuscular hemoglobin concentration (calculated)	

manual count

APTT and PT

There were no changes in hematology and coagulation parameters that could be attributed to the test article.

Clinical Chemistry

Parameters evaluated were:

A/G ratio (calculated)	Creatinine
Alanine aminotransferase	Globulin (calculated)
Albumin	Glucose
Alkaline phosphatase	Phosphorus (inorganic)
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Total protein
Chloride	Triglycerides
Cholesterol (total)	Urea

There were no changes in clinical chemistry parameters that could be attributed to the test article.

Urinalysis

NA

Gross Pathology

All surviving study animals were euthanized upon completion of the treatment/recovery periods i.e. at Days 183 and 211. Animals No. 2503A and 2012D which were found dead on Day 183 and 195, respectively, were subjected to necropsy as soon as feasible.

There were no changes at necropsy that could be attributed to the test article.

Organ Weights

Absolute organ weights were reported, and body and brain weight relative organ weights were calculated and reported (terminal body weight was used for body weight relative organ weights). Paired organs were weighed together.

There were no changes in organ weights that could be attributed to the test article.

Histopathology

The organs/tissues retained, weighted and examined microscopically are listed below:

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (N/M)	ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (N/M)
Adrenals	•	√	N/M	Skin & subcutis (inguinal)	•		N/M
Animal identification	•			Duodenum	•		N/M
Aorta (thoracic)	•		N/M	Jejunum	•		N/M
Blood				Ileum	•		N/M
Bone marrow smears (3)	•			SC, cervical	•		N/M
Brain	•	√	N/M	Spleen	•	√	N/M
Cecum	•		N/M	Sternum & marrow	•		N/M
Colon	•		N/M	Stomach	•		N/M
Epididymides	•d		N/M	Testes	•d	√	N/M
Esophagus	•		N/M	Thymus	•	√	N/M
Eyes	•aeg	√	N/M	Thyroid gland/parathyroids	•	√	N/M
Femur & marrow	•		N/M	Tongue	•		N/M
Gallbladder	•		N/M	Trachea	•c		N/M
Heart	•	√	N/M	Urinary bladder	•		N/M
Kidneys	•	√	N/M	Uterus	•	√	N/M
Liver (2 lobes)	•	√	N/M	Vagina	•		N/M
Lungs (2 lobes)	•b	√c	N/M				
LN, mandibular	•		N/M	Gross lesions	•		N/M
LN, mesenteric	•		N/M				
Mammary gland (inguinal)	•		N/M				
Optic nerves	•ae		N/M	**Additional Tissues presented below**			
Ovaries	•	√	N/M	ID microchip	•		
Pancreas	•		N/M	Lacrimal glands	•		N/M
Pituitary	•	√	N/M	Eyelids with conjunctiva and meibomian glands	•		N/M
Prostate	•	√	N/M				
Rectum	•		N/M				
SG, mandibular	•		N/M				
Sciatic nerve	•		N/M				
Seminal vesicles	•		N/M				
Skeletal muscle	•		N/M				

a	Davidson's fluid (euthanized animal only)		
b	Lungs were infused with 10% neutral buffered formalin (euthanized animal only)		
c	Lungs were weighed with trachea		
d	Bouin's fluid (euthanized animal only)		
e	Eyes were weighed with approximately 5 mm of the Optic nerve attached		
g	5 transverse sections/globe		
N	Necropsy examination	M	Microscopic examination
LN	Lymph node	SG	Salivary gland
SC	Spinal cord		
Notes:	Paired organs weighed together Parathyroids and mammary gland were only examined histologically if present in routine sections		

Femoral bone marrow smears were prepared but not examined.

Histopathological examination was performed on:
1) Tissues from animals found dead (Animals No. 2012D and 2503A)
2) Tissues from all control and high dose animals
3) All gross lesions from all animals
4) Ocular tissues from all animals
The microscopic examination was conducted by a Board-Certified Veterinary Pathologist.

Adequate Battery
Yes
Peer Review
No

Histological Findings
There were no microscopic changes that could be attributed to the test article.

Special Evaluation

NA

Toxicokinetics

Blood was collected from each rabbit on Days 1 and 182 at pre-dose, and at 0.25, 0.5, 1, 3, 6 (prior to the second dose), 6.25, 6.5, 7, 9, 12 (prior to the 3rd dose for Group 1 and 3), 12.25, 12.5, 13, 15 and 24 hours after the first treatment of the day.

AUC on Day 1 increased in an approximately dose proportional manner for both males and females. On Day 182, the exposure increased in a proportional manner for males and higher than dose proportional manner for females.

C_{max} did not increase with dose for either sex on Day 1 or for males on Day 182; C_{max} increased in an approximately dose proportional manner for females on Day 182.

Exposures within the 0-6 and the 6-12-hour sampling intervals following successive doses on each of the sampling days were comparable, indicating that there was no significant accumulation after administration of the first two doses on either Day 1 or Day 182.

Exposure values obtained on Day 182 were comparable (≤ 1.5x) to those obtained on Day 1 for Group 2 and for males in Group 3, suggesting that there was no appreciable accumulation over the dosing period in these animals. There was a tendency for the

compound to accumulate in Group 3 females (2 folds) after dosing 3 times a day for 182 days (Table 19).

