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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-516

Pharmacology Review(s)

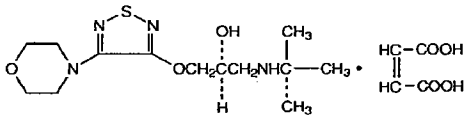
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **21-516**
Review number: 000
Sequence number/date/type of submission: 000/September 25, 2002/Commercial
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Senju Pharmaceutical Co., Ltd., 5-8 Hiranomachi 2-chome, Chuo-ku, Osaka 541-0046, Japan
Manufacturer for drug substance: [redacted]

Reviewer name:	Zhou Chen
Division name:	Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #:	HFD-550
Review completion date:	November 30, 2002

Drug:

Trade name: Unknown
Generic name (list alphabetically): Timolol maleate ophthalmic solution 0.5%
Code name: Timolol-LA
Chemical name: 2-Propanolol, 1-[(1,1-dimethylethyl)amino]-3-[(4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl)oxy]-,(S)-,(Z)-2-butenedioate (1:1) (salt)
CAS registry number: 26921-17-5
Mole file number: Not indicated
Molecular formula/molecular weight: $C_{13}H_{24}N_4O_3S\text{-}C_4H_4O_4$, MW: 432.49
Structure:



Relevant INDs/NDAs/DMFs: IND 59,046, DMFs [redacted]

Drug class: β-adrenoceptor antagonist

Indication: Treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma.

Clinical formulation:

Component	Function	Quantity (% w/v)
Timolol maleate, USP	Active ingredient	0.68 (= 0.5 g of timolol)
Potassium sorbate, NF		0.47
Monobasic sodium phosphate monohydrate, USP		
Sodium chloride, USP		
Benzalkonium chloride solution, NF	Preservative	0.005
Sodium hydroxide, NF		
Purified water, USP	Solvent	Qs to 100 ml

Route of administration: Topical, Ocular

Proposed use: 1 drop (50 µl) per eye, once daily (for a 50 kg adult, the total dose can reach 0.5 mg/patient/day or 0.01 mg/kg, 0.37 mg/m^2.)

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Executive Summary

I. Recommendations

A. Recommendation on Approvability

There are no pharmacology/toxicology objections to the approval of this NDA.

B. Recommendation for Nonclinical Studies

No recommendation is necessary.

C. Recommendations on Labeling

The labeling for the Carcinogenesis, Mutagenesis, Impairment of Fertility section and the Pregnancy section is identical to the labeling for Timoptic®, with the exception that the name TIMOPTIC is replaced with BRANDNAME. No modification is necessary at this time.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Sorbic acid in Timolol-LA formulation had no influence on the binding activity of timolol to β-receptor. Following ocular administration, the new formulation of timolol maleate ophthalmic solution, with sorbate, showed increased ocular bioavailability of timolol. However, the systemic bioavailability was not changed. The improvement in ocular bioavailability of timolol with sorbic acid was possibly due to the improved partitioning of timolol in the corneal epithelium. The drug product was well-tolerated in animal toxicity studies with the duration up to 4 weeks in rabbits and 6 months in monkeys, respectively.

B. Pharmacologic Activity

Timolol maleate is a β_1 and β_2 (non-selective) adrenergic receptor blocking agent. Timolol maleate ophthalmic solution has the action of reducing elevated as well as normal intraocular pressure, whether or not accompanied by glaucoma. The precise mechanism of the ocular hypotensive action of timolol maleate is not clearly established at this time. Clinical studies suggested that its predominant action may be related to reduced aqueous formation. In some studies, a slight increase in outflow facility was also observed. In this NDA application, the sponsor developed a new formulation of timolol maleate ophthalmic solution with sorbate in order to enhance the ocular bioavailability of timolol.

C. Nonclinical Safety Issues Relevant to Clinical Use

There are no nonclinical safety issues in this NDA relevant to the clinical use of the drug product.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

NDA 21-516/Division File
NDA 21-516/Original NDA
HFD-550/CSO/Puglisi
HFD-550/MO/Boyd
HFD-550/TL Pharm/Yang
HFD-550/Pharm/ChenZ

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

Reviewer's Comments: Timolol maleate ophthalmic solution has been marketed in the United States for more than 20 years. The nonclinical pharmacology, toxicology and PK studies have been previously conducted by several sponsors and reviewed in several NDAs. The drug product in this NDA application (timolol maleate ophthalmic solution 0.5%) is a new formulation of timolol with potassium sorbate. In this NDA submission, the sponsor only submitted several nonclinical studies related to the new formulation. For the information of other nonclinical studies, the sponsor asked for the reference of NDA 18-086 for Timoptic.

I. PHARMACOLOGY:

Only two studies were submitted. For other pharmacology studies, please refer to NDA 18-086.

