

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-398**

**PHARMACOLOGY REVIEW(S)**

**Division of Anti-Infective and Ophthalmology Products**

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Memorandum to the file for NDA 21-398

Date: September 26, 2007

From: Zhou Chen, MD, PhD

To: Alison Rodgers

Through: Amy Ellis, PhD, Acting Pharmacology/Toxicology Team Leader

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**Sponsor:** Allergan Inc., 2525 Dupont Drive, P.O.Box 19534, Irvine, CA 92623-9534

**Drug:** Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%) ophthalmic solution

**Drug Class:** Brimonidine tartrate:  $\alpha_2$ -agonist

Timolol:  $\beta$ -adrenoceptor antagonist

The following are changes recommended by the reviewing pharmacologist to the label for Combigan™ .

**CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY:**

With brimonidine tartrate, no compound-related carcinogenic effects were observed in either mice or rats following a 21-month and 24-month study, respectively. In these studies, dietary administration of brimonidine tartrate at doses up to 2.5 mg/kg/day in mice and 1.0 mg/kg/day in rats achieved 150 and 210 times, respectively, the plasma  $C_{max}$  drug concentration in humans treated with one drop COMBIGAN™ into both eyes twice daily, *the recommended daily human*  *dose.*

In a two-year study of timolol maleate administered orally to rats, there was a statistically significant increase in the incidence of adrenal pheochromocytomas in male rats administered 300 mg/kg/day [*approximately 25,000 times the maximum recommended human ocular dose of 0.012 mg/kg/day on a mg/kg basis (MRHOD)*]. Similar differences were not observed in rats administered oral doses equivalent to approximately 8,300 times *the*

In a lifetime oral study of timolol maleate in mice, there were statistically significant increases in the incidence of benign and malignant pulmonary tumors, benign uterine polyps and mammary adenocarcinomas in female mice at 500 mg/kg/day, (approximately 42,000 times *the MRHOD*), but not at 5 or 50 mg/kg/day (approximately 420 to 4,200 times higher, respectively, than *the MRHOD*). In a subsequent study in female mice, in which post-mortem examinations were limited to the uterus and the lungs, a statistically significant increase in the incidence of pulmonary tumors was again observed at 500 mg/kg/day.

The increased occurrence of mammary adenocarcinomas was associated with elevations in serum prolactin which occurred in female mice administered oral timolol at 500 mg/kg/day, but not at doses of 5 or 50 mg/kg/day. An increased incidence of mammary adenocarcinomas in rodents has been associated with administration of several other therapeutic agents that elevate serum prolactin, but no correlation between serum prolactin levels and mammary tumors has been established in humans. Furthermore, in

adult human female subjects who received oral dosages of up to 60 mg of timolol maleate (the maximum recommended human oral dosage), there were no clinically meaningful changes in serum prolactin.

Brimonidine tartrate was not mutagenic or clastogenic in a series of in vitro and in vivo studies including the Ames bacterial reversion test, chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells, and three in vivo studies in CD-1 mice: a host-mediated assay, cytogenetic study, and dominant lethal assay.

Timolol maleate was devoid of mutagenic potential when tested in vivo (mouse) in the micronucleus test and cytogenetic assay (doses up to 800 mg/kg) and in vitro in a neoplastic cell transformation assay (up to 100 µg/mL). In Ames tests the highest concentrations of timolol employed, 5,000 or 10,000 µg/plate, were associated with statistically significant elevations of revertants observed with tester strain TA100 (in seven replicate assays), but not in the remaining three strains. In the assays with tester strain TA100, no consistent dose response relationship was observed, and the ratio of test to control revertants did not reach 2. A ratio of 2 is usually considered the criterion for a positive Ames test.

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Reproduction and fertility studies in rats with timolol maleate demonstrated no adverse effect on male or female fertility at doses up to approximately 100 times the systemic exposure following the maximum recommended human ophthalmic dose.

#### **PREGNANCY:**

Pregnancy Category C: Teratogenicity studies have been performed in animals.

Brimonidine tartrate was not teratogenic when given orally during gestation days 6 through 15 in rats and days 6 through 18 in rabbits. The highest doses of brimonidine tartrate in rats (1.65 mg/kg/day) and rabbits (3.33 mg/kg/day) achieved AUC exposure values 580 and 37-fold higher, respectively, than similar values estimated in humans treated with COMBIGAN™

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Teratogenicity studies with timolol in mice, rats, and rabbits at oral doses up to 50 mg/kg/day (4,200 times *the MRHOD*) demonstrated no evidence of fetal malformations. Although delayed fetal ossification was observed at this dose in rats, there were no adverse effects on postnatal development of offspring. Doses of 1000 mg/kg/day (83,000 times *the MRHOD*) were maternotoxic in mice and resulted in an increased number of fetal resorptions. Increased fetal resorptions were also seen in rabbits at doses 8,300 times *the MRHOD* without apparent maternotoxicity.

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this page is the manifestation of the electronic signature.**  
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/s/  
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Zhou Chen  
9/26/2007 01:37:24 PM  
PHARMACOLOGIST

Amy, This is the memo we discussed this afternoon.  
Thanks. Zhou

Amy Ellis  
9/26/2007 02:40:23 PM  
PHARMACOLOGIST  
Acting TL on behalf of Wendelyn Schmidt



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number: NDA 21-398  
Review number: 001  
Sequence number/date/type of submission: 000/June 29, 2006 and October 3, 2006/Commercial  
Drug name: Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%) ophthalmic solution  
Indication: For reduction of elevated intraocular pressure in patients with glaucoma or ocular hypertension  
Sponsor and/or agent: Allergan Inc., 2525 Dupont Drive, P.O.Box 19534, Irvine, CA 92623-9534  
Division name: Anti-Infective and Ophthalmology Products

Pharm/Tox reviewer: Zhou Chen, MD, PhD  
Pharm/Tox supervisor: Terry Peters, DVM  
Division director: Janice Soreth, MD  
Project manager: Alison Rodgers

Date of review submission to Division File System (DFS): October 18, 2006

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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

#### **A. Recommendation on approvability**

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

#### **B. Recommendation for nonclinical studies**

No additional studies are necessary.

#### **C. Recommendations on labeling**

Several modifications of labeling in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section are recommended.

### **CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY:**

With brimonidine tartrate, no compound-related carcinogenic effects were observed in either mice or rats following a 21-month and 24-month study, respectively. In these studies, dietary administration of brimonidine tartrate at doses up to 2.5 mg/kg/day in mice and 1.0 mg/kg/day in rats achieved 150 and 210 times, respectively, the plasma  $C_{max}$  drug concentration in humans treated with one drop COMBIGAN™ into both eyes twice daily, *the recommended daily human dose.*

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Teratogenicity studies with timolol in mice, rats, and rabbits at oral doses up to 50 mg/kg/day (4,200 times *the MRHOD*) demonstrated no evidence of fetal malformations. Although delayed fetal ossification was observed at this dose in rats, there were no adverse effects on postnatal development of offspring. Doses of 1000 mg/kg/day (83,000 times *the MRHOD*) were maternotoxic in mice and resulted in an increased number of fetal resorptions. Increased fetal resorptions were also seen in rabbits at doses 8,300 times *the MRHOD* without apparent maternotoxicity.

## **II. Summary of nonclinical findings**

### **A. Brief overview of nonclinical findings**

Nonclinical data with the combination drug product indicated that the ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination and the single drug formulations. No biologically significant toxic effects were observed in animal studies, and no toxicological interaction between these two drugs was noted. Transient sedation was the only

systemic effect observed in animals topically treated with the combination (brimonidine tartrate 0.2%/timolol 0.5%) or with brimonidine 0.2% alone, and was considered an exaggerated pharmacological effect of brimonidine. In conclusion, nonclinical data support the safety of the combination drug product.

**B. Pharmacologic activity**

Brimonidine tartrate is a selective  $\alpha$ -2 adrenergic agonist, while timolol is a non-selective  $\beta$ -adrenergic receptor antagonist. With different sites and mechanisms of action, both compounds can lower intraocular pressure with a rapid onset of action. It is suggested that brimonidine tartrate decreases IOP by reducing aqueous humor production and increasing nonpressure-dependent uveoscleral outflow, while timolol decreases IOP predominantly by reducing aqueous humor formation. It is expected that there will be an added IOP-lowering effect when timolol and brimonidine are used together.

**C. Nonclinical safety issues relevant to clinical use**

Summarized from nonclinical studies, both combination and individual drug formulations were well tolerated. The nonclinical safety profile is similar between the combination and single-entity products. No toxicologically significant side effects were noted. There are no nonclinical safety concerns relevant to clinical use.