Table 19: Group Mean Toxicokinetic Parameters of Oxymetazoline Repeat Dose in New Zealand White Rabbits (copied from Applicant's report)

At Day1

Group	Sex (Dose level) (mg/animal/day)		$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (pg/mL)	AUC_{0-6} (hr*pg/mL)	AUC_{6-12} (hr*pg/mL)	AUC_{12-24} (hr*pg/mL)	AUC_{0-24} (hr*pg/mL)	AUC_{INF} (hr*pg/mL)
2	Male (0.14)	Mean	3.51	4.88	1137	2499	2237	495	5232	5277
		SD	0.535	2.586	928.5	2132.0	1296.8	387.2	3189.0	3207.0
		CV%	15.2	53.0	81.7	85.30	58.0	78.1	61.0	60.8
		N	15	15	15	15	15	15	15	15
2	Female (0.14)	Mean	3.48	3.68	921	1975	2097	482	4551	4396
		SD	0.920	2.986	362.4	990.4	985.0	335.9	2090.1	2198.2
		CV%	26.4	81.1	39.3	50.1	47.0	69.7	45.9	50.0
		N	13	15	15	15	15	15	15	13
3	Male (0.21)	Mean	2.67	6.98	980	1816	2473	2645	6935	7044
		SD	0.443	4.771	314.4	890.0	1542.4	1503.6	2937.6	3346.8
		CV%	16.6	68.3	32.1	49.0	62.4	56.8	42.4	47.5
		N	13	15	15	15	15	15	15	13
3	Female (0.21)	Mean	3.32	6.95	990	2111	2401	2661	7174	7468
		SD	0.310	3.657	325.8	865.0	992.6	1396.6	2799.9	2353.9
		CV%	12.5	52.6	32.9	41.0	41.3	52.5	39.0	34.5
		N	13	15	15	15	15	15	15	13

At Day 182

Group	Sex (Dose level) (mg/animal/day)		$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (pg/mL)	AUC_{0-6} (hr*pg/mL)	AUC_{6-12} (hr*pg/mL)	AUC_{12-24} (hr*pg/mL)	AUC_{0-24} (hr*pg/mL)
2	Male (0.14)	Mean	4.28	5.65	1473	2479	3269	1287	7035
		SD	0.884	5.869	397.0	767.4	1183.0	1301.9	1944.0
		CV%	20.6	103.9	27.0	31.0	36.20	101.2	27.6
		N	11	15	15	15	15	15	15
2	Female (0.14)	Mean	4.72	4.60	1196	2471	3070	995	6265
		SD	1.350	2.838	305.6	786.5	1198.3	456.5	1917.0
		CV%	28.6	61.7	25.6	31.8	39.0	45.9	30.60
		N	13	15	15	15	14	14	15
3	Male (0.21)	Mean	3.00	7.63	1516	2849	2869	4766	10484
		SD	0.392	6.836	632.3	1739.1	1464.4	3457.7	5231.2
		CV%	13.1	89.6	41.7	61.0	51.0	72.5	49.9
		N	6	15	15	15	15	15	15
3	Female (0.21)	Mean	3.98	7.35	1984	3574	4761	6555	14889
		SD	2.453	6.311	1090.0	1650.5	2959.1	3553.3	6814.7
		CV%	61.7	85.9	54.9	46.2	62.2	54.2	45.8
		N	9	15	15	15	15	15	15

Dosing Solution Analysis
Adequate.

6.2.2 Study No. 12C145Q2R3G25: Evaluation of the Ocular Toxicity of the Applicant's Test Article, Oxymetazoline, After Repeat Topical Administration in the Eyes of New Zealand White Rabbits

Design for Study No. 12C145Q2R3G25

Group	Treatment	Route	Dosing Regimen*	Concen-tration	Dosing Volume	Number of Animals Per Sex	Termination Day**
1	Vehicle Control	Bilateral Topical	QID	0	50 µL	5	Day 29
2	Oxymetazoline	Bilateral Topical	TID	0.1%	50 µL	5	Day 29
3		Bilateral Topical	QID	0.1%	50 µL	3	Day 29

*QID: four times per day; TID: three times per day.
**Termination Day: The last day of dosing was Day 28.

This study was reviewed by Maria Rivera, PhD, and her conclusion is summarized below:

"No adverse ocular findings were observed after treatment of rabbits with oxymetazoline 0.1% 3x/day (TID) or 4x/day (QID) for 28 days based on ophthalmology evaluations and ocular histopathology of the eye. Mydriasis was not reported in this study. Effects on IOP were not measured. No adverse systemic effects were observed based on clinical signs, clinical chemistry, urinalysis, and histopathological evaluation of selected organs."

Dr. Rivera has indicated that at the time of submission, the draft report did not include an adequate QA statement or the analysis of dosing solution. This reviewer certifies that the missing information has been included in this NDA submission.

Further, Dr. Rivera has noted the following deficiencies in Study No. 12C145Q2R3G25:

- The evaluation of the eyelids, optic nerve, accessory glands were not conducted;

- A limited list of tissues from only 3 animals per dose per sex were evaluated under the microscope whereas at least 5 animals per dose per sex are recommended; organs were not weighed;

- Toxicokinetic measurements were not conducted.

