CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-963

PHARMACOLOGY REVIEW(S)

THE DIVISION OF ANTI-INFLAMMATORY, ANALGESIC,
AND OPHTHALMIC DRUG PRODUCTS

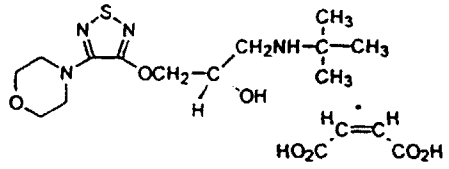
PHARMACOLOGY/TOXICOLOGY REVIEW
INITIAL REVIEW

NDA 20-963

DRUG:	Timolol Maleate Ophthalmic Gel Forming Solution (0.25% and 0.5%)
SPONSOR:	Alcon Laboratories Fort Worth, TX 76134-2099
SUBMISSION:	February 16, 1998
DATE RECEIVED:	February 18, 1998
AMENDMENT:	July 2, 1998
REVIEW COMPLETED:	July 15, 1998

DRUG CATEGORY: Non-selective β adrenergic receptor blocker

STRUCTURAL FORMULA:



$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ Mol. Wt. 432.50

Chemical Names:
a) (S)(-)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol, (Z)-2-butenedioate (1:1) salt

USAN and USP Name: Timolol Maleate

CAS Registry Nº: 26921-17-5

FORMULATION:

TIMOLOL OPHTHALMIC GEL FORMING SOLUTIONS (%)		
INGREDIENT	**PERCENT CONCENTRATION**	
Timolol Maleate, USP		0.68
Xanthan Gum, NF		
Mannitol, USP		
Boric Acid, NF		
Tromethamine, USP		
Benzododecinium Bromide, NOC		0.012
Polysorbate 80, NF		
Purified Water, USP	qs 100	qs 100

PROPOSED MARKETING INDICATION:

This product is indicated for the treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma. The dose is one drop, either 0.25% or 0.5%, in the affected eye(s) once daily.

RELATED DRUGS/INDs/NDAs:

NDA 18-086 TIMOPTIC® (Merck)
NDA 20-330 TIMOPTIC-XE® (Merck)

PRECLINICAL STUDIES/REVIEWS/REFERENCES: **PAGE**

PHARMACOLOGY: 3

Thom J. Zimmerman; Topical Ophthalmic Beta Blockers: A Comparative Review; J. Ocular Pharm. 9:373-384,1993.

Brian B. Hoffman and Robert J. Lefkowitz; Adrenergic Receptor Antagonists; Goodman and Gilman's The Pharmacological Basis of Therapeutics, Eight Edition; edited by Gilman, Rall, Nies and Taylor, Pergamon Press 1990.

Physicians' Desk Reference for Ophthalmology, 25th Edition, pp. 286-289.

Chapter 25, Adrenergic Antagonists; Becker-Shaffer's Diagnosis and Therapy of the Glaucomas, Sixth Edition; edited by H. Dunbar Hoskins, Jr. and Michael Kass, Mosby Company, 1989.

Guenter Krieglstein; Adrenergic Drugs in Management of Intraocular Pressure; Glaucoma: Applied Pharmacology in Medical Treatment; edited by Stephen M. Drance and Arthur H. Neufeld, Grune and Stratton, 1984.

TOXICOLOGY:

Three-Month Topical Ocular Irritation Evaluation of Timolol Gel Forming Solution in Rabbits.
Technical Report Nº 008:38520:0297

Six-Month Interim Report From the One-Year Topical Ocular Irritation and Systemic Toxicity Evaluation of Timolol Gel Forming Solution in primates.
Technical Report Nº 083:38520:0797

One-Year Topical Ocular irritation and Systemic Toxicity Evaluation of Timolol Gel Forming Solution in Primates.
Technical Report Nº 031:38520:0398 In the July 2, 1998 amendment

Agar Diffusion Test With Gamma Sterilized Dupont 20-6064 LDPE White DROP-TAINERs.
Technical Report Nº 004:38520:0195

Elution Test With Gamma Sterilized Dupont 20-6064 LPDE White DROP-TAINERs.
Technical Report Nº 005:38520:0195

Acute Systemic Toxicity in Mice With Extracts of Gamma Sterilized Dupont 20-6064 LPDE White DROP-TAINERs.
Technical Report Nº 006:38520:0195

Intracutaneous Reactivity Test in Albino Rabbits With Extracts of Gamma Sterilized Dupont 20-6064 LDPE White DROP-TAINERs.
Technical Report Nº 007:38520:0195

Agar Diffusion Test With Gamma Sterilized Dupont 20-6064 LDPE Natural DROP-TAINERs.
Technical Report Nº 039:38520:0495

Elution Test With Gamma Sterilized Dupont 20-6064 LDPE Natural DROP-TAINER.
Technical Report Nº 040:38520:0495