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On Original**

**2.6 PHARMACOLOGY/TOXICOLOGY REVIEW****2.6.1 INTRODUCTION AND DRUG HISTORY**

NDA number: NDA 21-398

Review number: 001

Sequence number/date/type of submission: 000/June 29, 2006 and October 3, 2006/Commercial

Information to sponsor: Yes ( X ) No ( )

Sponsor and/or agent: Allergan Inc., 2525 Dupont Drive, P.O.Box 19534, Irvine, CA 92623-9534

Manufacturer for drug substance: Allergan-Waco's physical facilities

--Brimonidine tartrate:

--Timolol maleate:

Reviewer name: Zhou Chen, MD, PhD

Division name: Division of Anti-Infective and Ophthalmology Products

Review completion date: October 18, 2006

Drug:

Trade name: Combigan™

Generic name (list alphabetically): Brimonidine tartrate 0.2%/Timolol 0.5% Ophthalmic Solution

Code name: Brimonidine tartrate: AGN-190342-LF, UK-14,304-18

Timolol maleate: AGN-196156-H

Chemical name: Brimonidine tartrate: 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate

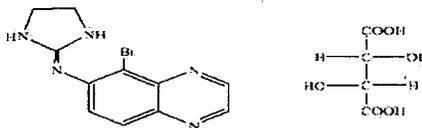
Timolol maleate: (-)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)-oxyl]-2-propanol maleate (1:1) salt

CAS registry number: Brimonidine tartrate: 59803-98-4

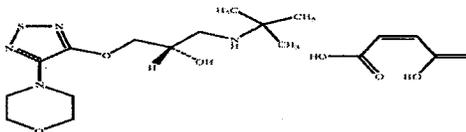
Timolol maleate: 26921-17-5

Molecular formula/molecular weight: Brimonidine tartrate: C<sub>15</sub>H<sub>16</sub>N<sub>5</sub>O<sub>6</sub>Br, MW: 442.24Timolol maleate: C<sub>17</sub>H<sub>28</sub>N<sub>4</sub>O<sub>7</sub>S, MW: 432.49

Structure: Brimonidine tartrate:



Timolol maleate:



Relevant INDs/NDAs/DMFs: INDs 58,460, 32,392/NDAs 20-490, 20-613, 21-262, 21-275/ANDAs 74-746, 74-747/

Drug class: Brimonidine tartrate:  $\alpha_2$ -adrenergic agonist  
 Timolol:  $\beta$ -adrenergic receptor antagonist

Indication: For reduction of elevated intraocular pressure in patients with glaucoma or ocular hypertension

Clinical formulation:

Ingredients	Concentration (% w/v)
Brimonidine tartrate	0.2
Timolol maleate, USP	0.68 (equal to 0.5% timolol free base)
Benzalkonium chloride, NF	0.005
Sodium phosphate, monobasic, USP	
Sodium phosphate, dibasic, USP	
Sodium hydroxide, NF	
Hydrochloric acid, NF	
Purified water, USP	

Route of administration: Topical, ocular

Proposed dosage: 1 drop (35  $\mu$ l), bid [For a 60 kg adult, total dose can reach 0.28 mg/patient/day or 0.0047 mg/kg (0.17 mg/m<sup>2</sup>) for brimonide tartrate, and 0.7 mg/patient/day or 0.0117 mg/kg (0.43 mg/m<sup>2</sup>) for timolol.]

Studies reviewed within this submission:

Toxicology:

- 1-month interim report: Combigan™ (brimonidine-tartrate 0.2%/timolol 0.5%): 3-month ocular toxicity study with impurity in rabbits
- Combigan™ (brimonidine tartrate 0.2 %/ timolol 0.5 %): Bacterial reverse mutation assay with an independent repeat assay
- Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%): *In vitro* mammalian chromosome aberration test

**Reviewer's comments:** The original NDA was submitted in September 2001. The pharmacology/toxicology review was finished in December 2001. In June 2006 and October 2006, the sponsor submitted new supplements. Three study reports for impurity were included in the pharmacology/toxicology section. For other nonclinical studies, please see the original Pharmacology/Toxicology Review for NDA 21-398 (Review number: 000).

2.6.2 PHARMACOLOGY

Brimonidine and timolol in this combination formulation are individually marketed drugs for lowering IOP in patients with glaucoma or ocular hypertension. The pharmacological studies for individual timolol and bromonidine have been reviewed under NDAs 20-613 and 21-262, and ANDA 74-747. Currently, the two marketed medications are often prescribed and used together clinically to achieve an added IOP-lowering effect. Therefore, no new nonclinical pharmacological studies were requested.

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the single drug formulations. The ocular distribution study showed that the distribution patterns of combination and single drug formulations were similar with the high concentrations of radioactivity in surface tissues and in the iris-ciliary body. Systemic concentrations of the radioactivity were low. For detailed study reviews, please see the original Pharmacology/Toxicology Review for NDA 21-398 (Review number: 000).

### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable.

### 2.6.6 TOXICOLOGY

#### Combigan™ (brimonidine-tartrate 0.2%/timolol 0.5%): 3-month ocular toxicity study with impurity in rabbits

**Key study findings:** Combigan™ ophthalmic solution containing exaggerated (a newly identified impurity with the proposed specification level of , produced neither drug- nor impurity-related ocular irritation or toxicity.

Study #: TX06003

Conducting laboratory and location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Date of study initiation: January 4, 2006

GLP compliance: Yes

QA reports: Yes ( X ) no ( )

The purpose of this study was to evaluate the ocular effects of Combigan™ ophthalmic solution containing high levels of ) when given topically 3 times daily in one eye for 3 months to female New Zealand White (NZW) rabbits.

**Drug:** Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%) ophthalmic solution containing exaggerated ) degradant impurity, Formulation #: Lot #:

**Control article:** Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%) eye drops solution with a level of Formulation #: Lot #:

Strains/species: Female New Zealand White rabbits

Age: 4 months old

Weight: 2.77-3.38 kg

Number/sex/group or time point (main study): 12 females/group (6 animals/group were terminated as interim sacrifice animals after one month of treatment.)

Dose: One drop (40 µl), left eye only, 3 times per day (at 3-hr intervals) x 3 months. The contralateral right eye served as an untreated control. The first day of dosing was designated as Day 1.

Route: Ocular, topical

Group	N	Treatment	Dosing frequency
1	12 females (6 for 1-month treatment and 6 for 3-month treatment)	Combigan™ eye drops solution containing [redacted]	One drop, TID, left eye
2	12 females (6 for 1-month treatment and 6 for 3-month treatment)	Combigan™ eye drops solution containing impurity [redacted]	One drop, TID, left eye

**Observations and times:**

Mortality: Twice daily

Clinical observations: At least once daily

Ocular observations: Daily during Week 1 and weekly thereafter

Ophthalmic examinations (slit lamp biomicroscopy, pupillary reflex, IOP, and indirect ophthalmoscopy): Pretest and at the end of Month 1 and Month 3 of treatment

Body weight and food consumption: Weekly

Terminal sacrifice: 6/group, at the end of one-month treatment and 3-month treatment

Gross pathology: Only ocular tissues from all animals terminated at the end of 1 or 3 months of treatment were examined.

Histopathology: Only ocular tissues from all animals terminated at the end of 1 or 3 months of treatment were examined.

**Results:**

Mortality: No mortality occurred in any group during the study.

Clinical observations: Neither drug- nor impurity-related effects on clinical observations were noted during the three months of the study.

Ocular examinations: Neither clinically significant drug- nor impurity-related effects on conjunctival congestion, ocular discharge, or swelling were observed during the study.

Ophthalmic examinations: No treatment-related, toxicologically significant findings were noted with slit lamp biomicroscopy, tonometry, pupillary reflex, or indirect ophthalmoscopic examinations.

Body weight and food consumption: No drug- or impurity-related effects on body weight and food consumption were observed during this study.

Gross pathology: There were no drug- or impurity-related abnormal findings.

Histopathology: No toxicologically significant findings were noted in the eye or ocular adnexal structures.

In summary, to evaluate the safety of impurity [redacted] female NZW rabbits were given one drop of Combigan™ ophthalmic solution containing exaggerated [redacted] topically in the left

eye 3 times daily for 3 months. No drug-or impurity-related, toxicologically significant ocular or systemic toxicity occurred in the study.

**Combigan™ (brimonidine tartrate 0.2 %/ timolol 0.5 %): Bacterial reverse mutation assay with an independent repeat assay**

**Key findings:** Combigan™ ophthalmic solution containing exaggerated [redacted] degradant impurity was not considered mutagenic under the present testing conditions.

**Study #:** TX06017

**Conducting laboratory and location:** [redacted]

**Date of study initiation:** 2/15/2006

**GLP compliance:** yes

**QA reports:** yes ( X ) no ( )

**Drug:** Combigan™ (brimonidine tartrate 0.2%/timolol maleate as timolol 0.5%) ophthalmic solution containing exaggerated [redacted] degradant impurity, Lot #: [redacted]

**Reference article:** Combigan™ eye drop solution with a level of [redacted] below the qualification threshold  
Lot #: [redacted]

The purpose of this study was to evaluate the mutagenic potential of a combination drug product containing brimonidine, timolol and exaggerated levels of the impurity [redacted]

**Methods**

**Strains/species/cell line:** *Salmonella typhimurium* strains TA1535, TA98, TA100, TA102, and TA1537

**Doses used in definitive study:** 3.3, 10, 33, 100, 333 and 1000 µl per plate. At 1000 µl/plate, the dose of [redacted] was 136 µg per plate

**Basis of dose selection:** Toxicity noted in preliminary toxicity assay

**Negative controls:** Phosphate buffer saline

**Positive controls:** 2-aminoanthracene (AA), Mitomycin C, sodium azide, 9-aminoacridine (9A), 2-nitrofluorene (NF)

**Treatment protocol of positive control**

Bacteria	Strain	dose µg/plate (w/S9)	dose µg/plate (w/o S9)
<i>Salmonella typhimurium</i>	TA1535	AA 1 µg	Sodium azide 1 µg
	TA1537	AA 1 µg	9A 75 µg
	TA98	AA 1 µg	NF 1 µg
	TA100	AA 1 µg	Sodium azide 1 µg
	TA102	AA 10 µg	Mitomycin C 1 µg

**Incubation and sampling times:** 48-72 hr

**Metabolic activation system:** Rat liver S9-mix

**Results:**

Preliminary toxicity assay: The preliminary toxicity assay was used to establish the dose-range. The dose levels tested were 0.33, 1.0, 3.3, 10, 33, 100, 333 and 1000 µl per plate. Toxicity was observed at 1000 µl per plate with most test conditions. No precipitate was observed. Based on the findings of the preliminary toxicity assay, the maximum dose tested in the mutagenicity assay was 1000 µl per plate (redacted per plate).

Study validity: The solvent control data were within the range of historical control data. The positive control chemicals induced a positive mutagenic effect. The study was valid.

Study outcome: In the initial assay, dose levels used were 10, 33, 100, 333 and 1000 µl per plate. Toxicity was observed beginning at 333 or at 1000 µl per plate with most test conditions. In the independent repeat mutagenicity assay, the dose levels tested were 3.3, 10, 33, 100, 333 and 1000 µl per plate. Toxicity was observed beginning at 100, 333 or at 1000 µl per plate all conditions. No precipitate was observed. In both assays, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. In conclusion, under the conditions of this study, test article Combigan™ ophthalmic solution containing exaggerated (redacted) degradant impurity did not cause a positive response in either the presence or absence of S9 up to a maximum concentration of 1000 µl per plate (redacted per plate).

**Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%): *In vitro* mammalian chromosome aberration test**

Key study findings: Combigan™ ophthalmic solution containing exaggerated (redacted) degradant impurity did not induce chromosome aberrations in the presence or absence of S9 in CHO cells.

Study #: TX06016

Conducting laboratory and location: (redacted)

Date of study initiation: 2/16/2006

GLP compliance: yes

QA reports: yes ( X ) no ( )

Drug: Combigan™ ophthalmic solution containing exaggerated (redacted) degradant impurity, Lot #: (redacted) (The equivalent of (redacted))

Reference article: Combigan™ eye drop solution with a level of (redacted) below the qualification threshold (redacted), Lot #: (redacted)

The purpose of this study was to evaluate the clastogenic potential of a combination drug product containing brimonidine, timolol and exaggerated levels of the impurity (redacted)

**Methods**

Strains/species/cell line: Chinese hamster ovary (CHO-K<sub>1</sub>) cells

Doses used in definitive study: 12.5, 25, 50, 75, 100, and 150 µl/ml

Basis of dose selection: Toxicity in preliminary toxicity assay

Negative controls: Phosphate buffer

Positive controls: Mitomycin C (MMC) 1 and 2 µg/ml without S9  
Cyclophosphamide (CP), 0.1 and 0.2 mg/ml with S9.

Incubation and sampling times: 4 hr and 20 hr without S9 and 4 hr with S9

Metabolic activation system: Rat liver S9-mix

In the preliminary toxicity assay for dose selection, CHO cells were exposed to solvent or 9 concentrations ( ) of the test article for 4 hr with or without S9 activation, and for 20 hr without S9 activation. The presence of drug precipitate was assessed visually, and cell viability was determined by trypan blue dye exclusion.

#### Results:

**Preliminary toxicity assay:** The drug was soluble at all concentrations tested. Substantial toxicity (at least 50% cell growth inhibition, relative to the solvent control) was observed at test article concentrations  $\geq 100$  µl/ml in both the non-activated 4- and 20-hr exposure groups, and at concentrations  $\geq 50$  µl/ml in the S9-activated 4-hr exposure group. Based upon the results of the toxicity study, the concentrations selected for testing in the chromosome aberration assay ranged from 12.5 to 150 µl/ml.

Treatment condition	Treatment time	Recovery time	Dose levels (µl/ml)
Non-activated	4 hr	16 hr	12.5, 25, 50, 75, 100, 150
Non-activated	20 hr	0 hr	12.5, 25, 50, 75, 100, 150
S9 activated	4 hr	16 hr	12.5, 25, 50, 75, 100, 150

**Study validity:** The solvent control data were within the range of historical control data. The positive control chemicals induced a positive effect. The study was valid.

**Study outcome:** Selection of concentrations for microscopic analysis was based on mitotic index (the lowest concentration with at least 50% reduction in mitotic index and two lower concentrations: 25, 50 or 75 µl/ml for 4 hr exposure without S9, 25, 75 or 150 µl/ml for 4 hr group with S9 and 12.5, 25 or 50 µl/ml for 20 hr continuous exposure without S9). The percentage of cells with structural or numerical aberrations in the test article-treated [Combigan™ containing exaggerated ( ) degradant impurity] groups was not significantly increased above that of the solvent control at any concentration. All values were within the historical range. In conclusion, Combigan™ ophthalmic solution containing exaggerated ( ) degradant impurity was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

#### Discussion and Conclusions

( ) is a newly identified impurity in the drug product. In June 29, 2006 submission, the sponsor proposed a specification of ( ). In the current NDA supplement dated October 3, 2006, the sponsor lowered the specification level to ( ) which is still higher than the qualification threshold listed in the

ICH Q3B document. Three nonclinical studies with Combigan™ ophthalmic solution containing exaggerated [REDACTED] were conducted for the qualification of [REDACTED]. In the 3-month ocular toxicity study, topical ocular administration of Combigan™ ophthalmic solution containing exaggerated [REDACTED] to female NZW rabbits 3 times a day for three month was well tolerated. There were neither drug- nor impurity-related ocular and extra ocular changes in this study. Two *in vitro* genotoxicity studies also showed negative results. The [REDACTED] levels in the three studies [REDACTED] were higher than the proposed specification level, [REDACTED].

The sponsor indicates that the source of [REDACTED] appears to be related to the migration of an [REDACTED] (trace amount) from a [REDACTED] into the formulation. This product uses a standard Allergan label and bottle configuration with a well-proven safety profile after many years of commercial use. The compound structure supports the negative toxicology findings listed in the accompanying 3-month toxicology study report for [REDACTED].

Based on the toxicity study results, the reviewer concluded that [REDACTED] does not present significant safety concerns.

#### 2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable.

#### OVERALL CONCLUSIONS AND RECOMMENDATIONS

##### Conclusions:

Both timolol and brimonidine are approved drugs. These two drugs have been used separately or concomitantly in clinical practice for a long period of time and are considered safe, effective and well-tolerated. Nonclinical data with the combination product indicated that the ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the single drug formulations. No additional toxicologically significant effects were observed in animal studies, and no toxicological interaction between timolol and brimonidine tartrate was noted. In toxicity studies with the combination product containing exaggerated concentrations of impurities, neither ocular irritation nor pathological findings were noted, suggesting that the impurities do not pose a human safety concern. In summary, nonclinical data support the safety of the combination drug product.

##### Recommendations:

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy sections.

#### CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY:

With brimonidine tartrate, no compound-related carcinogenic effects were observed in either mice or rats following a 21-month and 24-month study, respectively. In these studies, dietary administration of brimonidine tartrate at doses up to 2.5 mg/kg/day in mice and 1.0 mg/kg/day in rats achieved 150 and 210 times, respectively, the plasma  $C_{max}$  drug concentration in humans treated with one drop COMBIGAN™ into both eyes twice daily, the recommended daily human [REDACTED] dose.



Brimonidine tartrate was not teratogenic when given orally during gestation days 6 through 15 in rats and days 6 through 18 in rabbits. The highest doses of brimonidine tartrate in rats (1.65 mg/kg/day) and rabbits (3.33 mg/kg/day) achieved AUC exposure values 580 and 37-fold higher, respectively, than similar values estimated in humans treated with COMBIGAN™ ~~\_\_\_\_\_~~

Teratogenicity studies with timolol in mice, rats, and rabbits at oral doses up to 50 mg/kg/day (4,200 times *the MRHOD*) demonstrated no evidence of fetal malformations. Although delayed fetal ossification was observed at this dose in rats, there were no adverse effects on postnatal development of offspring. Doses of 1000 mg/kg/day (83,000 times *the MRHOD*) were maternotoxic in mice and resulted in an increased number of fetal resorptions. Increased fetal resorptions were also seen in rabbits at doses 8,300 times *the MRHOD* without apparent maternotoxicity.

Signatures:

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

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/s/

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Zhou Chen  
10/18/2006 01:40:11 PM  
PHARMACOLOGIST

Terry Peters  
10/18/2006 02:28:57 PM  
PHARMACOLOGIST

**Division of Anti-Infective and Ophthalmology Products**

Memorandum to the file for NDA 21-398  
Date: July 31, 2006  
From: Zhou Chen, MD, PhD  
To: Alison Rodgers  
Through: Terry Peters, DVM

**Sponsor:** Allergan Inc., 2525 Dupont Drive, P.O.Box 19534, Irvine, CA 92623-9534  
**Drug:** Combigan™ (brimonidine tartrate 0.2%/timolol 0.5%) ophthalmic solution  
**Drug Class:** Brimonidine tartrate:  $\alpha_2$ -agonist  
Timolol:  $\beta$ -adrenoceptor antagonist

**Chemical Name:** Brimonidine tartrate: 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate  
Timolol maleate: (-)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)-oxyl]-2-propanol maleate (1:1) salt

**Molecular Formula:** Brimonidine tartrate: C<sub>15</sub>H<sub>16</sub>N<sub>5</sub>O<sub>6</sub>Br, MW: 442.24  
Timolol maleate: C<sub>17</sub>H<sub>28</sub>N<sub>4</sub>O<sub>7</sub>S, MW: 432.49

**Related IND/NDA/BB-IND/BLA:** INDs 58,460, 32,392/NDA 20-490, 20-613, 21-262, 21-275/ANDAs 74-746, 74-747/

**Indication:** For reduction of elevated intraocular pressure in patients with glaucoma or ocular hypertension

**Route of Administration:** Topical ocular

**Formulation:**

Ingredients	Concentration (% w/v)
Brimonidine tartrate	0.2
Timolol maleate, USP	0.68 (equal to 0.5% timolol free base)
Benzalkonium chloride, NF	0.005
Sodium phosphate, monobasic, USP	
Sodium phosphate, dibasic, USP	
Sodium hydroxide, NF	
Hydrochloric acid, NF	
Purified water, USP	

Both timolol and brimonidine are approved drugs. With different sites and mechanisms of action, both compounds have been shown to lower intraocular pressure with a rapid onset of action. These two drugs have been used separately or together in clinical practice for a long period of time and are considered anecdotally as safe, effective and well tolerated. The sponsor submitted NDA 21-398 in September 2001, seeking the FDA's approval for Combigan™, a combination drug product.