Despite these drawbacks, in the conditions of Study No. 12C145Q2R3G25, the NOAEL was 0.1 % oxymetazoline QID/eye (50 µL drop) or 200 µg/eye/day which provided a safety margin of 5.7X based on a MRHD of 35 µg/eye/day.

7 Genetic Toxicology

Genotoxicity studies have not been conducted with RVL-1201.

The Applicant referred to RHOFADE™ PI 2017 indicating that "Oxymetazoline hydrochloride revealed no evidence of mutagenic or clastogenic potential based on the results of two *in vitro* genotoxicity tests (Ames assay and human lymphocyte chromosomal aberration assay) and one *in vivo* genotoxicity test (mouse micronucleus assay).

8 Carcinogenicity

Carcinogenicity studies have not been conducted with RVL-1201.

The Applicant referred to RHOFADE™ PI 2017 indicating that Oxymetazoline hydrochloride was not associated with an increased incidence of neoplastic or proliferative changes in transgenic mice given oral doses of 0.5, 1.0, or 2.5 mg/kg/day oxymetazoline hydrochloride for 6 months. The proposed clinical dose of RVL-1201 is approximately 1 µg/kg/day or approximately 2500-times less than the high dose in this transgenic mouse study.

In addition, carcinogenicity studies are not required for therapeutics intended for short term use (less than a month).

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicity studies have been conducted with RVL-1201.

The Applicant referred to RHOFADE™ PI 2017, KOVAZANE™ PI 2016, and to a published study by Johns, 1075.

9.1 Fertility and Early Embryonic Development

- RHOFADE™ PI, 2017

Effects on fertility and early embryonic development were evaluated in rats following oral administration of 0.05, 0.1, or 0.2 mg/kg/day oxymetazoline hydrochloride prior to and during mating and through early pregnancy.

Decreased number of corpora lutea and increased post-implantation losses were noted at 0.2 mg/kg/day oxymetazoline hydrochloride (3 times the MRHD for RHOFADE™ on an AUC comparison basis). However, no treatment related effects on fertility or mating parameters were noted at 0.2 mg/kg/day oxymetazoline hydrochloride (3 times the MRHD for RHOFADE™ on an AUC comparison basis).

- Johns, 1975

The effect of oxymetazoline on fertility was tested in mice. Female BDF1 mice were placed with fertile males and beginning on Day 1 of pregnancy received oxymetazoline (0.1 mL) IP for 5 days at 1 µg (n = 9), 4 µg (n = 8), 50 µg (n = 9), and 200 µg (n = 8) BID, or vehicle (n = 10). Mice were sacrificed at Day 12.

Oxymetazoline resulted in statistically significant fewer pregnancies and fewer fetuses at the 2 highest doses tested (Table 20) [the dose of 50 µg for a 20 g mouse corresponded to an HED of 0.21 mg/kg in mg/M^2 equivalent compared to a MRHD of 1µg/kg/day for RVL-1201which therefore allowed a safety margin of 21 with the human dose].

Table 20: Effects of Adrenergic Agonists on the Fertility of Mice (copied from Johns, 1975)

Drug	Individual Dose (µg)	Number in group	Number pregnant	Average Number of fetuses
Oxymetazoline	1	9	7	9.0
	4	8	5	7.2
	50	9	2*	8.5
	200	8	0**	-
Methoxamine	50	10	7	8.1
	200	9	8	8.9
	500	10	7	9.3
Salbutamol	2	9	9	8.4
	50	10	8	9.4
	200	10	4	7.0
	500	13	9	9.7
Isoproterenol	50	10	7	8.7
	200	10	10	8.3
	500	8	6	7.8
Control	(0.1 ml 0.1N HCl)	10	7	8.3
	(0.1 ml 1% ascorbic acid in 0.9% NaCl)	10	8	8.1

* $p < 0.05$, compared to control and ** $p < 0.001$, compared to control (Chi square).

- KOVAZANE™ PI, 2016

Oxymetazoline may reduce fertility in males and females of reproductive potential.

In fertility studies in male and female rats, SC oxymetazoline was administered prior to and during mating at 0.1 mg/kg/day alone, at doses of 0.01, 0.03, and 0.1 mg/kg/day in combination with tetracaine 7.5 mg/kg/day, and subcutaneous tetracaine at 7.5 mg/kg/day was administered alone.

In male rats, oxymetazoline at ≥ 0.03 mg/kg/day reduced the percentage of motile sperm and sperm counts. No effects on male mating behavior were observed at any dose tested. The no-effect level for sperm effects was 0.01 mg/kg/day.

In female rats, the number of viable embryos was reduced when oxymetazoline was given alone or in combination with tetracaine. Reduced numbers of corpora lutea and

implantation sites were observed. A no-effect level for fertility in female rats was not established in this study. The effects were attributed to oxymetazoline because they were not present in rats given tetracaine alone.

9.2 Embryonic Fetal Development

- RHOFADE™ PI, 2017

Effects on embryo-fetal development were evaluated in rats and rabbits following oral administration of oxymetazoline hydrochloride during the period of organogenesis.