Studies reviewed:
Interaction between timolol and sorbic acid on the binding to beta-adrenoceptor in rabbit ciliary body *in vitro*. Vol. 11, Page 046
Analysis of corneal permeation mechanism of timolol based on bilayer diffusion model. Vol. 11, Page 060

Interaction between timolol and sorbic acid on the binding to beta-adrenoceptor in rabbit ciliary body *in vitro*. Vol. 11, Page 046
Study #: B99-B06

The purpose of this competition binding assay was to determine if sorbic acid affected the binding of timolol to β-adrenoceptor. *In vitro* binding of timolol or timolol/sorbic acid to the β-receptor in the ciliary body dissected from albino rabbit eyes was measured using [^{3}H]-alprenolol as a tracer. The results showed that in the presence and absence of sorbic acid (0.13 mg/ml, ten times Cmax value in aqueous humor in Study 5990812), the IC_{50} values (timolol concentration, at which the binding of tracer, [^{3}H]-alprenolol, to receptor was inhibited by 50%) were 0.501±0.017 nM and 0.493±0.046 nM, respectively. The corresponding Ki values were 0.230±0.020 nM and 0.226±0.023 nM, respectively. It was concluded that sorbic acid in Timolol-LA formulation had no influence on the affinity of timolol to β-receptor.

Analysis of corneal permeation mechanism of timolol based on bilayer diffusion model. Vol. 11, Page 060
Study #: D98-B09

The purpose of this study was to understand the mechanism of improvement in the transcorneal absorption of timolol with sorbic acid. This *in vitro* permeation study was designed to observe the steady-state permeation rate, and measure the diffusion and partition coefficients across the intact and de-epithelialyzed cornea using the side-by-side diffusion chamber. The results showed that sorbic acid increased the permeation rate and the partition coefficient in corneal epithelium of timolol but did not affect the diffusion coefficient (see table below). In conclusion, the improvement in ocular bioavailability of timolol with sorbic acid was possibly due to the improved partitioning of timolol in the corneal epithelium.

Steady state permeation rate and diffusion and partition coefficients of timolol in rabbit cornea

	Permeation rate (mg/cm^2/hr)		Diffusion coefficient cm^2/sec		partition coefficient	
	Intact cornea	De-epithelialyzed	Epithelium	Stroma	Epithelium	Stroma
Control	81.48	319.38	8.724×10^{-9}	6.022×10^{-7}	2.438	1.179
+ Sorbic acid	161.40	576.66	8.765×10^{-9}	9.570×10^{-7}	4.972	1.339

Pharmacology summary and conclusions:

Two *in vitro* pharmacology studies were conducted. The results indicated that sorbic acid in Timolol-LA formulation had no influence on the binding activity of timolol to β-receptor, and that the improvement in ocular bioavailability of timolol with sorbic acid was possibly due to the improved partitioning of timolol in the corneal epithelium.

II. SAFETY PHARMACOLOGY:

Please refer to Pharmacology/Toxicology reviews for NDA 18-086.

III. PHARMACOKINETICS/TOXICOKINETICS:

Studies reviewed:
Evaluation of sorbic acid concentration on timolol bioavailability in rabbits. Vol. 11, Page 004
Comparison of various timolol formulations on ocular bioavailability in rabbits. Vol. 11, Page 015
Comparison of timolol bioavailability between sorbic acid and potassium sorbate formulation. Vol. 11, Page 026
Plasma levels of timolol after topical instillation in rabbits. Vol. 11, Page 035

Evaluation of sorbic acid concentration on timolol bioavailability in rabbits. Vol. 11, Page 004

Study Nº: D98-B11
Report Nº: 5990810
Test facility: Research Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4 Murotani, Nishiku Kobe, Hyogo 651-2241, Japan
Study initiation: November 24, 1998
Date of final report: August 18, 1999
Compound: Timolol-LA (Batch #: 75107002) with sorbic acid at different concentrations (see table below)
Route: Ocular
Dose level: 50 µl, single dose, one eye only
Time of sampling: Aqueous humor samples were collected 30 min and 3 hr after the instillation.
Animal: Male Japanese white rabbits, 1.8-2.3 kg
GLP: No

The purpose of this study was to determine an appropriate content of sorbic acid by quantifying transcorneal absorption of timolol after instillation with timolol ophthalmic solution with different sorbic contents. Timoptol 0.5% was used as control. Six formulations of timolol ophthalmic solution 0.5% were prepared with sorbic acid concentrations ranging from 0.046 to 1.76%. Each of the test solutions was instilled into one eye of rabbits in a single dose of 50 µl. The animals were sacrificed at 0.5 or 3 hr after dosing. Aqueous humor samples were collected for the determination of timolol and sorbic acid concentrations by HPLC.

Results:

The results, summarized in the following table, indicated that sorbic acid added at 0.35% or higher improved the transcorneal absorption of timolol at 30 min after instillation. The sorbic acid concentration in the aqueous humor was increased with increased content of sorbic acid in the ophthalmic solution, and it was quickly eliminated from aqueous humor.