A pharmacology/toxicology review was finished in December 2001 with the original NDA submission, and "approvable" was recommended with some minor modifications of labeling in the Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy sections. In the current NDA supplement dated June 29, 2006, an interim ocular toxicity

Zhou Chen

study with a degradant, [REDACTED], was included. Although the study results are all negative, the reviewer is concerned with the high proposed specification level of the impurity [REDACTED]. The sponsor mentioned that "although currently reported values for [REDACTED] appear quite high, this is due to the conventional assignment of a [REDACTED] relative to AGN 190342 (brimonidine) for unidentified impurities. The reported % (w/w) AGN 190342 concentrations on Combigan lots are all approximately [REDACTED]. Mass balance of AGN 190342 indicates that, at most, the combined concentrations of all impurities on a weight basis, cannot be more than approximately [REDACTED]. The high proposed specification level of the impurity [REDACTED] is too high. If the sponsor believes the method overestimates the impurity levels, they need to refine the method and provide actual impurity levels.

Message to be conveyed to the sponsor:

The high proposed specification level of the impurity [REDACTED] is too high. If you believe the method overestimates the impurity levels, you need to refine the method and provide actual impurity levels.

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/s/

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Zhou Chen  
7/31/2006 09:43:17 AM  
PHARMACOLOGIST

Terry Peters  
7/31/2006 11:10:44 AM  
PHARMACOLOGIST

## PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: NDA 21-398  
Review number: 000  
Sequence number/date/type of submission: 000/September 17, 2001/Commercial  
Information to sponsor: Yes ( X ) No ( )  
Sponsor and/or agent: Allergan Inc., 2525 Dupont Drive, P.O.Box 19534, Irvine, CA 92623-9534  
Manufacturer for drug substance :

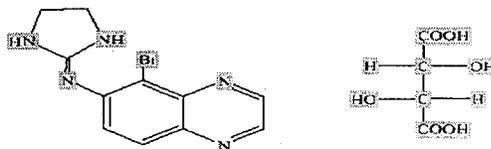
--Brimonidine tartrate:

--Timolol maleate:

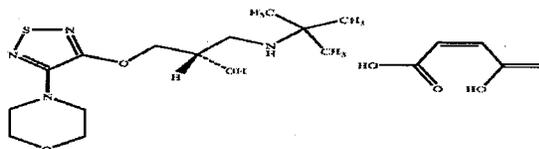
Reviewer name: Zhou Chen, Ph.D.  
Division name: Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products  
HFD #: HFD-550  
Review completion date: December 31, 2001

Drug:

Trade name: Not indicated  
Generic name (list alphabetically): **Brimonidine tartrate 0.2%/Timolol 0.5%  
Ophthalmic Solution**  
Code name: Brimonidine tartrate: AGN-190342-LF, UK-14,304-18  
Timolol maleate: AGN-196156-H  
Chemical name: Brimonidine tartrate: 5-bromo-6-(2-imidazolidinylideneamino)  
quinoxaline L-tartrate  
Timolol maleate: (-)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-  
thiadiazol-3-yl)-oxyl]-2-propanol maleate (1:1) salt  
CAS registry number: Brimonidine tartrate: 59803-98-4  
Timolol maleate: 26921-17-5  
Mole file number: Not indicated  
Molecular formula/molecular weight: Brimonidine tartrate: C<sub>15</sub>H<sub>16</sub>N<sub>5</sub>O<sub>6</sub>Br, MW: 442.24  
Timolol maleate: C<sub>17</sub>H<sub>28</sub>N<sub>4</sub>O<sub>7</sub>S, MW: 432.49  
Structure: Brimonidine tartrate:



Timolol maleate:



Relevant INDs/NDAs/DMFs: INDs 58,460, 32,392/NDAs 20-490, 20-613, 21-262, 21-275/ANDAs 74-746, 74-747//

Drug class: Brimonidine tartrate:  $\alpha_2$ -agonist  
Timolol:  $\beta$ -adrenoceptor antagonist

Indication: For reduction of elevated intraocular pressure in patients with glaucoma or ocular hypertension

Clinical formulation:

Ingredients	Concentration (% w/v)
Brimonidine tartrate	0.2
Timolol maleate, USP	0.68 (equal to 0.5% timolol free base)
Benzalkonium chloride, NF	0.005
Sodium phosphate, monobasic, USP	
Sodium phosphate, dibasic, USP	
Sodium hydroxide, NF	
Hydrochloric acid, NF	
Purified water, USP	

Route of administration: Topical, ocular

Proposed use: 1 drop (35  $\mu$ l), bid (For a 60 kg adult, total dose can reach 0.28 mg/patient/day or 0.0047 mg/kg, 0.17 mg/m<sup>2</sup> for brimonide tartrate, and 0.7 mg/patient/day or 0.0117 mg/kg, 0.43 mg/m<sup>2</sup> for timolol.)

## *Executive Summary*

### I. Recommendations

#### A. Recommendation on Approvability

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy sections.

#### B. Recommendation for Nonclinical Studies

No recommendation is necessary.

#### C. Recommendations on Labeling

Recommend minor modifications of labeling in the Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy sections.

### II. Summary of Nonclinical Findings

#### A. Brief Overview of Nonclinical Findings

Nonclinical data with the combination drug product indicated that the ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination and the single drug formulations. No biologically significant toxic effects were observed in animal studies, and no toxicological interaction between these two drugs was noted. Transient sedation was the only systemic effect observed in animals treated with the combination or with brimonidine alone, and was considered an exaggerated pharmacological effect of brimonidine. In conclusion, nonclinical data support the safety of the combination drug product.

#### B. Pharmacologic Activity

Brimonidine tartrate is a selective  $\alpha$ -2 adrenergic agonist, while timolol is a non-selective  $\beta$ -adrenergic receptor antagonist. With different sites and mechanisms, both compounds can lower intraocular pressure with a rapid onset of action. It is suggested that brimonidine tartrate decreases IOP by reducing aqueous humor production and increasing nonpressure dependent uveoscleral outflow, while timolol decreases IOP predominantly by reducing aqueous humor formation. It is expected that there will be an added IOP-lowering effect when timolol and brimonidine are used together.

#### C. Nonclinical Safety Issues Relevant to Clinical Use

Summarized from nonclinical studies, both combination and individual drug formulations were well tolerated. The nonclinical safety profile is similar between the combination and single-entity products. No toxicologically significant side effects were noted. There are no nonclinical safety concerns relevant to clinical use.

III. Administrative

A. Reviewer signature: \_\_\_\_\_

B. Supervisor signature:      Concurrence - \_\_\_\_\_

Non-Concurrence - \_\_\_\_\_  
(see memo attached)

C. cc: list:

NDA 21-398/Division File  
NDA 21-398/Original NDA  
HFD-550/CSO/Puglisi  
HFD-550/MO/Harris  
HFD-550/TL Pharm/Osterberg  
HFD-550/Pharm/ChenZ

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## PHARMACOLOGY/TOXICOLOGY REVIEW

### I. PHARMACOLOGY:

Brimonidine and timolol in this combination formulation are individually marketed drugs for lowering IOP in patients with glaucoma or ocular hypertension. The pharmacological studies for individual timolol and bromonidine have been reviewed under NDAs 20-613 and 21-262, and ANDA 74-747. Currently, the two marketed medications are often prescribed and used together to achieve an added IOP-lowering effect. Therefore, no new nonclinical pharmacological studies were performed.

### II. SAFETY PHARMACOLOGY:

None submitted. The safety pharmacological studies for individual timolol and bromonidine have been reviewed under NDAs 20-613 and 21-262, and ANDA 74-747.

### III. PHARMACOKINETICS/TOXICOKINETICS:

Timolol and brimonidine are marketed drugs. The ADME studies for individual drugs were reviewed under different NDAs or ANDAs. Only several ocular absorption and distribution study reports with the combined drug product are submitted and reviewed under NDA 21-398.

#### Absorption:

**Comparison of ocular absorption of brimonidine, levobunolol and timolol after a single ophthalmic dose administration of the single drug formulation versus combination drug formulation in albino rabbits**

#### Key study findings:

Ocular absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the single drug formulations.

Protocol N<sup>o</sup>: XXXXXXXXXX

Study N<sup>o</sup>: PK-98-P016

Vol/Page: 1.10/p 103

Conducting laboratory/location: Allergan, 2525 Dupont Drive, Irvine, CA 92623

Date of study initiation: 9/14/98

GLP: No

QA report: Yes ( ) No ( X )

Drug: Brimonidine 0.2%, (Lot#: 4818DA), timolol 0.5% (Lot#: 7380CA) and Brimonidine 0.2%-timolol 0.5% (Formulation reference #: R1998-4469-180)

Methods: LC-MS/MS; LOQ: 100 ng/ml for aqueous humor, 0.2 ng/ml for plasma, 10 ng/ml for tears, and 1 ng/g for iris-ciliary body

#### Dosing

Species/strain: New Zealand white rabbits

N: 6 females/group. Two animals were used as untreated control to obtain pre-dose concentrations.

Age/weight: Age data were not provided. Weight: 2.5-2.7 kg

The objective of this study is to determine the ocular absorption of brimonidine and timolol at 0.5 and 1 hr after the administration of a single dose of different formulations to rabbits. Study design is listed in the following table.

Group	Formulation	0.5 hr sampling	1 hr sampling
1	Brimonidine 0.2%-timolol 0.5%	3 rabbits, 6 eyes	3 rabbits, 6 eyes
2	Brimonidine 0.2%	3 rabbits, 6 eyes	3 rabbits, 6 eyes
3	Timolol 0.5%	3 rabbits, 6 eyes	3 rabbits, 6 eyes
4	Brimonidine 0.2%, timolol 0.5% (adjunctive dosing)*	3 rabbits, 6 eyes	3 rabbits, 6 eyes
5	Timolol 0.5%, brimonidine 0.2% (adjunctive dosing)*	3 rabbits, 6 eyes	3 rabbits, 6 eyes

\* There was a 10 min dosing interval between each drug

Frequency: single dose

Route: ocular topical

Volume: Groups 1-3: 1 drop (35 µl, a single drug formulation) each eye; Groups 4 and 5: combined drug formulation in each eye (35 µl of each drug with 10 min dosing interval)

Observations and times: Plasma, tears, aqueous humor and iris-ciliary body samples were collected from 3 animals in each group at 30 and 60 min after dosing.