Oxymetazoline hydrochloride did not cause adverse effects to the fetus at oral doses up to 0.2 mg/kg/day in pregnant rats during the period of organogenesis (3 times the MRHD for RHOFADE™ on an AUC comparison basis).

Oxymetazoline hydrochloride did not cause adverse effects to the fetus at oral doses up to 1 mg/kg/day in pregnant rabbits during the period of organogenesis (73 times the MRHD for RHOFADE ™ on an AUC comparison basis). Maternal toxicity, such as decreased maternal body weight, was produced at the high dose of 1 mg/kg/day in pregnant rabbits and was associated with findings of delayed skeletal ossification.

- KOVAZANE™ PI, 2016

In an embryo-fetal development study, pregnant rats were administered subcutaneous doses of oxymetazoline HCl only at 0.1 mg/kg, tetracaine HCl only at 7.5 mg/kg, or oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg/day in combination with 7.5 mg/kg tetracaine HCl during the period of organogenesis (Gestational Days [GD] 7-17).

Oxymetazoline HCl treatment at 0.1 mg/kg/day caused reduced fetal weight and structural abnormalities including external and skeletal malformations (e.g., short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs), and variations (e.g., irregularly shaped arches and increased bifid centra in thoracic vertebrae, and unossified forelimb phalanx) in the presence of maternal toxicity (reduced food consumption, body weight gain, and absolute body weight); however, the structural abnormality findings cannot be clearly attributed to the maternal toxicity. Adverse developmental effects were not observed when pregnant rats were co-administering the same dose of oxymetazoline HCl in combination with 7.5 mg/kg/day tetracaine HCl, or with 7.5 mg/kg/day tetracaine HCl alone. The no-observed-adverse-effect-level (NOAEL) for fetal effects was 0.03 mg/kg/day oxymetazoline HCl.

9.3 Prenatal and Postnatal Development

- RHOFADE™ PI, 2017

In a rat peri- and post-natal development study, oxymetazoline hydrochloride was orally administered to pregnant rats once daily from gestation day 6 through lactation day 20.

Maternal toxicity was produced at the high dose of 0.2 mg/kg/day (3 times the MRHD for RHOFADE™ on an AUC comparison basis) in pregnant rats and was associated with an increase in pup mortality and reduced pup body weights.

Delayed sexual maturation was noted at 0.1 and 0.2 mg/kg/day (2 times and 3 times the MRHD for RHOFADE™ on an AUC comparison basis, respectively). Oxymetazoline hydrochloride did not have any adverse effects on fetal development at a dose of 0.05 mg/kg/day (one-half of the MRHD for RHOFADE™ on an AUC comparison basis).

Oxymetazoline was detected in the milk of lactating rats.

- KOVAZANE™ PI, 2016

In a peri- and post-natal development study, pregnant rats were given subcutaneous doses of oxymetazoline HCl only at 0.1 mg/kg/day, tetracaine HCl only at 7.5 mg/kg/day, and oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg/day in combination with 7.5 mg/kg/day tetracaine HCl from GD 7 to Lactation Day [LD] 20 (corresponding to the beginning of organogenesis through parturition and subsequent pup weaning).

Oxymetazoline HCl treatment decreased the mean number of implant sites/litter at $\geq$ 0.03 mg/kg when administered with 7.5 mg/kg tetracaine HCl (approximately 9%) and without tetracaine HCl (5.5%), which resulted in a reduction in live litter sizes in these groups.

At the end of the lactation period, fetal body weights were significantly decreased at 0.1 mg/kg oxymetazoline HCl when administered alone (19%) and co-administered with 7.5 mg/kg/day tetracaine HCl (11%).

Maternal toxicity (e.g., mortality and reduced body weight gain, absolute body weight and food consumption) occurred in groups administered 0.1 mg/kg/day oxymetazoline HCl; however, the adverse developmental findings observed at this dose could not clearly be attributed to the maternal toxicity. There were no adverse effects on sexual maturation, neurobehavioral, or reproductive function in the offspring at any maternal dose. The no-effect level for oxymetazoline HCl for maternal reproduction was 0.01 mg/kg/day and for pup growth and development was 0.03 mg/kg/day.

Concentrations of oxymetazoline was measured in the milk of lactating rats at approximately 2 hours post-dose on LD 15. The concentrations of oxymetazoline were generally dose dependent reaching levels of 2.5, 7.0, and 33.8 ng/mL at 0.01, 0.03, and 0.1 mg/kg/day, respectively.

10 Special Toxicology Studies

Local tolerance

- Brånemark, 1975
The local tissue effects of oxymetazoline (Nezeril nasal spray) were assessed in hamsters and rabbits via topical application to intact epithelium, to a micro-wound with exposed connective tissue capillaries, *via* local injection (0.05 mL in the hamster cheek pouch), and *via* sub-perichondrial injection (0.1 mL in the connective tissue of the ear in the rabbit). The dosages were not indicated.

The authors concluded that oxymetazoline produced little microcirculatory disturbance unless there was already previous injury to the tissue. Injected into the sub-perichondrium of the rabbit ear, oxymetazoline produced minor microvascular damage.