Acute Systemic Toxicity in Mice With Extracts of Gamma Sterilized Dupont 20-6064 Natural DROP-TAINEs.
Technical Report Nº 041:38520:0495

Intracutaneous Reactivity Test in Albino Rabbits With Extracts of Gamma Sterilized Dupont 20-6064 LDPE Natural DROP-TAINERs.
Technical Report Nº 042:38520:0495

Primary Ocular Irritation in Rabbits With Extracts of Gamma Sterilized Dupont 20-6064 LDPE Natural DROP-TAINERs.
Technical Report Nº 043:38520:0495

Agar Overlay of Gamma Irradiated Himont PF-511 Yellow and Light Blue 15 mm Polypropylene Closures.
Technical Report Nº 070:38520:0990

Acute Systemic Toxicity in Mice and Primary Ocular Irritation in Rabbits of Extracts of Yellow and Light Blue 15 mm PF-511 Yellow and Light Blue 15 mm Polypropylene Closures.
Technical Report Nº 071:38520:0990

Acute Systemic Toxicity in Mice and Primary Ocular Irritation in Rabbits of Extracts of 15 mm (Red, Blue, Green and White) Closures.
Technical Report Nº 043:38520:0689

Agar Overlay Test of Red, Blue, Green and White Polypropylene Closures.
Technical Report Nº 044:38520:0690

ABBREVIATIONS USED IN THIS REVIEW

D = day(S) W = week(s) ↑ = increase ↓ = decrease
BAB = benzyldimethyldodecylammonium bromide - also indicated as benzododecinium bromide (preservative)
TGFS = Timolol Gel Forming Solution

PHARMACOLOGY:

Timolol is a nonselective β_1 and β_2 adrenergic receptor blocking compound (blocks epinephrine stimulation of the heart) having no local anesthetic activity. It is essentially lacking in β-agonist and anticholinergic activity.

TOXICOLOGY:

Three-Month Topical Ocular Irritation Evaluation of Timolol Gel Forming Solution in Rabbits.
Report Nº 008:38520:0297; Protocol Nº N-96-226
NDA Vol. 1.12, p. 5-0129

Compound: Timolol Gel Forming Solution (GFS)0.5%, lot Nº 96-16594
Formulation: Timolol Maleate, USP 0.68%
Xanthan Gum, NF/AR,
Polysorbate 80, NF
Boric acid, NF
Tromethamine, USP
Mannitol, USP
Benzododecinium bromide (BAB), NF
Purified water, USP qs to 100 w/v%
Route: Topical, ocular - one drop to both eyes bid for 3 months
Diet: One cup (250 mL) Certified Rabbit Chow/day - water *ad libitum*.
Strain: NZW, body weight 3.0 to 3.3 kg

Dose:		Test Article	Approx Volume (µL)	Treatments/Day
Group:	1	Untreated Control	0	2
	2	Vehicle	80	2
	3	Timolol GFS 0.5%	80	2
	4	Timoptic XE 0.5%	80	2

Vehicle: Xanthan gum NF/AR polysorbate 80 NF boric acid NF tromethamine USP, manitol USP BAB NF
purified water USP qs to 100%
Number: 7/sex/group
Study Site: Alcon Laboratories, Inc., Fort Worth, TX.
Date: October 16, 1996 - January 10, 1998
GLP/QAU Statements: Both present and signed.

The purpose of this study was to determine the ocular irritation potential of 0.5% Timolol Gel Forming Solution when administered at one drop topically to both eyes of rabbits twice a day for three months.

Prior to study initiation, animal eyes were said to be within normal limits, with 0 scores for all parameters except conjunctival congestion (scores of 0 or +1). Three rabbits/group were sacrificed at Day 35. **Observations** of all animals occurred twice a day for morbidity, moribundity, and general health. A detailed **examination** was done twice a week about one hour after the first dose. **Body weight** was determined prior to the first treatment (Day 0), weekly for the first five weeks, then biweekly. **Ophthalmoscopic examinations** were carried out prior to study initiation and at one and three months of treatment [fundus with respect to optic head nerve characteristics-fundic vascular pattern (retinal and choroidal)-pigmentation and coloration-corneal pachymetry] **Necropsy** included examination of eyes and adnexa. **Microscopic examinations** were conducted on eyes, adnexa, and nasal lacrimal tissue of all animals. **Biostatistics** (means and SD) were determined for body weight and corneal pachymetry at measured intervals.