Timolol and sorbic acid concentration in rabbit aqueous humor after instillation (mean ± SD)

Preparation			Aqueous humor concentration (μg/ml)					
		Sorbic acid	Timolol			Sorbic acid		
Group	Treatment	(%)	N	0.5 hr	3 hr	N	0.5 hr	3 hr
Control	Timoptol	0	5	3.3±1.4	0.6±0.2	5		
A-1-0.5	Timolol-LA	0	3	2.8±1.4	0.6±0.2	3		
A-2-0.5	Timolol-LA	0.046	2	2.8±0.1	0.9±0.1	3	0.9±0.2	0.0
A-3-0.5	Timolol-LA	0.18	3	3.2±1.6	1.0±0.4	3	2.3±0.9	0.04±0.02
A-4-0.5	Timolol-LA	0.25	3	4.1±1.5	0.6±0.1	3	3.7±1.4	0.1±0.1
A-5-0.5	Timolol-LA	0.35	5	7.6±3.4	1.0±0.7	6	7.5±3.4	0.04±0.02
A-6-0.5	Timolol-LA	0.71	3	8.2±2.5	1.0±0.8	3	10.4±5.2	0.1±0.1
A-7-0.5	Timolol-LA	1.76	3	6.2±0.6	1.5±0.2	3	11.4±1.1	0.1±0.1

Comparison of various timolol formulations on ocular bioavailability in rabbits. Vol. 11, Page 014

Study Nº: D98-B03
Report Nº: 5990809
Test facility: Research Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4 Murotani, Nishiku Kobe, Hyogo 651-2241, Japan
Study initiation: June 29, 1998
Date of final report: August 26, 1999
Compound: Timolol-LA (Batch #: 75107002) with sorbic acid 0.35%
Route: Ocular
Dose level: 50 μl, single dose, one eye only
Time of sampling: Aqueous humor, cornea and iris-ciliary body samples were collected at 15 min, 30 min, 1 hr and 3 hr after the instillation.
Animal: Male Japanese white rabbits, about 2 kg
GLP: No

The purpose of this study was to determine if a sorbic acid-containing timolol maleate ophthalmic solution could increase transcorneal absorption and duration of timolol in ocular tissues. Three drugs (Timoptol 0.5%, Timoptic-XE 0.5% and Timolol-LA 0.5%) were compared in this assay. Timoptol is a conventional timolol ophthalmic solution, while Timoptic-XE is a sustained released timolol maleate ophthalmic solution with gellan gum that is dosed once daily clinically. Fifty μl of each drug was instilled into one eye of rabbits. The animals were sacrificed at 15 min, 30 min, 1 hr and 3 hr after instillation and ocular tissues (cornea, aqueous humor, and iris-ciliary body) were collected for determination of timolol concentrations by the HPLC.

Results:

The results are summarized in the table below. Sorbic acid 0.35% increased timolol transcorneal absorption in rabbit aqueous humor by 3.15 times in Cmax and 2.17 times in AUC

relative to Timoptic 0.5%. Three hr after instillation, the AUC and Cmax between Timolol-LA and Timoptic-XE, a once daily formulation of timolol, were almost equivalent. In conclusion, addition of 0.35% sorbic acid to timolol ophthalmic solution increased ocular absorption of the drug. In animals treated with Timolol-LA or Timoptic-XE, the transcorneal absorption and the duration of timolol concentrations in ocular tissues appeared equivalent.

Timolol concentration in ocular tissues after instillation in rabbits (n = 5)

Tissue	Drug	Concentration (μg/g or ml, mean ± SD) Time after instillation				AUC_{0-3} (μg-hr/g or ml)
		15 min	30 min	1 hr	3 hr	
Corneal	Timoptol	32.2±12.7	21.9±7.8	21.9±8.1	6.2±3.7	49.88
	Timolol-LA	94.1±49.5	81.0±38.9	43.6±14.9	13.1±7.1	121.4
	TIMOPTIC-XE	73.9±24.3	80.9±23.6	52.1±40.9	12.2±4.9	126.2
Aqueous humor	Timoptol	2.2±0.7	2.5±1.1	3.0±1.3	0.7±0.4	5.9
	Timolol-LA	7.9±4.2	9.4±3.3	4.8±1.5	1.3±0.7	12.8
	TIMOPTIC-XE	4.3±0.6	8.4±2.8	6.0±3.8	1.5±0.5	13.1
Iris-ciliary body	Timoptol	5.2±1.2	4.5±1.1	4.9±1.8	1.7±0.9	10.7
	Timolol-LA	16.0±9.0	15.3±6.1	8.4±2.5	2.6±1.4	22.8
	TIMOPTIC-XE	9.9±1.1	14.8±2.8	10.1±4.0	2.6±0.9	23.2

Comparison of timolol bioavailability between sorbic acid and potassium sorbate formulation. Vol. 11, Page 026

Study Nº: D99-B07
Report Nº: 5990812
Test facility: Research Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4 Murotani, Nishiku Kobe, Hyogo 651-2241, Japan
Study initiation: June 8, 1999
Date of final report: August 31, 1999
Compound: Timolol-LA (Batch #: 75108003) in different formulations including reduction of benzalkonium chloride content and substitution of potassium sorbate for sorbic acid
Route: Ocular
Dose level: 50 μl, single dose, one eye only
Time of sampling: Aqueous humor samples were collected at 30 min and 3 hr after the instillation.
Animal: Male Japanese white rabbits, 1.8-2.2 kg, 5/group
GLP: No

The purpose of this study was to determine if the formulation modifications including reduction of benzalkonium chloride content and replacement of sorbic acid by potassium sorbate might cause any effect on transcorneal absorption of timolol. Four formulations of Timolol-LA were prepared (see table below). Each of the 4 different Timolol-LA 0.5% was instilled into one eye of rabbits in a single dose of 50 μl. The animals were sacrificed at 30 min or 3 hr after dosing. Aqueous humor samples were collected for determination of timolol and sorbic acid concentrations by the HPLC.