Results:

Plasma drug concentrations were not reported due to "assay selectivity or injection carry-over problems with the LC-MS methodology". Plasma drug analysis was performed in other studies.

The concentrations of brimonidine and timolol in ocular tissues are summarized in the table below. In aqueous humor, at 1 hr after dosing, the timolol and brimonidine concentrations were 2-3 times higher in the animals dosed with the combination drugs than the animals dosed with single drug.

**Tissue brimonidine and timolol concentrations (mean ± SE, n = 5-6 eyes/group)**

Group	Formulation	Concentration at 0.5 hr after dose			Concentration at 1 hr after dose		
		Tears (µg/ml)	A. humor (µg/ml)	Iris-ciliary body (µg/g)	Tears (µg/ml)	A. humor (µg/ml)	Iris-ciliary body (µg/g)
<b>Brimonidine</b>							
1	Brimonidine 0.2%-timolol 0.5%	13.1± 2.9	0.77± 0.07	0.08± 0.02	2.88± 1.17	0.92± 0.24	0.05± 0.02
2	Brimonidine 0.2%	8.1± 4.6	0.66± 0.08	0.26± 0.06	1.76± 1.45	0.34 ± 0.06	0.07± 0.01
4	Brimonidine 0.2%, timolol 0.5% (adjunctive dosing)	10.1± 4.5	0.45± 0.06	0.07± 0.01	13.7± 10.5	0.40± 0.05	0.21± 0.06
5	Timolol 0.5%, brimonidine 0.2% (adjunctive dosing)	48.7± 33.6	0.91± 0.18	0.46± 0.18	7.57± 4.50	1.00± 0.20	0.48± 0.09
<b>Timolol</b>							
1	Brimonidine 0.2%-timolol 0.5%	58.6± 8.38	2.46± 0.39	1.70± 0.24	24.7± 5.60	2.73± 0.57	1.84± 0.42
3	Timolol 0.5%	28.6± 9.78	1.53± 0.08	1.68± 0.25	5.64± 1.12	0.88± 0.26	1.39± 0.39
4	Brimonidine 0.2%, timolol 0.5% (adjunctive dosing)	171± 40	3.27± 0.61	5.87± 0.80	66.4± 32.6	2.03± 0.22	5.11± 0.94
5	Timolol 0.5%, brimonidine 0.2% (adjunctive dosing)	78.3± 45.9	2.49± 0.29	4.77± 0.65	19.3± 6.45	2.66± 0.33	5.10± 0.53

In conclusion, no compromise on timolol and brimonidine absorption was observed in animals treated with the combination product when comparing with the rabbits receiving a single drug.

**Effect of dosing sequence on the ocular observation of brimonidine and timolol in white rabbits**

## Key study findings:

Ocular absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the adjunctive drug dosing.

The dosing sequence of the individual drugs may affect the absorption.

Document N<sup>o</sup>: PK-99-022

Protocol N<sup>o</sup>:                     

Vol/Page: 1.10/p 130

Conducting laboratory/location: Allergan, 2525 Dupont Drive, Irvine, CA 92623

Study Period: 11/17/98-12/18/98

GLP: No

QA report: Yes ( ) No ( X )

Drug: Brimonidine 0.2%, (Lot#: 4818DA), timolol 0.5% (Lot#: 7380CA) and brimonidine 0.2%-timolol 0.5% (Formulation reference #: R1998-4469-180)

Vehicle: Timolol vehicle

Methods: LC-MS/MS and GCMS; LOQ 100 ng/ml for aqueous humor and 0.2 ng/ml for plasma

Dosing

Species/strain: Female New Zealand white rabbits

N: 12 females/group. Two undosed animals were used as controls to obtain blank tissue concentrations.

Age/weight: Age data were not provided. Weight: 2.36-3.06 kg

Frequency: single dose

Route: ocular topical

Volume: 35 µl, in each eye

Observations and times: Samples were collected at the times listed in the following table. Aqueous humor samples were collected from 2 rabbits (4 eyes) per time point. Blood samples were collected from 4 animals per group.

The objective of this study is to determine the ocular bioavailability and PK profiles of brimonidine and timolol after a single dose of different formulations to rabbits. Study design is listed in the following table.

Study design for                     

Group	Formulation	Blood sampling time (min)	Aqueous humor sampling time (min)
A	Brimonidine 0.2%-timolol 0.5%	5, 15, 20, 30, 45, 60, 120	15, 30, 45, 60, 120** and 240**
B	Brimonidine, timolol (adjunctive)*	5, 15, 20, 30, 45, 60, 120	15, 30, 45, 60, 120** and 240**
C	Timolol, brimonidine (adjunctive)*	15, 25, 30, 40, 55, 70, 130	25, 40, 55, 70, 130** and 250**
D	Brimonidine, timolol vehicle (adjunctive)*	5, 15, 20, 30, 45, 60, 120	15, 30, 45, 60, 120** and 240**
E	Timolol vehicle, brimonidine (adjunctive)*	15, 25, 30, 40, 55, 70, 130	25, 40, 55, 70, 130** and 250**

\* There was a 10 min dosing interval between each drug

\*\*Animals used to collect blood sample (4/group)

## Results:

The concentrations of timolol and brimonidine with different formulations are summarized in the table below. Plasma timolol concentrations were not reported due to "assay selectivity or injection carry-over problems with the LC-MS methodology". Similar to Study PK-98-P016, at 30 min, aqueous humor timolol concentrations and AUC of Group B animals were higher than the animals treated with the combination drugs, suggesting dosing sequence in adjunctive therapy may affect the extent of ocular absorption of timolol.

**Brimonidine and timolol concentrations in aqueous humor and plasma after a single ophthalmic dose**

Group	Formulation	Brimonidine			Timolol		
		Cmax (µg/ml)**	Tmax (min)	AUC <sub>0-4 hr</sub> (µg-min/ml)*	Cmax (µg/ml)**	Tmax (min)	AUC <sub>0-4 hr</sub> (µg-min/ml)*
	<b>Aqueous humor</b>						
A	Brimonidine 0.2%-timolol 0.5%	0.47±0.09	45	66.3±4.3	1.24±0.41	45	171±4
B	B + T, adjunctive dosing	0.94±0.38	30	76.9±10.3	3.18±0.22	30	260±19
C	T + B, adjunctive dosing	0.69±0.06	45	95.4±12.7	1.57±0.07	55	174±12
D	B + T vehicle, adjunctive dosing	0.68±0.32	30	89.0±12.3			
E	T vehicle + B, adjunctive dosing	0.79±0.14	60	82.5±7.3			
	<b>Plasma</b>						
A	Brimonidine 0.2%-timolol 0.5%	4.61±2.00	37.5±19.4	267±40			
B	B + T, adjunctive dosing	3.54±0.68	7.50±5.00	182±8			
C	T + B, adjunctive dosing	4.18±3.39	22.5±5.00	239±169			
D	B + T vehicle, adjunctive dosing	3.08±1.22	8.75±7.50	182±23			
E	T vehicle + B, adjunctive dosing	4.79±1.21	22.5±5.00	287±45			

\* mean ± SE; \*\* mean ± SD

In conclusion, The brimonidine AUC and Cmax values in aqueous humor and plasma were comparable between the combination product and adjunctive dosing. The aqueous humor timolol AUC was higher in Group B, suggesting that dosing sequence in conjunctive therapy may affect timolol absorption.

**Toxicokinetic analysis for Study No. TX99070 entitled "0.2% Brimonidine tartrate/0.5% timolol maleate combination: a 6-month ocular toxicity study in rabbits"**

## Key study findings:

Brimonidine and timolol were absorbed in the systemic circulation in male and female rabbits following 6-month ocular administration (35 µl, tid, left eye) with the combination and single drug formulations. The systemic exposures to the drugs with different formulations were comparable.

Study No: TX99070

Document #: PK-00-057

Vol/Page: 1.10/p 153

Conducting laboratory/location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Study Period: 1/6/2000 to 10/23/2000

GLP: Yes

QA report: Yes ( X ) No ( )

Drug: Brimonidine 0.2%, (Lot#: 11601B, purity = ██████████, timolol 0.5% (Lot#: 11712 and 11725, purity = ██████████, and brimonidine 0.2%-timolol 0.5% (Lot#: 11604, purity = ██████████, for brimonidine and ██████████ for timolol)

Vehicle: Vehicle of brimonidine 0.2%-timolol 0.5%

Methods: LC-MS/MS for timolol and GC-MS/MS for brimonidine; LOQ 0.05 ng/ml

## Dosing

Group	Treatment	Frequency	Dosing period	N/sex
1	Vehicle	Tid (left eye only)	6 months	12
2	Brimonidine tartrate 0.2%/timolol 0.5%	Tid (left eye only)	6 months	12
3	Brimonidine tartrate 0.2%	Tid (left eye only)	6 months	12
4	Timolol 0.5%	Tid (left eye only)	6 months	12

Species/strain: New Zealand white rabbits

N: 12/sex/group

Frequency: tid (3 hr 15 min between doses) x 6 months

Route: ocular topical

Volume: 35 µl, in left eye

Observations and times: Blood samples were collected from 3 animals/sex/group during Weeks 5 and 25 at the time points listed in the table below.

Group	Formulation	Blood sampling time (min)
1	Vehicle	0 (pre-3 <sup>rd</sup> dose), 5, 15, 30, 45 min and 1, 2, and 3 hr post-3 <sup>rd</sup> dose
2	Brimonidine tartrate 0.2%/timolol 0.5%	0 (pre-3 <sup>rd</sup> dose), 5, 15, 30, 45 min and 1, 2, and 3 hr post-3 <sup>rd</sup> dose
3	Brimonidine tartrate 0.2%	0 (pre-3 <sup>rd</sup> dose), 5, 15, 30, 45 min and 1, 2, and 3 hr post-3 <sup>rd</sup> dose
4	Timolol 0.5%	0 (pre-3 <sup>rd</sup> dose), 5, 15, 30, 45 min and 1, 2, and 3 hr post-3 <sup>rd</sup> dose

### Results:

The plasma concentrations of timolol and brimonidine with different formulations are summarized in the table below. Both brimonidine and timolol were absorbed into the systemic circulation following ocular administration with different formulations. The systemic exposures to brimonidine and timolol with combination and single drug formulations were comparable.