RESULTS

- ocular discharge: high incidence with some hair loss and crusting around eyes with Timoptic XE during study (G4)- one vehicle group developed OD discharge D48-
- body weight: no significance between control and treated groups-
- conjunctival congestion was minimal/moderate in G3 and G4-
- ophthalmoscopic exam: all animals were within normal range-
- gross observations: no adverse treatment related findings-
- pachymetry ♂ (♀) µm: (from Vol. 1.12, pp. 5-0226 and 5-0228)

	G1	G2	G3	G4
Mean D-1:	356(366)	360(346)	359(355)	357(370)
Mean D34:	363(374)	358(342)	360(359)	371(381)

Mean D90:	382(393)	380(365)	375(374)	377(390)
Δ D-1(-)D90:	26(27)	20(19)	16(19)	20(20)

- histopathology: no treatment-related changes occurred in any tissue or between ♂ and ♀ of treated groups - incidental changes were present in some tissues-

The results of this study did not produce any changes in the eyes, adnexa, or nasal lacrimal tissues from topical administration of Timolol Gel Forming Solution 0.5%. Ocular discharge was rather widespread in the Timoptic XE treated group.

Six-Month Interim Report From the One-Year Topical Ocular Irritation and Systemic Toxicity Evaluation of Timolol Gel Forming Solution in Primates.
Report Nº 083:38520:0797; Protocol Nº N-97-020
NDA Vol. 1.13, p. 5-0317

Technical Report Nº: 083:38520:0797
Protocol Nº: N-97-020
Compound: Timolol Gel Forming Solution, Lot Nº AQE-2866
Formulation: Timolol Maleate, USP 0.68 w/v%
Polysorbate 80, NF
Xanthan Gum, NF
Mannitol, USP
Boric Acid, NF
Tromethamine, USP
BAB
Purified Water, USP QS to 100 w/v%
Route: Topical, ocular
Diet: Each animal was given a measured quantity (not indicated) of food/day, supplemented with apples, oranges, and other fruit. Water was available *ad libitum*.
Strain: Cynomolgus monkey, body weight 2.6-4.9 kg on Day 0.
Dose Levels: Group: 1 2
Treatment Volume: vehicle 40 µL (one drop bid OD only)
Control Treatment: 40 µL of the following vehicle: polysorbate 80 xanthan gum mannitol boric acid tromethamine BAB purified water qs to 100%-
Number: 8/sex/group - 3/sex/group assigned for interim sacrifice.
Study Site: Alcon Laboratories, Inc., Fort Worth, TX
Date: February 12, 1997 to February 10, 1998
GLP/QAU Statements: Both present and signed.

The purpose of this study was to determine the ocular irritation and systemic toxicity potential of 0.5% Timolol Gel Forming Solution

(TGFS) when administered to monkeys bid for one year. This report is the interim six month evaluation.

Three/sex/group were evaluated in this interim report, the remainder will be evaluated after one year on study (see the 7/2/98 amendment). The left eye served as the contralateral untreated control. **Daily observations** were done twice a day. **Detailed examinations** were done prior to the second daily dose on Tuesday and Friday of each week. **Body weight** was recorded prior to the treatment on D0, W1, 2, 4, 6, 8, 12, then monthly thereafter, and at necropsy. **Biomicroscopic examination** of both eyes (conjunctiva, cornea, anterior chamber, light reflex, lens, iris) were evaluated prestudy (OD and OS within normal limits) and during W1, 2, 4, 6, 8, and 12, and monthly thereafter. **Indirect ophthalmoscopic examinations** (fundus/optic nerve head characteristics-fundic vascular pattern (retinal and choroidal)-pigmentation/coloration) were done prestudy and after 3, 6, 9, and 12 months of treatment on both eyes. **Pachymetry** (central corneal thickness, 3 readings/eye/examination) was determined prior to study initiation and at 3, 6, 9, and 12 months. **Specular microscopy and photography** of the central corneal endothelium were done prestudy, at approximately 6 month and one year of treatment. **Hematology** [Hct-Hb-RBC/WBC platelet counts-mean corpuscular Hb concentration/mean corpuscular volume/mean corpuscular Hb-prothrombin time (PT)-activated partial thromboplastin time (APTT)] analyses were performed prestudy, after 6 months and at one year. **Serum chemistry** (albumin-A/G ratio-ALT-AST-amylase-alanine aminotransferase-bilirubin-BUN/creatinine ratio-Ca-cholesterol-creatine phosphokinase-creatinine-globulin-γ glutamyl transpeptidase-glucose- LDH-phosphorus-K-Na-total protein-triglycerides-urea nitrogen-uric acid) analyses were determined prior to study, after 6 months and at one year. **Gross pathology** was conducted on about 49 tissues and organs. **Organ weights** (absolute and relative to final body weight) were determined for liver, gonads, adrenals, heart, kidneys, brain, and spleen. All tissues collected from all animals were submitted for **histopathologic examination** (Experimental Pathology Laboratories). **Biostatistics** (mean and S.D.) were calculated (Kruskal-Wallis) for body weights, clinical pathology parameters, organ weights, pachymetry, and specular microscopy measurements.