Results:

The results are summarized in the table below. The concentrations of timolol and sorbic acid in the animals treated with the 4 different formulations were similar. The formulation modification had little effect on transcorneal absorption of timolol.

Aqueous humor concentrations of timolol and sorbic acid (mean ± SD) after ocular instillation of Timolol-LA(n=5)

Treatment group	Benzalkonium chloride (g/100 ml)	Sorbic acid (g/100 ml)	Potassium sorbate (g/100 ml)	Timolol (µg/ml)		Sorbic acid (µg/ml)	
				0.5 hr	3 hr	0.5 hr	3 hr
1		0.35		8.4±4.9	1.0±0.6	8.9±5.5	0.05±0.02
2	0.005	0.35		5.6±1.6	0.9±0.4	5.3±.4	0.04±0.02
3	0.002	0.35		6.8±.9	1.1±0.7	6.1±2.6	0.05±0.02
4	0.005		0.47	8.2±3.7	0.9±0.3	7.7±4.9	0.04±0.02

Plasma levels of timolol after topical instillation in rabbits. Vol. 11, Page 035

Study N^{o}: D99-B04
Report N^{o}: 5990811
Test facility: Research Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4 Murotani, Nishiku Kobe, Hyogo 651-2241, Japan
Study initiation: March 23, 1999
Date of final report: August 27, 1999
Compound: Timolol-LA (Batch #: 75107002) and Timoptic (Lot #: 1418H)
Route: Ocular
Dose level: 50 µl, single dose, both eyes
Time of sampling: Blood samples were collected at 0.25, 0.5, 1 and 3 hr after the instillation.
Animal: Male Japanese white rabbits, 1.8-2.0 kg, 5/group
GLP: No

The purpose of this study was to examine the effect of sorbic acid on systemic absorption of timolol by comparing plasma timolol concentrations following a single instillation of Timolol-LA or Timoptic (50 µl) into both eyes of Japanese white rabbits. Blood samples were collected at 0.25, 0.5, 1 and 3 hr after instillation. Plasma concentrations of timolol were determined by the HPLC assay.

Results:

The results are summarized in the table below. The two treatments showed very similar bioavailability. In conclusion, the increased ocular bioavailability of timolol with the Timolol-LA formulation did not affect the systemic absorption of timolol.

Plasma concentration of timolol (mean ± SD) after ocular instillation in rabbits (ng/ml)

	Time (hr)				AUC_{0-3}
Treatment	0.25	0.5	1	3	ng-hr/ml
Timoptic (n=4)	36.4±9.4	25.2±3.3	14.5±1.2	3.7±0.5	40.3
Timolol-LA (n=5)	39.8±7.9	26.3±.3	16.2±.5	3.8±.1	43.9

PK/TK summary and conclusions:

The inclusion of sorbic acid increased the ocular bioavailability of timolol compared to other timolol drug products. This increase did not come at the expense of increased systemic bioavailability.

IV. GENERAL TOXICOLOGY:

Please refer to Pharmacology/Toxicology reviews for NDA 18-086.

V. GENETIC TOXICOLOGY:

Please refer to Pharmacology/Toxicology reviews for NDA 18-086.

VI. CARCINOGENICITY:

Please refer to Pharmacology/Toxicology reviews for NDA 18-086.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Please refer to Pharmacology/Toxicology reviews for NDA 18-086.

VIII. SPECIAL TOXICOLOGY STUDIES:

Ocular Toxicity Studies:

Studies reviewed:
The ocular irritation study of 0.5% Timolol-LA ophthalmic solution in a 7-day repeated administration in rabbits. Vol. 11, page 073
An ocular irritation study of 0.5% Timolol-LA ophthalmic solution by 7 days repeated instillation in rabbits. Vol. 11, Page 087
An ocular toxicity study of 0.5% Timolol-LA ophthalmic solution by 28 days repeated instillation in rabbits. Vol. 11, Page 116
An ocular toxicity study of 0.5% Timolol-LA ophthalmic solution by 26 weeks repeated ocular instillation in Cynomolgus monkeys. Vol. 12, Page 001
Additional information on the Timolol-LA 6 month monkey toxicology study. Vol. 12, Page 245
Effects of ketamine anesthesia and training for measurement procedures on the intraocular pressure-reducing action of timolol maleate ophthalmic solution in monkeys. Vol. 12, Page 292

The ocular irritation study of 0.5% Timolol-LA ophthalmic solution in a 7-day repeated administration in rabbits. Vol. 11, page 073

Key study finding: Timolol-LA 0.5% or sorbic acid (0.25-0.45%) produced no ocular irritation in rabbits with ocular treatment for 7 days.