11 Integrated Summary and Safety Evaluation

RevitaLid, Inc. has developed RVL-1201 (oxymetazoline hydrochloride ophthalmic solution, 0.1%) for the treatment of acquired blepharoptosis. The proposed clinical dose is 1 drop of 35 µL per eye once daily, equivalent to approximately 35 μg/eye/day (or 1.16 μg/kg/day i.e. 43 μg/M^2/day for a 60 kg subject).

RevitaLid is pursuing the 505(b)(2) approval pathway proposing RHOFADE™ (oxymetazoline HCl) Cream as the RLD.

The Applicant has fulfilled the preclinical regulatory requirements with 2 original rabbit studies that include ocular toxicity, updated the pharmacology and toxicology of the compound based on the literature, and relied on the LD for the genotoxicity, carcinogenicity, reproductive and developmental toxicity findings. The clinically relevant information pertaining to RVL-120 is summarized below.

Pharmacology

In vitro binding studies with human cells have found that oxymetazoline acts as an α1A- and α2A-adrenoceptor agonist with IC_{50} values of 0.02, and 0.58 μM (or 5.94 ng/mL and 172 ng/mL, respectively). These receptors are widely distributed throughout the body in all animal species. There is species specificity in the abundance and location of receptors in different tissues.

In the human eye, α2 adrenergic receptors are the predominant subtype in the Mueller's muscle of the upper eyelid, and both agonist binding of the α1 and α2 adrenergic receptors at this location can mediate muscle contraction. The α1 adrenergic receptor was also found to play a role in canine and murine models of ptosis.

At clinically relevant dosages, oxymetazoline produced mydriasis and decreased IOP in different animal models:

1) Oxymetazoline induced a contractile response in albino and pigmented rabbits, and in human iris dilator muscle *in vitro* with EC_{50s} of 42, 62, and 56 ng/mL, respectively. *In vivo* it also caused a dose-related increase in pupil diameter of up to a maximum of 71% at a dose of 44.5 μg/eye in an albino rabbit study.

2) In the albino rabbit study, oxymetazoline caused an initial dose-related rapid IOP increase of up to 4.5 mm Hg at 4.45 μg/eye, lasting for 15-30 minutes, followed by IOP decrease from baseline over 8 hours. The effects on mydriasis and IOP in the rabbit was thought to be mediated by 2 different types of α adeno-receptors.

3) Decrease in IOP was also observed *in vivo* in monkeys with as little as 1 drop of 60 μg oxymetazoline *per* eye. The effect was time and dose dependent. In both rabbit and monkey, the maximum effect tended to reach a plateau at higher dosages.

Mydriasis or decrease IOP have not been observed in clinical studies, so far.

As an α-adrenergic agonist, oxymetazoline is an arterial vasoconstrictor with high degree of tissue selectivity and species-specificity in potency. For example, oxymetazoline induced contractions in the aorta *in vitro* with an EC_{50} of 3.27 ng/mL in the rabbit compared to the rat's EC_{50} of 65.3 ng/mL.

Other clinically relevant effects of α-adrenergic agonists (including oxymetazoline) on the respiratory and cardiovascular systems were observed *in vivo* in animal models:

1) In the respiratory system, oxymetazoline decreased nasal and sinus mucosa blood flow in the New Zealand White rabbit, when administered intranasally, with 50% reduction

observed at 0.1 mg/mL (equivalent to commercial nasal spray) compared to saline. Blood pressure and heart rate were unchanged. The authors hypothesized that it was due to oxymetazoline-induced vasoconstriction of the arteries penetrating the maxillary sinus ostium. Also, oxymetazoline dose-dependently constricted the bronchial mucosa vasculature and decreased pulmonary resistance when administered IV in anesthetized dogs at the doses of 0.5, 1.0 and 2.0 µg/kg.

2) In the cardiovascular system, oxymetazoline administered IV at 1, 5, and 10 µg/kg significantly increased blood pressure and decreased heart rate in normal conscious rats. Dr. Maria Rivera noted in her review that the dose of 1 µg/kg in the rat was equivalent to a human dose of 0.16 µg/kg (based on BSA conversionn). Assuming 100% systemic absorption after ocular administration, this dose was 6 times less that the MRHD of 1 µg/kg for RVL-120 (based on a 60 kg body weight and a 35 µL drop/eye oxymetazoline 0.1%). No effects on blood pressure and heart rate have been observed in clinical studies with the test compound so far.

3) In addition, RHOFADE ™ label contains a cautionary language for patients with cardiovascular disease, orthostatic hypotension, uncontrolled hypertension or hypotension. Increased blood pressure and bradycardia has been observed with oxymetazoline in a variety of animal species due to central and peripheral effects on α-adrenergic receptors.

PK/ADME/TK

After a single dose of 50 µL 0.025% ^{14}C-labeled oxymetazoline administered topically bilaterally to the eyes of 12 New Zealand White rabbits, 0.006% of the original drug concentration was found in the aqueous humor 30 minutes after instillation. Radioactivity was also low in other intraocular tissues including the ciliary body, iris, choroid, retina, lens, and vitreous humor. The highest concentrations of radiolabeled material were found in the external ocular tissues (cornea, conjunctiva, sclera, nictitating membrane) with radioactivity reaching a pic of 0.55% of the dose in the cornea after 1 hour and gradually decreasing after that. Thirty per cent of the dose was excreted in the urine unchanged after 24 hours.