RESULTS

- clinical signs: sporadic diarrhea in each group - minor mechanical injuries to fingers (5/16G1, 2/16G2)-
- sacrifice: ♀ G1 (№ 1597) in 1 year group terminated at 6 months - no explanation given-

- body weight: no significance between G1 and G2 over 6 months - Δkg (control body weight minus treated body weight) varied from 0.45 kg D0 to 0.63 kg D28, then decreased to 0.35 at D182 - this was a significant (p = 0.0001) time effect - both groups showed a mean ↑ in body weight gain-
- biomicroscopic exam: no conjunctival congestion/swelling/discharge - no light reflex change/flares/iritis or fluorescein staining - no iritis or additional lenticular changes from prescrean - no additional corneal cloudiness - no observed neovascularization-
- ophthalmoscopy: optic nerve head and major retinal and choroidal vessels within normal limits D0, D84, and D182-
- pachymetry: no significant effects of G2, time effect, or time/group interaction-
- specular microscopy: no significant difference in cell density, coefficient of variation for cell density, or % hexagons from corneal endothelial cells of treated eyes-
- hematology: mean values
  - platelets: significant low mean values D8 in ♀G2-
  - monocytes: ↑ in ♀G2 D176 (p=0.0039)-
  - polysegmented neutrophils: significant low mean values D8 in G2♂♀-
  - prothrombin time: significantly higher mean value D8 in ♂ G2-
- serum chemistry:
  - alkaline phosphatase: (p=0.0391) elevated in ♂G2 D176-
- organ weights: no significant differences reported for absolute or relative to final body organ weights-
- gross observations (correlated microscopic findings): 3/sex/group
  - G1 ♂(Nº 1563) kidneys moderately pitted (mononuclear cell infiltrate) - mesenteric lymph nodes markedly enlarged (lymphoid hyperplasia) - stomach fundus focal gastric ulcer (acute mucosal hemorrhage/acute erosion - spleen, prominent lymphoid follicles (lymphoid hyperplasia)-
    ♂(Nº 1560) white firm plaques in mesentery (cartilaginous metaplasia)-
    ♀(Nº 1610) right kidney pale cortical foci-
  - G2 no gross lesions reported-
- histopathology: no ocular lesions seen in G1 sexes-
  - G2 rectum: ♀ Nº 1601 acute serosal hemorrhage, moderately severe-
    kidney: ♀ minimal mononuclear cell infiltrate (Nº 1583, 1601)-
    lung: ♀ minimal chronic interstitial inflammation-
    skin: ♀ Nº 1595 minimal mononuclear dermal cell infiltrate-
    ♂ Nº 1623 minimal mononuclear dermal cell infiltrate-

skeletal muscle: ♂ Nº 1562 slight/mild acute hemorrhage and moderate acute inflammation-

There were no ocular irritation or ocular lesions that appeared to be related to the six-month treatment of these monkeys. Other organ lesions appeared to be of random occurrence and not related to drug treatment.

Final Study Report For The One-Year Topical Ocular Irritation And Systemic Toxicology Evaluation Of Timolol Gel Forming Solution In Primates
Report Nº 031:38520:0398
In amendment dated July 2, 1998

Technical Report Nº: 031:38520:0398
Protocol Nº: N-97-020
Compound: Timolol Gel Forming Solution
Formulation: Timolol Maleate, USP 0.68 w/v%
Polysorbate 80,
Xanthan Gum, NF
Mannitol, USP
Boric Acid, NF
Tromethamine, USP
BAB
Purified Water, USP QSS to 100 w/v%
Route: Topical, ocular
Diet: Each animal was given a measured quantity (not indicated) of food per day, supplemented with apples/oranges, and other fruit
Water: Available *ad libitum*.
Strain: Cynomolgus monkeys, body weight 2.6 to 4.9 kg on Day 0.
Number: 8/sex/group - 3/sex/group were assigned interim sacrifice.
Dose Levels: Group 1 2
Treatment Volume: 0 40 µL (1 drop b.i.d. OD only) for 371 days.
Control Treatment: 40 µL of the following: polysorbate xanthane gum mannitil boric acid tromethamine BAB purified water qs to 100%-
Study Site: Alcon Laboratories, Inc., Fort Worth, TX
Date: Testing initiated 2/12/97 - In-life phase completed 2/18/98.
GLP/QAU Statements: Both present and signed.