Study No: S99-B04
Report No: 3990802
Conducting laboratory and location: Research Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4 Murotani, Nishiku Kobe, Hyogo 651-2241 Japan
Date of study initiation: March 2, 1999
GLP compliance: No
QA report: No
Animal: Male Japanese white rabbits, 1.65-1.87 kg
Route: Ocular, topical

Dosage: 100 µl qid at intervals of about 2.5 hr x 7 days
Drug: 0.5% Timolol-LA ophthalmic solution (containing 0.35% sorbic acid) [Lot#: 99M111, purity: [ ] and saline solutions of 0.25%, 0.35% and 0.45% sorbic acid [Lot#: IDS-8]

Study design:

Animal	Right eye	Dose	Left eye	Dose
1	0.5% Timolol-LA	100 µl	0.25% sorbic acid solution	100 µl
2	0.5% Timolol-LA	100 µl	0.25% sorbic acid solution	100 µl
3	0.5% Timolol-LA	100 µl	0.35% sorbic acid solution	100 µl
4	0.35% sorbic acid solution	100 µl	0.45% sorbic acid solution	100 µl

The purpose of this study was to evaluate the ocular irritancy of 0.5% Timolol-LA ophthalmic solution and sorbic acid (0.25-0.45%) when instilled to rabbits 4 times daily for 7 days. The day of the first dosing was designated as Day 1. Ocular toxicity was assessed as shown below.

Ocular toxicity assessment for Study S99-B04

Parameter	Procedure
Macroscopic observations	Eyes were observed prior to the first dosing and 30 min following the last dosing on days 1, 4 and 7.
Fluorescein	Prior to the first administration and on Days 1, 4 and 7

Results:

There were no signs of ocular irritation in any eyes. Fluorescein examination showed slight diffuse stains in every group, and some of stains were observed before the study initiation. These changes were considered within a normal range.

In summary, rabbits were treated topically with Timolol-LA 0.5% or sorbic acid (0.25-0.45%) for 7 days (qid). No ocular irritation was observed in either Timolol-LA or sorbic acid treated eyes.

An ocular irritation study of 0.5% Timolol-LA ophthalmic solution by 7 days repeated instillation in rabbits. Vol. 11, Page 087

Key study finding: Timolol-LA 0.5% formulated with either sorbic acid or potassium sorbate produced no ocular toxicity in rabbits with ocular treatment for 7 days.

Study No: [ ] 50-67
Report No: 9939
Conducting laboratory and location: [ ]

Date of study initiation: August 4, 1999
GLP compliance: Yes
QA report: Yes
Animal: Male New Zealand white rabbits, 10-week old, 1.98-2.19 kg, 5/group
Route: Ocular, topical
Dosage: 50 µl qid at intervals of about 2 hr x 7 days
Drug: 0.5% Timolol-LA ophthalmic solution (The formulation was similar to the clinical formulation with the sorbic acid concentration of 0.35%.) [Lot#: 9T06,

purity: [ ]] for left eye and 0.5% Timolol-LA ophthalmic solution (with potassium sorbate) [Lot#: 9T06, purity: [ ]] for right eye

Study design:

Group	Test article	Formulation	N/group	Dosing regimen
1	0.5% Timolol-LA	Potassium sorbate	5	qid x 7 days, right eye
2	0.5% Timolol-LA	Sorbic acid	5	qid x 7 days, left eye

The purpose of this study was to evaluate the ocular irritancy of 0.5% Timolol-LA ophthalmic solution when instilled to rabbits 4 times daily for 7 days. Two formulations were used in this study: one with sorbic acid, the other with potassium sorbate. The day of the first dosing was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study [ ]. 50-67

Parameter	Procedure
Clinical observations	Once daily
Body weights	Once on Day 0 (prior to the first dosing) and once on the day of gross pathology
Ophthalmology	Gross examination: Once prior to the study initiation and on Days 0, 3 and 6 (following the fourth daily dosing) Slit-lamp microscope: Once prior to the study initiation and on Days 0, 3 and 6 (following the fourth daily dosing)
Gross pathology	Complete necropsy was performed on all animals at the end of the study.

Results:

Clinical observations: During the dosing period, no mortality and dosing-related clinical signs were noted.

Body weights: No abnormalities in body weights were observed.

Ophthalmology: No abnormalities were observed in gross and slit-lamp microscopic examinations.

Macroscopic examination: No treatment-related abnormalities were noted.

In summary, rabbits were treated topically with Timolol-LA 0.5% containing sorbic acid or potassium sorbate for 7 days (qid). No systemic or ocular toxicity was observed. Both formulations caused no ocular irritation.

An ocular toxicity study of 0.5% Timolol-LA ophthalmic solution by 28 days repeated instillation in rabbits. Vol. 11, Page 116

Key study finding: Timolol-LA 0.5% produced no ocular and systemic toxicity in rabbits with ocular treatment 4 times daily for 28 days.

Study No: [ ] 50-64
Report No: 9938
Conducting laboratory and location: [ ]

Date of study initiation: May 6, 1999
GLP compliance: Yes
QA report: Yes

Animal: New Zealand white rabbits, 10-week old, ♂: 2.04-2.33 kg, ♀: 1.97-2.49 kg, 5/sex/group
Route: Ocular, topical
Dosage: 50 µl qid at intervals of about 2.5 hr, right eye only
Drug: 0.5% Timolol-LA ophthalmic solution (Lot#: 9N23, purity: [ ]
Control: Physiological saline (PSS) and vehicle
Positive control: Tomoptic 0.5% (Lot#: 1623H)
Study design:

Group	Test article	N/sex/group	Dosing regimen
1	PSS	5	qid x 28 days
2	Vehicle	5	qid x 28 days
3	0.5% Timolol-LA	5	qid x 28 days
4	Timoptic 0.5%	5	qid x 28 days