#### Plasma brimonidine and timolol concentrations in plasma repeat ophthalmic dose

Sex	Week	Formulation	Brimonidine			Timolol		
			C <sub>max</sub> * (ng/ml)	T <sub>max</sub> (hr)	AUC <sub>0-3 hr</sub> ** (ng-hr/ml)	C <sub>max</sub> * (ng/ml)	T <sub>max</sub> (hr)	AUC <sub>0-3 hr</sub> ** (ng-hr/ml)
♂	5	Combination	2.97± 0.81	0.5	2.43± 0.20	12.7± 2.3	0.5	14.9± 1.0
♂	5	Single drug	2.89± 1.33	0.25	2.30± 0.25	12.0± 3.0	0.25	8.79± 0.74
♂	25	Combination	1.47± 0.15	0.083	1.22± 0.13	5.65± 2.96	0.75	8.31± 0.72
♂	25	Single drug	1.99± 1.17	0.5	1.99± 0.21	10.4± 4.8	0.083	9.21± 0.66
♀	5	Combination	6.07± 4.33	0.25	3.52± 0.68	12.8± 5.2	0.25	15.2± 1.4
♀	5	Single drug	3.72± 0.83	0.25	3.05± 0.27	12.6± 2.6	0.083	9.39± 0.44
♀	25	Combination	1.58± 0.48	0.25	1.34± 0.12	6.77± 2.54	0.25	9.15± 0.62
♀	25	Single drug	2.28± 0.85	0.5	1.92± 0.17	10.3± 3.7	0.083	7.71± 0.48
♀+♂	5	Combination	3.91± 3.69	0.25	2.97± 0.36	10.6± 4.4	0.25	15.1± 0.9
♀+♂	5	Single drug	3.22± 1.12	0.25	2.65± 0.19	11.6± 2.1	0.25	9.12± 0.43
♀+♂	25	Combination	1.39± 0.43	0.25	1.27± 0.09	5.86± 2.19	0.25	8.73± 0.49
♀+♂	25	Single drug	2.14± 0.93	0.5	1.96± 0.13	10.4± 3.8	0.083	8.49± 0.43

\* mean ± SD; \*\* mean ± SE

### Distribution:

Ocular distribution and pharmacokinetics of radioactivity following a single ophthalmic instillation of <sup>14</sup>C-brimonidine tartrate 0.2%/<sup>3</sup>H-timolol 0.5%, <sup>14</sup>C-brimonidine tartrate 0.2%, and <sup>3</sup>H-timolol 0.5% solution to female rabbit eyes

#### Key study findings:

Radioactivity was rapidly absorbed and distributed into ocular tissues of rabbits following a single 35 µl ocular administration with the combination and single drug formulations. The distribution of radioactivity in ocular tissues was similar with different formulations. The concentrations were generally higher in ocular surface tissues. Systemic concentrations of the radioactivity were low.

Study N<sup>o</sup>: PK-00-P017

Report N<sup>o</sup>: PK-01-014

Vol/Page: 1.10/p 183

Conducting laboratory/location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Study initiation: 12/6/2000

GLP: Yes

QA report: Yes ( X ) No ( )

Drug: Brimonidine tartrate, (Lot#: 950041), timolol maleate (Lot#: 11159), <sup>14</sup>C-Brimonidine tartrate (Lot#: 53H9207, 0.1 mCi/ml), <sup>3</sup>H-timolol (Lot#: TRQ9728, 1 mCi/ml). Test formulations: <sup>14</sup>C-Brimonidine tartrate 0.2% solution, (Lot#: 7831X-14C, 0.075 mCi/2mg/ml, 2.6  $\mu$ Ci/eye), <sup>3</sup>H-timolol 0.5% solution (Lot#: 8770X-3H, 0.8 mCi/5 mg/ml, 28  $\mu$ Ci/eye), and <sup>14</sup>C-brimonidine 0.2%/<sup>3</sup>H-timolol 0.5% solution (Lot#: 9262X-14C)

Methods: LC-MS/MS for plasma timolol concentrations and GC-MS/MS for plasma brimonidine concentrations, LOQ: 0.05 ng/ml; Liquid scintillation counting for radioactivity concentrations, LOQ: mean undosed tissue concentration + 2SD

## Dosing

Species/strain: Female New Zealand white rabbits, 3 months old, 1.4-2.2 kg

N: 12/group (2 rabbits were not treated as blank control.)

Frequency: Single dose

Route: Ocular topical

Volume: 35  $\mu$ l, both eyes

Observations and times: Animals were terminated at 10, 20, 40, 90, 180 and 360 min after dosing (2 animals/group/time point). Ocular tissues and blood samples were collected.

## Results:

The results are summarized in the table below. The absorption of <sup>14</sup>C-Brimonidine and <sup>3</sup>H-timolol radioactivity into ocular tissues was rapid in all drug treated groups. The ocular distribution of the radioactivity following treatment with the combination was comparable or higher than that with single drug products. High concentrations were observed in surface tissues and in the iris-ciliary body. Systemic concentrations of <sup>14</sup>C-Brimonidine and <sup>3</sup>H-timolol radioactivity and intact brimonidine and timolol were low. Tmax values of <sup>14</sup>C-Brimonidine and <sup>3</sup>H-timolol radioactivity in most tissues were 10-20 min after dosing.

Ocular distribution of <sup>14</sup>C-Brimonidine and <sup>3</sup>H-timolol radioactivity (Cmax: mean  $\pm$ SD; AUC: mean  $\pm$  SE)

Tissue	Brimonidine Cmax (ng-eq/g or ml)		Brimonidine AUC <sub>0-6hr</sub> (ng-hr/g or ml)		Timolol Cmax (ng-eq/g or ml)		Timolol AUC <sub>0-6hr</sub> (ng-hr/g or ml)	
	Combination	Brimonidine	Combination	Brimonidine	Combination	Timolol	Combination	Timolol
Tear	19300 $\pm$ 7200	10900 $\pm$ 9100	9550 $\pm$ 1180	6860 $\pm$ 1810	77700 $\pm$ 28500	53100 $\pm$ 59200	51100 $\pm$ 4700	70000 $\pm$ 17100
Lower conjunctiva	5140 $\pm$ 3920	1220 $\pm$ 980	2970 $\pm$ 390	1190 $\pm$ 130	17700 $\pm$ 13500	4750 $\pm$ 1050	9670 $\pm$ 1350	3990 $\pm$ 560
Upper conjunctiva	3650 $\pm$ 730	1430 $\pm$ 910	3800 $\pm$ 380	1680 $\pm$ 210	12000 $\pm$ 400	11500 $\pm$ 6200	11900 $\pm$ 1300	5790 $\pm$ 740
Cornea	5870 $\pm$ 2610	3110 $\pm$ 2700	6860 $\pm$ 720	3230 $\pm$ 240	23200 $\pm$ 9700	13500 $\pm$ 3900	29800 $\pm$ 3300	14300 $\pm$ 1100
Sclera	1190 $\pm$ 830	478 $\pm$ 124	1980 $\pm$ 170	663 $\pm$ 26	2950 $\pm$ 600	1640 $\pm$ 360	3980 $\pm$ 330	1840 $\pm$ 240
Aqueous humor	677 $\pm$ 19	505 $\pm$ 84	1230 $\pm$ 70	580 $\pm$ 19	2510 $\pm$ 660	1640 $\pm$ 620	4440 $\pm$ 360	2410 $\pm$ 280
Iris	1990 $\pm$ 1100	1300 $\pm$ 370	3010 $\pm$ 320	1470 $\pm$ 70	6650 $\pm$ 3120	4260 $\pm$ 570	10800 $\pm$ 1300	5780 $\pm$ 690
Ciliary body	1810 $\pm$ 950	1210 $\pm$ 50	2660 $\pm$ 270	1310 $\pm$ 90	5750 $\pm$ 2640	2920 $\pm$ 460	8920 $\pm$ 930	3880 $\pm$ 440
Retina	239 $\pm$ 194	63.8 $\pm$ 22.7	233 $\pm$ 45 <sup>a</sup>	106 $\pm$ 9	887 $\pm$ 748	358 $\pm$ 38	1200 $\pm$ 140	497 $\pm$ 73
Choroid	237 $\pm$ 16	98.5 $\pm$ 18.7	395 $\pm$ 50 <sup>a</sup>	173 $\pm$ 22 <sup>a</sup>	913 $\pm$ 92	876 $\pm$ 603	1810 $\pm$ 160	958 $\pm$ 164
Vitreous humor	38.7 $\pm$ 22.4	16.8 $\pm$ 9.4	52.0 $\pm$ 9.6	22.7 $\pm$ 3.3 <sup>a</sup>	137 $\pm$ 84	56.5 $\pm$ 34.3	267 $\pm$ 44	103 $\pm$ 20
Blood	16.1 $\pm$ NC	9.37 $\pm$ NC	24.9 $\pm$ 3.6 <sup>a</sup>	19.5 $\pm$ 1.2 <sup>a</sup>	46.3 $\pm$ NC	35.0 $\pm$ NC	213 $\pm$ 31	126 $\pm$ 12
Plasma*	4.97 $\pm$ NC	3.39 $\pm$ NC	5.29 $\pm$ 1.68 <sup>a</sup>	4.75 $\pm$ 0.45	28.7 $\pm$ NC	29.6 $\pm$ NC	41.5 $\pm$ 8.6	20.2 $\pm$ 4.0

a: AUC<sub>0-3 hr</sub>; NC = not calculable; \* intact brimonidine and timolol

## PK/TK summary and conclusions:

Ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the single drug formulations. Dosing sequence in adjunctive therapy may affect the extent of ocular absorption of timolol. Ocular distribution study showed that the distribution patterns of combination and single drug formulations were similar with the high concentrations of radioactivity in surface tissues and in the iris-ciliary body. Systemic concentrations of the radioactivity were low.