RESULTS

Clinical signs: both groups and sexes had minor injury to fingers, hands, or tail - diarrhea at times in both groups-

Mortality: 1G1♀ found dead D238 distended abdomen with two large holes - mottled liver -yellow bile - hemorrhage areas in colon - mesenteric lymph node hemorrhage - other tissues autolytic - cause of death not determined but considered incidental-

Body weight: no significant effects-

Ocular evaluation:

- biomicroscopic evaluation:
- conjunctival congestion: not observed in any animal
- conjunctival swelling: not observed in any animal
- conjunctival discharge: not observed in any animal
- light reflex: changes not observed in any treated or control animal
- flare: ♀G1 Day 210 in OS (score 1 out of 3)
  ♀G1 Day 210 in OS (score 1 out of 3)
  ♀G1 Day 210 in OD (score 1 out of 3)
- iritis: not observed in any animal
- corneal cloudiness: not observed in any animal
- fluorescein staining: not observed in any animal
- lense: all reported as normal
- neovascularization: all animals reported normal
- indirect examinations: all eyes reported within normal limits-
- pachymetry: no significant difference in either sex-
- specular microscopy: % hexagons seen in G2♂ OD was larger Day-5 but changed over time when compared to controls - at end of study the % was slightly smaller than controls-

Hematology: no significant changes in the hematology parameters-

Coagulation:

- prothrombin time: no significant change-
- activated partial thromboplastin time: ↓ 8.9% in ♀ over the study period - not significant-

Serum chemistry: G2 ALT ↓ 18.5% over study period - not significant-

Gross pathology: G1♂ spleen with multiple 0.2 cm white foci- animal that died had stomach, liver, gallbladder, and colon lesions - no other animal with reported lesions-

Histopathology:

SUMMARY INCIDENCE TABLE OF THE EYE
(from p. 9-250 of 7/2/98 amendment)

NUMBER EXAMINED (number observed with incidence)	G1 OS	G1 OD*	G2 OS	G2 OD*
ANTERIOR CHAMBER	10	10	10	10
CHOROID - infiltrate, mononuclear cell	10	10 (1)	10	10 (1)
CILIARY BODY -infiltrate, mononuclear cell	10 (2)	10 (1)	10 (3)	10 (3)
CORNEA Limbus, infiltrate, mononuclear cell	10	10	10 (1)	10
EYELID - infiltrate, mononuclear cell	10 (2)	10 (1)	10 (4)	10
IRIS	10	10	10	10
LACRIMAL GLAND - infiltrate, mononuclear cell	7 (3)	8 (4)	9 (7)	7 (5)
LENSE	10	10	10	10
NASAL TURBINATE	10	10	10	10
NASOLACRIMAL DUCT	9	8	4	4
OPTIC NERVE	10	10	10	10
RETINA	10	10	10	10
SCLELRA - infiltrate, mononuclear cell	10 (1)	10 (1)	10 (2)	10 (2)

*OD = the treated right eye

The following histopathology table includes only incidences occurring in greater number in the treated monkeys than were observed in control monkeys.

NONOCULAR HISTOPATHOLOGY INCIDENCE TABLE
(from pp. 9-266 to 9-273)

	♂ G1	♂ G2	♀ G1	♀ G2
Bone Marrow Sternum - mononuclear cell infiltrate		3 (1-3)*		
Brain Meninges - mononuclear cell infiltrate		2 (1)		
Kidney Cortex, Interstitium - fibrosis mononuclear cell infiltrate	2 (1-2)	1 (3) 5 (1-4)		
Liver Periportal - mononuclear cell infiltrate	2 (1)	3 (1)	3 (1)	4 (1)
Lymph Node, Cervical - lymphoid hyperplasia		3 (2-3)		1 (2)
Lymph Node, Mesenteric, lymphoid hyperplasia		1 (3)		1 (3)
Pancreas - mononuclear cell infiltrate		3 (1-2)		1 (1)
Parathyroid - mononuclear cell infiltrate		1 (2)		
Peripheral Nerve, Sciatic - mononuclear cell infiltrate		1 (1)		
Prostate - mononuclear cell infiltrate		1 (3)		
Salivary Gland - mononuclear cell infiltrate	3 (1-2)	5 (1-4)		

	♂ G1	♂ G2	♀ G1	♀ G2
Skeletal Muscle - mononuclear cell infiltrate - chronic inflammation - myofiber degeneration		2 (1-2)		 1 (2) 1 (1)
Skin Dermis mononuclear cell infiltrate		3 (1-3)		
Spleen - lymphoid hyperplasia		3 (2)		1 (2)
Testis Seminiferous Tubules - mineralization		1 (1)		
Thymus lymphoid hyperplasia		1 (2)		
Thyroid mononuclear cell infiltrate	1 (1)	4 (1-2)		
Urinary Bladder - mononuclear cell infiltrate		1 (1)		2 (1-2)

* The first number indicates the number on animals with the lesion. The number(s) in parenthesis indicate the degree of severity. 1 minimal, 2 slight/mild, 3 moderate, 4 moderately severe, and 5 severe/high.