The purpose of this study was to evaluate the ocular toxicity of 0.5% Timolol-LA ophthalmic solution when instilled to rabbits 4 times daily for 4 weeks. The day of the first dosing was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study [ ] 50-64

Parameter	Procedure
Clinical observations	Twice daily
Body weights	Once weekly
Food consumption	Weekly
Ophthalmology	Gross examination: Once prior to the study initiation and weekly thereafter. Slit-lamp microscope and ocular fundus examination: Prior to the study initiation and in Weeks 2 and 4. ERG: Prior to the study initiation and in Weeks 2 and 4.
Clinical pathology	Once prior to the study initiation and in Week 4
Gross pathology	All animals at the end of the study
Organ weights	The following organs from all animals were weighed at gross pathology. Eyeball (right), brain, heart, lungs, thymus, thyroids, submandibular glands, liver, spleen, kidneys, adrenals, tests, epididymides, ovaries and uterus.
Histopathology	The following tissues from all animals were examined microscopically. Eyeball (right, including optic nerves), upper and lower eyelids (right), nasolacrimal ducts (right), lacrimal glands (right), accessory lacrimal glands (right), heart, spleen, bone marrow and bone (right femur and sternum), lungs, liver, kidneys.

Results:

Clinical observations: During the dosing period, no mortality and dosing-related clinical signs were noted.

Body weights: No treatment-related changes in body weights were observed.

Food consumption: There were no differences in food consumption among treated and different control groups.

Ophthalmology: No treatment-related abnormal findings were noted during ophthalmic examinations.

Clinical pathology: No treatment-related, toxicologically significant differences from the negative or vehicle control groups were noted in the test article or positive control groups.

Macroscopic examination: No treatment-related abnormalities were noted.

Organ weights: No treatment-related abnormalities were observed.

Microscopic observations: No toxicologically significant, biologically relevant abnormalities were observed in histopathological examination.

In summary, rabbits were treated topically with Timolol-LA 0.5% for 4 weeks (qid, right eye only). No systemic and ocular toxicity was observed in either Timolol-LA treated group or different control groups.

An ocular toxicity study of 0.5% Timolol-LA ophthalmic solution by 26 weeks repeated ocular instillation in cynomolgus monkeys. Vol. 12, Page 001

Key study finding: Timolol-LA 0.5% produced no ocular and systemic toxicity in monkeys with ocular treatment 4 times daily for 26 weeks.

Study Nº: [redacted]50-76
Report Nº: S2001I0401
Conducting laboratory and location: [redacted]

Date of study initiation: April 4, 2000
GLP compliance: Yes
QA report: Yes
Animal: Cynomolgus monkeys, 3-6 years old, ♂: 2.83-4.35 kg, ♀: 2.60-3.47 kg
Route: Ocular, topical
Dosage: 50 µl qid at intervals of 3 hr, right eye only
Drug: 0.5% Timolol-LA ophthalmic solution (Lot#: 21066, purity = [redacted]. This is the clinical formulation)
Control: Vehicle
Positive control: Timolol Maleate Ophthalmic Solution USP, 0.5% (Lot#: 91389)
Study design:

Group	Test article	N/sex/group	Dosing regimen
1	Vehicle	5	qid x 26 weeks
2	0.5% Timolol-LA	5	qid x 26 weeks
3	Timolol maleate	5	qid x 26 weeks

The purpose of this study was to evaluate the ocular toxicity of 0.5% Timolol-LA ophthalmic solution when instilled to monkeys (5/sex/group) 4 times daily for 26 weeks. The drug was compared with timolol maleate ophthalmic solution USP, 0.5%. The day of the first dosing was designated as Day 0. The week of the first dosing was designated as week 1. Toxicity was assessed as shown below.

Toxicity assessment for Study [ ] 50-76

Parameter	Procedure
Clinical observations	Twice daily
Body weights	Weekly
Food consumption	Daily
Ophthalmology (right eye)	Gross examination of ocular anterior portion, slit-lamp microscope and ocular fundus examination, and intraocular pressure measurements: once prior to the study initiation and at Weeks 4, 13 and 26 ERG: Prior to the study initiation and in Weeks 13 and 26
Clinical pathology	Once prior to the study initiation and in Weeks 12 and 25
Gross pathology	All animals at the end of the study
Organ weights	The following organs from all animals were weighed: Eyeball (right), brain, heart, lungs, thymus, thyroids, submandibular glands, liver, spleen, kidneys, adrenals, testes, epididymides, ovaries, and uterus.
Histopathology	The ocular and systemic organs and tissues were prepared and stored. Histopathological examinations were not performed because no drug-related abnormalities were noted in gross pathology.*

***Reviewer's comments**: In the message conveyed to the sponsor in January 2000, the reviewer suggested that the histopathological examinations on the systemic organs and tissues could be put on hold. However, in this 6-month monkey study the sponsor also held the histopathological examinations on the ocular tissues, which was not recommended at that time. Considering that timolol is a well known drug, and that toxicity studies in rabbits and monkeys showed no drug-related toxicities, the reviewer believes that it is acceptable at this time not to ask the sponsor to perform the histopathological examinations on the ocular tissues.

Results:

Clinical observations: No mortality and dosing-related clinical signs were noted.

Body weights: No treatment-related changes in body weights were observed.

Food consumption: There were no differences in food consumption among treated and different control groups.