**IV. GENERAL TOXICOLOGY:**

None submitted. Please reference the individual drug's NDA reviews.

**V. GENETIC TOXICOLOGY:**

None submitted. Please reference the individual drug's NDA reviews.

**VI. CARCINOGENICITY:**

None submitted. Please reference the individual drug's NDA reviews.

**VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:**

None submitted. Please reference the individual drug's NDA reviews.

**VIII. SPECIAL TOXICOLOGY STUDIES:**

**OCULAR TOXICITY STUDIES**

**Study title: Brimonidine-tartrate 0.2%/timolol 0.5% combination: a 1-month ocular toxicity study with [redacted] impurity in rabbits**

**Key study findings:** Except for the transient slight discomfort, no significant ocular toxicity was observed in rabbits treated topically with stressed and non-stressed brimonidine-tartrate 0.2%/timolol 0.5% ophthalmic solutions or vehicles for 29 days.

Study #: TX01001

Study purpose: To evaluate the ocular effects of brimonidine tartrate 0.2%/timolol 0.5% combination containing impurities, when given topically, tid for 29 days to female rabbits

Volume #, and page #: Vol. 10, Page 240

Conducting laboratory and location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Date of study initiation: January 25, 2001

GLP compliance: Yes

QA reports: Yes (X) no ( ):

Drug, lot #, radiolabel, and % purity:

Brimonidine tartrate 0.2%/timolol 0.5% combination with [redacted] impurity, and [redacted] impurity, Formulation #: [redacted] Lot #: [redacted]

Brimonidine tartrate 0.2%/timolol 0.5% combination with [redacted] impurity, and [redacted] impurity. This formulation was stressed by storage at accelerated conditions (40 °C, 20%RH) for 7 months. Formulation #: [redacted] Lot #: [redacted]  
Formulation/vehicle:

Vehicle of brimonidine tartrate 0.2%/timolol 0.5% combination, Formulation #: \_\_\_\_\_, Lot #: \_\_\_\_\_

Vehicle of brimonidine tartrate 0.2%/timolol 0.5% combination. This formulation was stressed by storage at accelerated conditions (40 °C, 20%RH) for 7 months. Formulation #: \_\_\_\_\_, Lot #: \_\_\_\_\_

#### Methods:

Strains/species: Female New Zealand white rabbits

Age: 4-6 months old

Weight: 3-4 kg

#/sex/group or time point (main study): 6 females/group

Dose: 1 drop (35 µl), left eye only, 3 times per day (at 3 hr interval) x 1 month

Route: Ocular, topical

Test agent stability: Not indicated

#### Study design:

Group	N	Treatment	Frequency
1	6	Vehicle of brimonidine tartrate 0.2%/timolol 0.5% combination	Tid, left eye
2	6	Stressed vehicle of brimonidine tartrate 0.2%/timolol 0.5% combination	Tid, left eye
3	6	Brimonidine tartrate 0.2%/timolol 0.5% combination	Tid, left eye
4	6	Stressed brimonidine tartrate 0.2%/timolol 0.5% combination	Tid, left eye

#### Observations and times:

Mortality: Twice daily

Clinical observations: Daily

Ocular observations: Day 1 and weekly

Ophthalmic examinations: Pretest and Day 29 with slit lamp biomicroscopy and indirect ophthalmoscopy

Ocular discomfort: Weekly

Body weight: Weekly

Terminal sacrifice: Day 30

Gross pathology: All animals

Histopathology: All animals, ocular tissues

#### Results:

Mortality: No mortality occurred in any group during the treatment period.

Clinical observations: No drug-related effects were noted.

Ocular observations: One Group 3 animal showed minimal conjunctival hyperemia on Day 29.

This finding was considered incidental because it was noted only once in the study, and it occurred prior to the first dose of the day. No drug-related ocular irritation and or other ocular reactions occurred during the 1-month treatment period.

Ophthalmic examinations: No treatment-related, toxicologically significant findings were noted with slit lamp biomicroscopic and indirect ophthalmoscopic examinations.

Ocular discomfort: Transient slight ocular discomfort, ranging from 17% to 42% within a group, was noted in all groups (see table below). These findings were not considered treatment-related.

**Total and percent frequency of ocular discomfort following dosing**

Group	Test Article	n	Total # of Observations*	Severity					Duration				
				0	+1	+2	+3	+4	0	+1	+2	+3	+4
1	Vehicle of Brimonidine Comb. Formulations	6	24	20	4	0	0	0	20	3	1	0	0
			%	83	17	0	0	0	83	13	4	0	0
2	Stressed Vehicle of Brimonidine Comb. Formulations	6	24	14	10	0	0	0	14	10	0	0	0
			%	58	42	0	0	0	58	42	0	0	0
3	Brimonidine 0.2%/Timolol 0.5% Comb. Formulation	6	24	17	7	0	0	0	17	7	0	0	0
			%	71	29	0	0	0	71	29	0	0	0
4	Stressed Brimonidine 0.2%/Timolol 0.5% Comb. Formulation	6	24	16	8	0	0	0	16	7	1	0	0
			%	67	33	0	0	0	67	29	4	0	0

All observations are for the treated left eye.

**Grading Scale for Ocular Discomfort**

Severity	Duration
0 = No reaction	0 = No reaction
+1 = Slight	+1 = 1 to 30 sec.
+2 = Mild	+2 = 31 sec. to 60 sec.
+3 = Moderate	+3 = 61 sec to 120 sec.
+4 = Severe	+4 = 121 sec. to 5 min. or more

Body weight: There were no toxicologically significant differences in body weight among different groups during the treatment period.

Gross pathology: No abnormal findings were noted.

Histopathology: No toxicologically significant findings were noted in the eye or ocular adnexal structures.

**Summary and conclusion:**

To evaluate the safety of impurity [redacted] and impurity [redacted], female New Zealand white rabbits were topically treated with stressed or unstressed brimonidine tartrate 0.2%/timolol 0.5% combination solutions or vehicles (tid) for 29 days. No toxicologically significant findings were noted. Transient slight ocular discomfort was noted in all groups, and was not considered toxicologically significant.

**Study title: 0.2% Brimonidine-tartrate/ 0.5% timolol combination: a 6-month ocular toxicity study in rabbits**

**Key study findings:** No toxicologically significant ocular and systemic toxicity was observed.

Study no: TX99070

Study purpose: To evaluate the ocular and systemic toxicity of brimonidine tartrate 0.2%/timolol 0.5% combination ophthalmic solution when administered topically 3 times daily to rabbits for 6 months followed by a 2-month recovery period

Volume #, and page #: Vol. 11, Page 001

Conducting laboratory and location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Date of study initiation: January 11, 2000

GLP compliance: Yes

QA reports: Yes (X) No ( ):

Drug, lot #, radiolabel, and % purity:

Brimonidine 0.2%, (Lot#: [redacted], purity = [redacted], timolol 0.5% (Lot#: [redacted], purity = [redacted], and brimonidine 0.2%-timolol 0.5% (Lot#: [redacted], purity = [redacted] for brimonide and [redacted] for timolol)

The combination formulation is the same as the clinical formulation.

Vehicle: Vehicle of brimonidine 0.2%-timolol 0.5%

**Dosing**

Group	Treatment	Frequency	Dosing period	N/sex (main study)	N/sex (recovery)
-------	-----------	-----------	---------------	--------------------	------------------

1	Vehicle	Tid (left eye only)	6 months	8	4
2	Brimonidine tartrate 0.2%/timolol 0.5%	Tid (left eye only)	6 months	8	4
3	Brimonidine tartrate 0.2%	Tid (left eye only)	6 months	8	4
4	Timolol 0.5%	Tid (left eye only)	6 months	8	4

Species/strain: New Zealand white rabbits

Age: 4 months old

Weight: 2.3-3.0 kg

#/sex/group or time point: 8/sex/group for main study and 4/sex/group for recovery

Frequency: tid (dosing interval = 3 hr) x 6 months

Route: ocular topical, in left eye (The right eye remained untreated as a control.)

Volume: 35 µl

#### Observations and times:

Mortality: Once daily

Clinical observations: Daily

Sedative effects: Prior to the 1<sup>st</sup> and 2<sup>nd</sup> instillation and 1.5 hr following the 1<sup>st</sup> and 3<sup>rd</sup> instillations on Days 1-4 and 7-8, and once weekly at 1.5 hr after the 1<sup>st</sup> daily dose

Body weight: Weekly for the 1<sup>st</sup> month, once every 2 weeks thereafter

Ocular observations: Daily on the 1<sup>st</sup> week, and weekly thereafter

Ophthalmic examinations: Pretest, after 1, 3 and 6 months of treatment, and at the end of recovery period with slit lamp biomicroscopy and indirect ophthalmoscopy

Clinical pathology: Hematology and clinical chemistry examinations were conducted pretest, at 3 months, and at the end of the treatment and recovery periods.

Gross pathology: All animals in main study and recovery groups

Organ weights: The following organs from all animals were weighted: adrenal glands, brain, eyes with optic nerve and surrounding structures, kidneys, heart, liver, ovaries, spleen, testes.

Histopathology: The following organs from all animals were examined histologically: adrenal glands, aorta, bone, brain, cervix, diaphragm, epididymides, eyes with optic nerve and surrounding structures, esophagus, stomach, duodenum, jejunum, ileum, kidneys, cecum, colon, heart, liver, gall bladder, lungs, lymph nodes (mandibular and mesenteric), ovaries, pancreas, prostate, salivary glands (mandibular), sciatic nerve, seminal vesicle, spleen, skeletal muscle, skin with mammary area, spinal cord, sternum, testes, thymus, thyroid glands with parathyroids, pituitary gland, trachea, urinary bladder, uterus, vagina, gross lesions.

Toxicokinetics: Please see the Pharmacokinetics section.