Organ weights:

- liver: relative weight ♂ ↑ $p<0.05$
- ♂ spleen: relative weight ♂ ↑ $p<0.05$, ↓ $p<0.05$ (23%)-

Several of the nonocular lesions graded from 1 to 4 were considered to be congenital, and most occurred in the treated males. They were "considered incidental and unrelated to treatment." They were indicated as commonly occurring spontaneously in macaques. [Wojcinski, Z.W. et al., Proceedings of the 14th Annual Symposium of the Society of Toxicology Pathologists, San Diego CA, June 11-15, 1995, p. 56, and Yanai, T., et al., Vascular Mineralization in the Monkey Brain, Vet. Pathol., 31:546-552:1994.]

It is difficult, however, to understand why the above incidences of mononuclear cell infiltrates occurred more extensively in the treated groups. These mononuclear cells were described as infiltrates consisting of small, loosely to densely packed aggregates of lymphocytes and/or other small mononuclear cells. In addition to the above lesions, there were also lesions in the control animals that were not observed in the treated animals.

There were no ocular lesions that appeared to be related to the treatment. Slit-lamp biomicroscopy, specular microscopy of the corneal endothelium, and pachymetry measurements did not show any treatment related findings. Based on the data from the six month interim report and this final report, it is concluded that the product did not cause damage to the eye or adnexa when administered twice a day for one year.

Agar Diffusion Test With White DROP-TAINERs.
Report Nº 004:38520:0195
NDA Vol. 1.14, p. 5-0550

This study was conducted to determine if diffusible components from white droptainers (lot X-0118-G) are cytotoxic to L-929 mouse fibroblast cell monolayers. The study was done at and completed in the early part of 1995.

Under the conditions in which this USP XXIII study was conducted, slight reactivity of Grade 1 (slight-some malformed or degenerated cells under the specimen) was reported at 48 hours. Natural rubber, the positive control, produced Grade 2 (mild - 0.4 cm zone limited to the area under the specimen) and 3 (moderate, 0.7 cm - zone extends 0.5 to 1.0 cm beyond the specimen) at 24 and 48 hours, respectively. The silicon negative control produced no detectable zone around or under the specimen. According to the USP, samples meet the test if none of the cultures show greater than a mild reactivity (Grade 2).

The results of the study indicate white Drop-Tainers are suitable for use as ophthalmic containers for TGFS.

Elution Test With White Drop-Tainers.
Report Nº 005:38520:0195
NDA Vol. 1.14, p. 5-0564

This study was performed by under GLPs for the purpose of determining if diffusible components from white droptainers are cytotoxic to mouse fibroblast L-929 (Lot Nº X-0118-G) cell cultures. The study followed the USP XXII requirements. The results are indicated in the following table:

The positive control was natural rubber, the negative control was silicone. All extracts were incubated for 48 hours at 37°C and tested in duplicate.

Time		Test Article		Control Articles Negative		Positive	
Hours	Date	A	B	A	B	A	B
0	2/1/95	0	0	0	0	0	0
24	2/2/95	0	0	0	0	4	4
48	2/3/95	0	0	0	0	4	4

A grade of 0 reactivity indicated no cell lysis. Grade 4, which was observed for natural rubber, was the most reactive and indicated nearly complete destruction of cell layers.

The results of this study indicate that the white Drop-Tainers are suitable for use as ophthalmic containers for TGFS.

Acute Systemic Toxicity in Mice With Extracts of White DROP-TAINERs.
Report Nº 006:38520:0195
NDA Vol. 1.14, p. 5-0579

Saline and cottonseed oil extracts of White droptainers (Lot Nº X-0118-G) were injected IP into five mice/group at a dose volume of 50 mL/kg. Sodium and cottonseed oil were used as controls. All animals were observed for 72 hours for mortality and signs of toxicity. The study was done at and completed in February 1995.

No signs of toxicity or loss of weight in any of the four groups were noted. These containers meet the requirements of the USP XXIII test and appear to be safe for use as ophthalmic containers for TGFS.

Intracutaneous Reactivity Test in Albino Rabbits With Extracts of White DROP-TAINERs.
Report Nº 007:38520:0195
NDA Vol. 1.14, p. 5-0595

Local tissue response to intracutaneous administration of saline and cottonseed oil extracts of was evaluated in NZW rabbits. The study was performed by and signed in February 1998.

The results of the study did not indicate any local tissue skin reaction (erythema, edema, or eschar formation) from the control or test extracts. The study met the requirements of the USP XXIII Intracutaneous Reactivity Test and indicate that the white Drop-Tainer is suitable for use as an ophthalmic container for TGFS.

Agar Diffusion Test With Natural DROP-TAINERs.
Report Nº 039:38520:0495

NDA Vol. 1.14, p. 5-0613

This study gave results similar to the above study (Report Nº 004:38520:0195) conducted with White DROP-TAINERS, with the exception that at 48 hours no biological reactivity (Grade 0) was observed. The biological reactivity data are indicated below.