Ophthalmology: No treatment-related abnormal findings were noted in gross ophthalmic, slit-lamp biomicroscopic, funduscopic, IOP, and ERG examinations. No IOP decrease was noted. The sponsor indicated in this study report that there was no ready explanation for this finding.

Clinical pathology: No treatment-related, toxicologically significant differences in clinical chemistry and hematology examinations were noted between the treatment and control groups.

Urinalysis: An increase in protein concentrations was noted in 2 treated males (see table below). Since there were no abnormalities observed in other related parameters, and no abnormalities were noted in females in the same group, this change was not considered as toxicologically significant.

Protein concentration changes in urinalysis in male monkeys (mg%)

Group	Animal number	Pre-test	Week 12	Week 25
Vehicle	1	6	5	3
	2	3	4	15
	3	5	1	4
	4	7	5	6
	5	5	4	2
	Mean ± SD	**5.2 ± 1.5**	**3.8 ± 1.6**	**6.0 ± 5.2**
0.5% Timolol-LA	11	15	10	**60**
	12	8	4	6
	13	7	14	7
	14	6	2	**34**
	15	7	4	6
	Mean ± SD	**8.6 ± 3.6**	**6.8 ± 5.0**	**22.6 ± 24.1**
Timolol maleate	21	9	2	10
	22	8	2	5
	23	7	3	7
	24	5	5	8
	25	5	3	6
	Mean ± SD	**6.8 ± 1.8**	**3.0 ± 1.2**	**7.2 ± 1.9**

Macroscopic examination: No treatment-related abnormalities were noted.

Organ weights: No treatment-related abnormalities were observed.

In summary, monkeys were treated topically with Timolol-LA 0.5% for 6 months (qid, right eye only). No systemic and ocular toxicity was observed in either Timolol-LA treated group or different control groups.

Additional information on the Timolol-LA 6-month monkey toxicology study. Vol. 12, Page 245

Effects of ketamine anesthesia and training for measurement procedures on the intraocular pressure-reducing action of timolol maleate ophthalmic solution in monkeys. Vol. 12, Page 292

This was an evaluation made by the sponsor to figure out why in the 6-month monkey study (Study #: [redacted]-50-76) there was no difference in IOP between the vehicle and active treatment groups. The sponsor answered the following questions:

1. Was the study medication instilled qid as specified?
2. Were the study medications chemically stable throughout the treatment period?
3. Was the observation of no apparent treatment effect related to the applanation naivete in the monkeys?
4. The software package used for evaluation of inferential statistics.

In Study [redacted]-50-76 (An ocular toxicity study of 0.5% Timolol-LA ophthalmic solution by 26 weeks repeated ocular instillation in Cynomolgus monkeys), IOP was measured at four points during the study (baseline, and Weeks 4, 13 and 26). The results (see table below) showed that there was no difference between the vehicle and active treatment groups. Timolol was not effective in this study.

IOP changes in study [ ]-50-76 (mmHg, mean ± SD)

Group	N	Baseline	Week 4	Week 13	Week 26
Vehicle	10	18.7± 2.4	17.5± 3.0	17.4± 3.9	18.5± 2.7
		Change from baseline	**-1.2± 1.8**	**-1.3± 2.8**	**-0.2± 2.0**
Timolol-LA	10	19.0± 2.9	17.9± 2.1	18.8± 3.3	16.8± 2.4
		Change from baseline	**-1.1± 2.4**	**-0.2± 2.9**	**-2.2± 1.7**
Timolol	10	20.4± 2.9	18.7± 2.7	20.0± 2.9	19.2± 2.4
		Change from baseline	**-1.7± 1.9**	**-0.4± 3.1**	**-1.2± 2.0**

Instillation: After reviewing the instillation record, the sponsor concluded that failure to properly receive study medications was NOT an explanation for the observation regarding IOP.

Chemistry: The sponsor reviewed the analytical results for Timolol. The results showed that the concentration of active ingredient, timolol maleate, in the product was within specification at the end of the study (content = [ ] stability = [ ]. Therefore, loss of potency of the active test material was NOT an explanation for the observation regarding IOP.

Pneumatonometer naivete: The monkeys used in Study [ ]-50-76 were naïve to measurement of their IOP by pneumatonometer. During the measurement, the animals only received a topical anesthetic agent (Benoxyl 0.4% solution). No ketamine or other injectable agents were given. To investigate the effects of training for IOP measurement procedures and ketamine anesthesia on IOP-lowering action of timolol maleate, the sponsor conducted a study (Study 2002SJ016) in male cynomolgus monkeys. In this study, timolol maleate ophthalmic solution was instilled once into the left eye of monkeys and IOP was measured 2 hr after dosing. The results, as shown in the table below, demonstrated that IOP decrease was noted in all three treatment groups. The decrease was most marked in trained, anesthetized (ketamine chloride, 10 mg/kg, im) animals (-6.8 mmHg), followed by trained conscious animals (-3.6 mmHg). The study indicated that timolol IOP-reducing effect was clearly manifested in trained animals and was enhanced by ketamine-anesthesia in trained animals.