Pooled human, Sprague-Dawley rat, or NZW rabbit liver S9 fractions and microsomes were analyzed after incubation with 10 µ L of 50 µ M oxymetazoline. Four major metabolites (M1, M2, M6 and M7) and 7 minor metabolites (M3. M4, M5, M8, M9, M10, and M11) of oxymetazoline were detected with the most extensive metabolism observed in the rabbit. There were no unique human metabolites.

M6 and M7 were glutathione conjugates of oxymetazoline and M2, respectively, indicating the capability of oxymetazoline to undergo bioactivation to reactive intermediate species. M6 was identified as the glutathione conjugate of a p-quinone methide.

In humans CYP2C19 catalyzed the bioactivation of oxymetazoline to reactive intermediates. Considering that p-quinone methide is abundant in liver S9 incubations of oxymetazoline for the three animal species, the toxicological significance of this reactive intermediate cannot be overlooked completely.

In toxicokinetics, systemic blood parameters evaluated at Day 1 and Day 182 of a 26-week BID- or TID- ocular dose study of oxymetazoline in the rabbit indicated that on Day 1 AUC increased in an approximately dose proportional manner for both males and females. On Day 182, the exposure increased in a proportional manner for males and higher than dose proportional manner for females.

C_{max} did not increase with dose for either sex on Day 1 or for males on Day 182; C_{max} increased in an approximately dose proportional manner for females on Day 182.

There was no significant accumulation after BID administration on either Day 1 or Day 182 whereas the compound accumulated in females after TID dosing for 182 days.

Toxicology

In a pivotal 26-week BID- or TID- ocular dose study of oxymetazoline in the rabbit, the NOAEL for RVL-1201 was 0.21 mg/animal/day or 105 µg/eye/day which provided a local safety margin of 3X based on a MRHD of 35 µg/eye/day. In term of systemic toxicity, at the NOAEL of 0.117 mg/kg/day in a 1.8-kg rabbit (or 0.038 mg/kg HED based on BSA conversion), the safety margin was 32.4X based on a MRHD of 1.16 µg/kg/day for a 60 kg subject. At the NOAEL, systemic exposures achieved at Day 182 were 1516, and 1984 pg/mL for $C_{max,}$ and 10484 and 14889 pg.h/mL for AUC_{0-24} in males and female rabbits, respectively. Incidences of findings of fibrin in the anterior chamber of the eye were drug-treatment and procedural (ERG)-related. They were reversible.

Oxymetazoline was not found to be genotoxic or carcinogenic.

Oxymetazoline was a reproductive and developmental toxicant in the rat and a developmental toxicant in the rabbit. Male and female fertility in the rat was affected at doses as low as 0.03, and 0.01 mg/kg/day subcutaneously (HED of 5 and 1.7 µg/kg/day based on BSA conversion) in males and females, respectively. Developmental toxicity in the fetus was observed in the rabbit and the rat in the presence of maternal toxicity. However, in the rat, the structural abnormality findings in fetuses from dams treated with

0.1 mg/kg/day SC (HED of 17 µg/kg/day based on BSA conversion) could not be clearly attributed to the maternal toxicity. The NOAEL of oxymetazoline for postnatal pup growth and development in the rat was 0.05 mg/kg/day SC (HED of 8.3 µg/kg/day based on BSA conversion). Oxymetazoline was excreted into the milk of lactating rats.

References

Aboud R, Shafii M, Docherty JR. Investigation of the subtypes of α1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. Br J Pharmacol 1993; 109:80-87.

Akerlund A, Arfors KE, Bende M, Intaglietta M. Effect of oxymetazoline on nasal and sinus mucosal blood flow in the rabbit as measured with laser-Doppler flowmetry. Ann Otol Rhinol Laryngol. 1993 Feb;102(2):123-126.

Armstrong JM, Lefevre-Borg F, Scatton B, Cavero I. Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. J Pharmacol Exp Ther 1982; 223(2):524-535.

Bayorh MA, Ogbolu E, Socci RR. Cardiovascular effects of oxymetazoline and UK14,304 in conscious and pithed rats. Clin Exp Hypertens. 1997 May;19(4):445-460.

Beck-Speier I, Dayal N, Karg E, Maier K, Schumann G, Semmler M, Koelsch S. Oxymetazoline Inhibits Proinflammatory Reactions: Effect on Arachidonic Acid-Derived Metabolites. J Pharmacol Exp Ther 2006; 316(2):843-851.

Boudier HS, Smeets G, Brouwer G, van Rossum J. Central and peripheral alpha-adrenergic activity of imidazoline derivatives. Life Sci. 1974 Sep 1;15(5):887-899.

Brånemark PI, Hallén O, Nilsson K, Lindström J, Brunell J. Local tissue effects of surface-applied ENT drugs. Acta Otolaryngol.1975 Jul-Aug:80(1-2):128-36.

Briand R, Yamaguchi N, Gagne J, Nadeau R, de Champlain J. α2-adrenoceptor modulation of catecholamine and neuropeptide Y responses during hemorrhagic hypotension in anaesthetized dogs. J Autonomic Nervous Syst 1990; 30(2):111-122.