Dish	Time (hr)	Test Article Zone Size	Test Article Grade	Natural Rubber Positive Control Zone Size	Natural Rubber Positive Control Grade	Silicon Negative Control Zone Size	Silicon Negative Control Grade
A	0	0.0 cm	0	0.0 cm	0	0.0 cm	0
	24	0.0 cm	0	0.4 cm	2	0.0 cm	0
	48	0.0 cm	0	0.7 cm	3	0.0 cm	0
B	0	0.0 cm	0	0.0 cm	0	0.0 cm	0
	24	0.0 cm	0	0.4 cm	0	0.0 cm	0
	48	0.0 cm	0	0.7 cm	3	0.0 cm	0

From the results of the study, the test article meets the requirements of the USP XXIII Diffusion Test and is biocompatible for use as ophthalmic containers for TGFS.

Elution Test With
Natural DROP-TAINERs.
Report Nº 040:38520:0495
NDA Vol. 1.14, p. 5-0627

This report was evaluated at for the purpose of determining biological reactivity of extracts of natural Drop-Tainer (Lot Nº MD-2103-G). Twenty-four hour Minimum Essential Medium 10 mL extracts of 30 cm^2 were prepared at 37°C. The positive control was natural rubber and the negative control was silicone. The study was said to follow the USP XXIII requirements for this test. Results of the study are indicated below.

Time (hour)	Date	Test Article A	Test Article B	Control Articles Negative A	Control Articles Negative B	Control Articles Positive A	Control Articles Positive B
0	4/12/95	0	0	0	0	0	0
24	4/13/95	0	0	0	0	4	4
48	4/14/95	0	0	0	0	4	4

A grade of 0 indicated discrete intracytoplasmic granules - no cell lysis. A grade of 4 (severe) indicated nearly complete

destruction of the cell layers. Under the conditions of the study, these containers meet the requirements for the Elution Test and are biocompatible for use with TGFS.

Acute Systemic Toxicity in Mice With Extracts of Natural DROP-TAINERs.
Report Nº 041:38520:0495
NDA Vol. 1.14, p. 5-0641

This study was conducted by according to the USP XXIII Systemic Toxicity Test and signed February 1998. Animals were dosed with 50 mL/kg of saline or cottonseed oil extracts of natural Drop-Tainers or controls. The extracts were prepared at 70°C for 24 hours using 60 cm^2 of Drop-Tainer per 20 mL of 0.9% USP Sodium Chloride Injection or cottonseed oil.

The Study results indicated no adverse change in body weight. All animal groups showed similar weight increases. No signs of toxicity were reported for any animal. The natural Drop-Tainer is therefore compatible with TGFS.

Intracutaneous Reactivity Test in Albino Rabbits With Extracts of Natural DROP-TAINERs.
Report Nº 042:38520:0494
NDA Vol. 1.14, p. 5-0656

This study evaluated local tissue responses in NZW rabbits following intracutaneous injection of extracts of LDPE natural droptainers. Two animals per group were dosed at five different test sites with 0.2 mL aliquots of 0.9% sodium chloride control, cottonseed control, or test extracts from Drop-Tainers.

No overt signs of edema, erythema, or eschar formation were reported in any of the rabbits. Body weight changes were similar in all groups. These Drop-Tainers meet the USP XXIII Intracutaneous Reactivity Test, and are biocompatible for use with TGFS.

Primary Ocular Irritation in Rabbits With Extracts of Natural DROP-TAINERs.
Report Nº 043:38520:0495
NDA Vol. 1.14, p. 5-0674

The purpose of this study was to evaluate the ocular irritation

potential of saline and cottonseed extracts of natural DROP-TAINER. Three rabbits per group were administered 0.1 mL of extract into the conjunctival sac of the left eye. The corresponding control was instilled into the conjunctival sac of the right eye. Eyes were scored at 24, 48, and 72 hours following dosing. The test solutions were prepared by extracting 60 cm^2 of Drop-Tainer with 20 mL of USP 0.9% Sodium Chloride Solution or cottonseed oil for 24 hours at 70°C.

No body weights animals increased during the study, and there were no toxic signs reported. Macroscopic differences between OD and OS were not observed, and Draize scoring of ocular irritation indicated no differences in the controls vs extracts. Further, there was an absence of fluorescein staining in either control or treated eyes. These Drop-Tainers were compatible for use with the TGFS.

Agar Overlay of 15 mm Polypropylene Closures. **Yellow and Light Blue**
Report Nº 070:38520:0990
NDA Vol. 1.15, p. 5-0701

The purpose of this study was to determine any cytotoxicity to mouse fibroblast L929 cell from yellow 15 mm polypropylene closures. The study was conducted by and was based on the USP XXII 1990 procedure. Natural rubber was the positive control and silicone was the negative control. A monolayer of cells was overlayed with agar, stained with neutral red dye, and controls or test material then placed on the agar surface. The petri dishes were incubated in a 5% CO_2 atmosphere for 48 hours at 37°C. Results of the zone size (cm) and grade are indicated below.