#### Results:

**Mortality:** No mortality occurred in any group during the study period.

**Clinical observations:** No drug-related effects were noted.

**Sedative effects:** All rabbits administered brimonidine or brimonidine/timolol combination during the study sporadically displayed signs of sedation, which included a change in posture and/or decreased motor function at approximately 1.5 hours following instillation, resolving within 3 hours post-instillation (see table below). Frequency of sedation in the 0.2% brimonidine group was 46.2% in males and 38.4% in females. Frequency of sedation in the 0.2% brimonidine/0.5% timolol group was 42.5% in males and 39.0% in females. A very low frequency of sedation (equal to or less than 1.5%) was observed in one male and one female rabbit administered 0.5% timolol alone during the first week of treatment. Sedation was not detected in any rabbits administered vehicle only.

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Summary of sedative effects

Group No.	Sex	Incidence	Frequency of Sedative Effects <sup>a</sup> Observations				
			Week 1 <sup>b</sup>				Day 1-Day 15 <sup>c</sup>
			Prior to 1st Instillation	1.5 Hours Post 1st Instillation	Prior to 2 <sup>nd</sup> Instillation	1.5 Hours Post 3rd Instillation	1.5 Hours Post 1st Instillation
1 (vehicle)	M	0 / 12	0	0	0	0	0
	F	0 / 12	0	0	0	0	0
	Both	0 / 24	0	0	0	0	0
2 (combo)	M	12 / 12	0	58.3%	5.6%	38.9%	42.5%
	F	11 / 11	0	53.0%	25.8%	28.8%	39.0%
	Both	23 / 23	0	55.8%	15.2%	34.1%	40.8%
3 (brimonidine)	M	12 / 12	0	68.1%	11.1%	41.7%	46.2%
	F	12 / 12	0	51.4%	25.0%	29.2%	38.4%
	Both	24 / 24	0	59.7%	18.1%	35.4%	42.3%
4 (timolol)	M	1 / 12	0	0	1.4%	1.4%	0
	F	1 / 11	0	0	0	1.5%	0
	Both	2 / 23	0	0	0.7%	1.4%	0

- (a) Sedative effects were characterized by a change in posture and/or decreased motor activity.
- (b) Week 1 frequency is based on observations recorded on days 1-4 and on days 7-8. A total of 72 observations were recorded per timepoint for groups 1-4 males and for groups 1 & 3 females. A total of 66 observations were recorded per timepoint for groups 2 & 4 females.
- (c) Frequency is based on total observations recorded at the end of the treatment period. A total of 372 observations were recorded for groups 1-4 males and for groups 1 & 3 females. A total of 341 observations were recorded for groups 2 & 4 females.

**Body weights:** No drug-related abnormal findings were observed.

**Gross ocular observations:** There were no clinically relevant, drug-related gross ocular observations. Transient, slight ocular discomfort was observed in all dose groups, including the vehicle. Frequencies (combined male and female) of slight ocular discomfort lasting 1 to 60 seconds were 2.6%, 10.3%, 10.7%, and 17.2% in rabbits given vehicle, 0.2% brimonidine tartrate/0.5% timolol combination, 0.2% brimonidine tartrate alone, and 0.5% timolol alone, respectively. Transient and low frequency (<1%) slight ocular hyperemia was observed in all dose groups, including the vehicle. Gross ocular examination of rabbits during recovery in all four treatment groups revealed no evidence of ocular discomfort or hyperemia.

**Slit lamp examination:** A few ocular findings included positive fluorescein staining, corneal opacities, ocular flare, and pannus were observed in low incidence, sporadically among the vehicle and drug treated groups. These findings were usually resolved within a few days and were not seen in subsequent scheduled examinations, thus are not considered to be drug related.

**Ophthalmoscopy:** No drug-related abnormal findings were noted.

**Clinical pathology:** No toxicologically significant findings were noted in hematology and clinical chemistry examinations.

**Gross pathology:** No biologically relevant abnormal findings were noted.

**Organ weights:** Absolute liver weight and liver weight relative to body weight in the male combination and 0.2% brimonidine tartrate male rabbit groups were increased (15% and 31%, respectively) after 6 months of treatment (see table below). This difference was not observed in females

of the same treatment group, nor in males or females at the end of the recovery period. There were no concomitant findings in serum chemistry parameters related to liver function and no liver histopathological changes were observed. In addition, increased liver weight (absolute or relative to body weight) was not observed in previous studies with rabbits administered brimonidine topically to the eye for 6 months. Thus, the toxicological significance of the increased liver weight and liver weight relative to body weight cannot be determined.

**Absolute and relative liver weight in male rabbits (mean  $\pm$  SD)**

Group	1	2	3	4
N (main study group)	8	8	8	8
Treatment	Vehicle	Combination	Brimonide	Timolol
Absolute wt. (g)	89.37 $\pm$ 12.52	102.47 $\pm$ 18.69	117.50 $\pm$ 20.41	97.73 $\pm$ 12.42
Relative (%)	2.58 $\pm$ 0.29	3.02 $\pm$ 0.46	3.38 $\pm$ 0.63	2.87 $\pm$ 0.35
N (recovery group)	4	4	4	4
Absolute wt. (g)	105.66 $\pm$ 11.25	99.89 $\pm$ 4.74	96.14 $\pm$ 28.59	103.58 $\pm$ 6.16
Relative (%)	2.98 $\pm$ 0.35	2.78 $\pm$ 0.22	2.68 $\pm$ 0.51	2.85 $\pm$ 0.20

**Histopatology:** No toxicologically significant findings were noted in systemic and ocular tissues.

**Summary and conclusion:**

Male and female New Zealand white rabbits were topically treated in the left eye with brimonidine tartrate 0.2%, timolol 0.5%, brimonidine tartrate 0.2%/timolol 0.5% combination solutions or vehicles (tid) for 6 months followed by a 2-month recovery period. The drugs were generally well tolerated. No toxicologically significant findings in clinical observations, body weight, ocular examinations, clinical pathology, gross and histopathological examinations were noted. Sedative effects, observed in all rabbits treated with brimonidine or brimonidine/timolol combination, were likely caused by a pharmacological effect of  $\alpha$  2-adrenoreceptor activation in the central nervous system. Sedative effects were also observed in previous rabbit ocular studies with 0.2% Brimonidine-Purite™ or 0.2% Alphagan® (brimonidine tartrate ophthalmic solution). Transient, slight ocular discomfort was observed in all groups, and was not considered toxicologically significant. Absolute and relative liver weights in the male combination and 0.2% brimonidine tartrate male rabbit groups were increased after 6 months of treatment. Without corresponding clinical chemistry changes and histopathological findings, the increased liver weights are probably not toxicologically significant.

**IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:**

**Conclusions:**

Both timolol and bromonidine are approved drugs. These two drugs have been used separately or together in clinical practice for a long period of time and are considered as safe, effective and well-tolerated. Nonclinical data with the combination indicated that the ocular and systemic absorption of brimonidine and timolol in rabbits is comparable between the combination drug and the single drug formulations. No toxicologically significant effects were observed in animal studies, and no toxicological interaction between timolol and brimonidine tartrate was noted. In an ocular toxicity study with the combination containing exaggerated concentrations of [redacted] impurities, [redacted], no ocular irritation and pathological findings were noted, suggesting that the impurities do not pose a human safety concern. In summary, nonclinical data support the safety of the combination drug product.

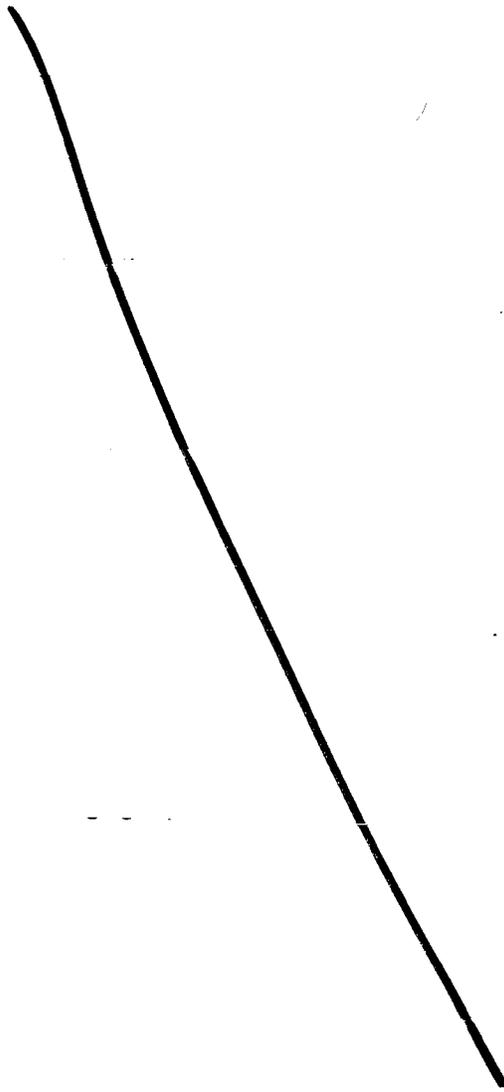
**General Toxicology Issues:**

No toxicologically significant issues were indicated.

**Recommendations:**

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy sections.

**Labeling with basis for findings:**



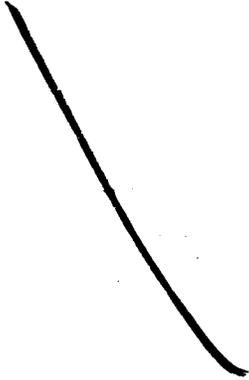
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       Trade Secret / Confidential

  ✓   Draft Labeling

       Deliberative Process

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**X. APPENDIX/ATTACHMENTS:**

**Addendum to review: No**

**Other relevant materials (Studies not reviewed, appended consults, etc.): No**

**Any compliance issues: No**

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Zhou Chen  
1/17/02 01:11:21 PM  
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Bob, Please sign this NDA review. Thanks! ZHou

Robert Osterberg  
1/23/02 03:03:07 PM  
PHARMACOLOGIST