Campbell WR, Potter DE. Potential role of imidazoline (I1) receptors in modulating aqueous humor dynamics. J Ocul Pharmacol 1994; 10(1):393-402.

Campbell WR, Potter DE. Centrally mediated ocular hypotension: potential role of imidazoline receptors. Ann N Y Acad Sci 1995; 763:463-485.

Carrillo L, Kishimoto T, Aviado DM. The nasal and bronchopulmonary effects of oxymetazoline and KB227. Ann Otol Rhinol Laryngol. 1969 Apr;78(2):415-424.

Chu TC, Ogidigben MJ, Potter DE. Oxymetazoline: potential mechanisms of inhibitory effects on aqueous humor dynamics. Pharmacology 1996; 53(4):259-270.

Deniard MJ, Meignen J, DeFeudis FV. Reversal of reserpine-induced ptosis in the mouse by α1- adrenoceptor agonists. Psychopharmacology 1983; 80:243-248.

Dowty ME, Dietsch CR. Improved prediction of in vivo peroral absorption from in vitro intestinal permeability using an internal standard to control for intra- and inter-rat variability. Pharm Res. 1997,14(12):1792-1797.

Doxey JC, Frank LW, Hersom AS. Studies on the pre- and post-junctional activities of alpha-adrenoreceptor agonists and their cardiovascular effects in the anaesthetized rat. J Auton Pharmacol. 1981 Mar;1(2):157-169.

Duzman E, Anderson J, Vita JB, Lue JC, Chen CC, Leopold IH. Topically applied oxymetazoline. Ocular vasoconstrictive activity, pharmacokinetics, and metabolism. Arch Ophthalmol. 1983, 101(7):1122-1126.

Esmaeli-Gutstein B, Hewlett BR, Pashby RC, Ostreicher J, Harvey JT. Distribution of adrenergic receptor subtypes in the retractor muscles of the upper eyelid. Ophthalmic Plast Reconstr Surg 1999; 15(2):92-99.

Fulop K, Zadori Z, Ronai AZ, Gyires K. Characterisation of α2-adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric mucosal defense. Eur J Pharmacol 2005; 528(1-3):150-157.

Garibaldi DC, Hindman HB, Grant MP, Iliff NT, Merbs SL. Effect of 0.5% apraclonidine on ptosis in Horner syndrome. Ophthalmic Plast Reconstr Surg 2006; 22(1):53-55.

Goldenthal EI. A compilation of LD_{50} values in newborn and adult animals. Toxicol Appl Pharmacol. 1971 Jan;18(1):185-207.

Haenisch B, Walstab J, Herberhold S, Bootz F, Tschaikin M, Ramseger R, Bonisch H. Alpha adrenoceptor agonistic activity of oxymetazoline and xylometazoline. Fundam Clin Pharmacol (2010); 24(6):729-739.

Halpin PA, Renfro JL. Renal organic anion secretion: evidence for dopaminergic and adrenergic regulation. Ann J Physiol 1996; 271: R1372-1379.

Hayes FJ, Baker TR, Dobson RL, Tsueda MS. Rapid liquid chromatographic-mass spectrometric assay for oxymetazoline in whole rat blood. J Chromatogr A. 1995, 692(1-2):73-81.

Hybbinette JC, Mercke U. Effects of sympathomimetic agonists and antagonists on mucociliary activity. Acta Otolaryngol. 1982 Jul-Aug;94(1-2):121-130.

Hikasa Y, Ogasawara S, Takase K. Alpha adrenoceptor subtypes involved in the emetic action in dogs. J Pharmacol Exp Ther 1992; 261(2):746-754.

Isaacson G, Buttaro B, Mazeffa V, Li G, Frenz D. Oxymetazoline Solutions Inhibit Middle Ear Pathogens and Are Not Ototoxic. Ann Otol Rhinol Laryngol. 2005;114(8):645-651.

Johns A, Chlumecky J, Cottle MK, Paton DM. Effect of chemical sympathectomy and adrenergic agonists on the fertility of mice. Contraception. 1975 May;11(5):563-70.

Kobinger W. Central blood pressure regulation. Involvement of presynaptic or postsynaptic, alpha 1- or alpha 2-adrenoceptors? Chest. 1983 Feb;83(2 Suppl):296-9.

Kruszewska H, Zareba T, Tyski S. Search of antimicrobial activity of selected nonantibiotic drugs. Acta Pol Pharm. 2002 Nov-Dec;59(6):436-439.

Liu L, Coupar IM. Evidence for functional α2-adrenoceptors in the rat intestine. Br J Pharmacol 1996; 117:787-792.

Loomis CW, Vyas D, Sherman SE. Intrathecal oxymetazoline does not produce neurotoxicity in the spinal cord of the rat. Brain Res 1992; 599:73-82.

Mahajan MK, Uttamsingh V, Daniels JS, Gan LS, LeDuc BW, Williams DA. In vitro metabolism of oxymetazoline: evidence for bioactivation to a reactive metabolite. Drug Metab Dispos, 2011b, 39(4):693-702.

Mahajan MK, Uttamsingh V, Gan LS, Leduc B, Williams DA. Identification and characterization of oxymetazoline glucuronidation in human liver microsomes: evidence for the involvement of UGT1A9. J Pharm Sci. 2011a,100(2):784-793.