Effects of timolol maleate ophthalmic solution in monkeys (mmHg, mean ± SD)

Group	Baseline	2 hr	Change
Trained conscious	21.2 ± 0.3	17.6± 0.8	-3.6± 0.7
Trained anesthetized	20.2± 0.9	13.4± 1.6	-6.8± 1.0
Untrained anesthetized	22.7± 1.7	21.2± 2.3	-1.5± 0.7

Software issue: The 6-month monkey study (Study [ ]-50-76) employed MITOX as a data management system. The statistical analysis was performed using MUSCOT. This system was commonly used in Japan, but not in the United States. Thus, the sponsor re-analyzed the data using PC-SAS. The results from PC-SAS and MUSCOT analyses were similar.

In conclusion, the sponsor concluded that the lack of ocular hypotensive efficacy of timolol maleate ophthalmic solution and Timolol-LA in the 6-month monkey study was related to the naivete of the monkeys.

Summary and Conclusions:

The sponsor submitted four toxicity studies with Timolol-LA with the duration up to 28 days in rabbits and 6 months in monkeys. The drug was very well tolerated. No drug-related systemic or ocular toxicity was observed. In monkey study, there was no IOP decrease following

the administration of timolol. The sponsor indicated that it is possibly due to the naivete of the monkeys to the IOP measurement by pneumatonometer.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Timolol maleate is a non-selective β adrenergic receptor blocking agent. The drug has the action of reducing elevated as well as normal intraocular pressure, whether or not accompanied by glaucoma. Timolol maleate ophthalmic solution has been marketed for many years in many countries for the treatment of ocular hypertension and glaucoma. The drug is generally considered as safe and well tolerated.

In this NDA submission, the sponsor proposed a new formulation of timolol maleate ophthalmic solution with potassium sorbate (0.47%) in order to enhance the ocular bioavailability of timolol instilled. In animal PK studies, following ocular administration, the new formulation of timolol maleate ophthalmic solution, with potassium sorbate, showed increased ocular bioavailability of timolol. On the other hand, the systemic bioavailability was not changed. The improvement in ocular bioavailability of timolol with sorbic acid in Timolol-LA was likely due to the improved partitioning of timolol in the corneal epithelium.

In toxicity studies, the drug product was well-tolerated with the duration up to 4 weeks in rabbits and 6 months in monkeys, respectively. No ocular and systemic toxicity was observed. There were no differences in safety profile between Timolol-LA and the comparator product, 0.5% timolol maleate ophthalmic solution.

Based on the nonclinical study results, it is concluded that there are no safety concerns over this new drug product. No new nonclinical toxicology studies are necessary. No toxicological issues are indicated.

General Toxicology Issues: No new nonclinical toxicology studies are necessary. No toxicological issues are indicated.

Recommendations: There are no pharmacology/toxicology objections to the approval of this NDA.

Labeling with basis for findings: The labeling for the Carcinogenesis, Mutagenesis, Impairment of Fertility section and the Pregnancy section is identical to the labeling for Timoptic, with the exception that the name TIMOPTIC is replaced with BRANDNAME.

X. APPENDIX/ATTACHMENTS:

Addendum to review: No.

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

Any compliance issues: No.

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

/s/

Zhou Chen
12/13/02 10:17:33 AM
PHARMACOLOGIST

Josie, Please sign this review. Thanks for your corrections!
Zhou

Josie Yang
12/13/02 10:51:10 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA Number: NDA 21-516 Applicant: Senju Pharmaceutical Co., Ltd. Stamp Date: September 27, 2002
Drug Name: Timolol maleate ophthalmic solution, 0.5%

IS THE PHARM/TOX SECTION OF THE APPLICATION FILABLE? (Yes or No) Yes

The following parameters are necessary in order to initiate a full review, i.e., complete enough to review but may have deficiencies.

	Parameter	Yes	No	Comment
1	On its face, is the pharmacology section of the NDA organized in a manner to allow substantive review to begin?	XX		
2	Is the pharmacology section of the NDA indexed and paginated in a manner to allow substantive review to begin?	XX		
3	On its face, is the pharmacology section of the NDA legible so that substantive review can begin?	XX		
4	Are ALL required and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute adult studies, chronic adult studies, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal PK studies, etc)?	XX		The drug product submitted with this NDA application is a reformulation of the approved drug product. Most nonclinical studies have been performed by other sponsors. Only several nonclinical studies related to the new formulations are submitted.
5	If the formulation to be marketed is different from that used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies with the to be marketed product or to explain why such repetition should not be required?	XX		
6	Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	XX		
7	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?		XX	No special studies were requested by the Division during pre-submission discussions.
8	On its surface, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	XX		
9	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	XX		
10	Has the sponsor submitted a statement(s) that the pharm/tox studies have been performed using acceptable, state-of-art protocols which also reflect agency animal welfare concerns?	XX		
11	From pharmacology perspective, is this NDA fileable? If "no", please state below why it is not.	XX		

Reviewing Pharmacologist: Date:

Team Leader: Date:

cc:
Original NDA 21-516
HFD-550/Division File
HFD-550/Pharmacology/ChenZ
HFD-550/PM/TL/Yang
HFD-550

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

/s/

Zhou Chen
11/13/02 09:46:58 AM
PHARMACOLOGIST

Josie, Please sign this checklist. Thanks, Zhou

Josie Yang
11/13/02 09:52:31 AM
PHARMACOLOGIST