Malta E, McPherson GA. Raper C. Comparison of pre-junctional α-adrenoceptors at the neuromuscular junction with vascular post-junctional α-receptors in cat skeletal muscle. Br J Pharmacol 1979; 65:249-256.

Min YG, Kim HS, Suh SH, Jeon SY, Son YI, Yoon S. Paranasal sinusitis after long-term use of topical nasal decongestants. Acta Otolaryngol. 1996 May;116(3):465-471.

Mucinex Nasal Spray [material safety data sheet]. Parsippany, NJ: Reckitt Benckiser Inc.; 2009.

Murray DL, Leopold IH. Alpha-adrenergic receptors in rabbit eyes. J Ocul Pharmacol. 1985 Spring;1(1):3-18.

Nasal A, Frackowiak T, Petrusewicz J, Bucińska A, Kaliszan R. Mydriasis elicited by imidazol(in)e alpha 2-adrenomimetics in comparison with other adrenoceptor-mediated effects and hydrophobicity. Eur J Pharmacol. 1995 Feb 14;274(1-3):125-132.

Nickenig G, Ko Y, Nettekoven W, Appenheimer M, Schiermeyer B, Vetter H, Sachinidis A. Oxymetazoline enhances epidermal- and platelet-derived growth factor-induced DNA synthesis. Atherosclerosis. 1994 Jan;105(1):71-77.

Patil PN, Ishikawa H. Antimuscarinic action of oxymetazoline on human intraocular muscles. J Ocul Pharmacol Ther 2004; 20(4):328-32.

O'Rourke M, Kearns S, Docherty JR. Investigation of the actions of chloroethylclonidine in rat aorta. Br J Pharmacol 1995; 115:1399-1406.

RHOFADE ™ (oxymetazoline hydrochloride) cream, 1% (Prescribing Information), Allergan, Irvine, CA, 2017.

Ruffolo R, Waddell JE. Receptor interactions of imidazolines: α-adrenoceptors of rat and rabbit aortae differentiated by relative potencies, affinities and efficacies of imidazoline agonists. Br J Pharmacol 1982; 77:169-176.

Sherman S, Loomis C, Milne B, Cervenko F. Prolonged spinal analgesia in the rat with the alpha-adrenoceptor agonist oxymetazoline. Eur J Pharmacol. 1987 Aug 4;140(1):25-32.

Shujaa N, Al-Khrasani M, Zadori AS, Rossi M, Matyus P, Nemeth J, Hein L, Gyires K. α2-adrenoceptor agonist-induced inhibition of gastric motor activity is mediated by α2-adrenoceptor subtype in the mouse. Neurochem Int 2011; 58(6):708-713.

Sugden D, Anwar N, Klein D. Rat pineal α1-adrenoceptor subtypes: studies using radioligand binding and reverse transcription-polymerase chain reaction analysis. Br J Pharmacol 1996; 118:1246-1252.

Suh SH, Chon KM, Min YG, Jeong CH, Hong SH. Effects of topical nasal decongestants on histology of nasal respiratory mucosa in rabbits. Acta Otolaryngol. 1995 Sep;115(5):664-671.

Timmermans PB, Fluitman PH, Mackaay JCJ, van Zwieten PA. Hypotensive and bradycardiac effects of classical alpha-sympathomimetic drugs upon intravenous administration to pentobarbitone-anaesthetized rats. Arch Int Pharmacodyn Ther. 1978; 231(1):98-103.

Wang RF, Lee PY, Taniguchi T, Becker B, Podos SM, Serle JB, Mittag TW. Effect of oxymetazoline on aqueous humor dynamics and ocular blood flow in monkeys and rabbits. Arch Ophthalmol 1993; 111(4):535-538.

Westerveld G, Scheeren R, Dekker I, Griffioen D, Voss H, Bast A. Anti-oxidant actions of oxymethazoline and xylomethazoline. Eur J Pharmacol.1995 Apr;291(1):27-31.

Wikberg-Matsson A, Simonsen U. Potent alpha(2A)-adrenoceptor-mediated vasoconstriction by brimonidine in porcine ciliary arteries. Invest Ophthalmol Vis Sci. 2001 Aug;42(9):2049-2055.

Xuan B, Chiou GC. Efficacy of oxymetazoline eye drops in non-infectious conjunctivitis, the most common cause of acute red eyes. J Ocul Pharmacol Ther. 1997 Aug;13(4):363-367.

Yano S, Hirose M, Nakada T, Nakayama J, Matsuo K, Yamada M. Selective α1A adrenoceptor stimulation induces Mueller's smooth muscle contraction in an isolated canine upper eyelid preparation. Curr Eye Res 2010; 35(5):363-369.

Zadori Z, Shujaa N, Fulop K, Dunkel P, Gyires K. Pre- and postsynaptic mechanisms in the clonidine- and oxymetazoline-induced inhibition of gastric motility in the rat. Neurochem Int 2007; 51(5):297-305.

APPEARS THIS WAY ON ORIGINAL

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MURIEL J SAULNIER
06/15/2020 04:36:58 PM

LORI E KOTCH
06/16/2020 02:53:20 AM