		Positive Control		Negative Control		Test Article	
Dish	Hours	Zone Size	Grade	Zone Size	Grade	Zone Size	Grade
A	0	0.0	0	0.0	0	0.0	0
	24	0.6	3	0.0	0	0.0	0
	48	1.2	4	0.0	0	0.0	0
B	0	0.0	0	0.0	0	0.0	0
	24	0.5	3	0.0	0	0.0	0
	48	1.1	4	0.0	0	0.0	0

The area around or under the test article or negative control produced no detectable cytotoxic zone. Natural rubber, the positive control, produced Grade 3 (moderate) and 4 (severe) zones.

Under the condition of the test, the yellow 15 mm polypropylene closures is considered to not be cytotoxic.

Acute Systemic Toxicity in Mice and Primary ocular Irritation in Rabbits of Extracts of Yellow and Light Blue 15 mm Polypropylene Closures.
Report Nº 071:38520:0990
NDA Vol. 1.15, p. 5-0719

This study was done to evaluate the acute systemic toxicity and eye irritation of extracts of yellow and light blue polypropylene closures.

Extracts of yellow and light blue closures were prepared by extracting 4 g of the test substance with 20 mL of 0.9% Sodium Chloride USP or cottonseed oil at 70°C for 24 hr. Female Swiss CD-1® mice, 17-23 g body weight, 4-5 weeks old, 5/group were administered a single 50 mL/kg intraperitoneal injection of cottonseed oil extract or control vehicle. A second group was administered a single 50 mL/kg iv dose of the saline extract or control vehicle. The mice were observed up to 72 hr following extract administration.

In the ocular toxicity irritation study, groups of three NZW rabbits were administered 0.2 mL of extract into the conjunctival sac of the left eye and the control vehicle administered in the right eye. Irritation and slit-lamp scoring, and fluorescein staining were done at 0, 24, 48, and 72 hr following treatment.

All mice and rabbits gained weight, and signs of toxicity were not reported. The results indicated that these polypropylene closures met the requirements of the USP XXII.

Acute Systemic Toxicity in Mice and Primary Ocular Irritation in Rabbits of Extracts of 15 mm Polypropylene (red, blue, green, and white) Closures.
Report Nº 043:38520:0689
NDA Volume 1.15, p. 5-0791

The purpose of this study was to evaluate ocular irritation and systemic toxicity in mice using saline and cottonseed oil extracts of red, blue, green and white plypropylene closures. conducted the study in compliance with GLP/QAU regulations. The study is based on the biological test recommended in the USP XXI for the evaluation of plastic components

and containers for ophthalmic products. This study was completed subsequent to June 20, 1979.

The closure extracts did not produce any noticeable acute systemic toxicity or adverse body weight changes. Primary ocular toxicity scores (Draize scores and slit-lamp bimicroscopic scores) were all 0.

Pharmacokinetics and Metabolism.

The data in this section were obtained from the four literature references:

P. Vermeij, M. El Sherbini-Schepers and P. A. van Zweiten, The Disposition of Timolol in Man, J. Pharm. Pharmacol., 1978, 30, 53-55.

This study examined the pharmacokinetics of oral timolol maleate and Blocadren Tablets (10 mg timolol maleate = 7.4 mg timolol) in three female and 2 male volunteers (22-35 years old, 52-85 Kg body weight).

RESULTS

Mean Plasma Timolol Concentrations Following 0.1 mg/kg PO Administration (n = 5)

TIME (h)	TIMOLOL CONCENTRATION (μg/L)
0.75	17.0 ± 3.6
1.0	23.4 ± 5.4
1.5	26.0 ± 4.5
2.0	24.2 ± 4.5
3.0	18.3 ± 3.5
4.0	15.0 ± 4.0
5.0	11.3 ± 3.0
6.0	9.2 ± 1.7

- availability of tablet with respect to the solution was 0.84±0.09-
- linear kinetics was observed in each subject-
- volume of distribution = 1.33 L/kg-
- plasma $t_{1/2}$ = 2.5 h-
- accumulation following multiple dosing was not large-
- bioavailability was about 50%-

D. J. Tocco, A. E. W. Duncan, F. A. Deluna, H. B. Hucker, V. F. Gruber and W. J. A. Vandenheuvel, Physiological Disposition and Metabolism of Timolol in Man and Laboratory Animals, Drug Metab. Disp., 1975, 3, 361-370.

This report evaluated the absorption, distribution, and excretion of timolol in rats, dogs, and human subjects. Five human subjects received a single oral dose of 4 mg ^{14}C-timolol maleate and three received an oral dose of 10 mg/kg. Two dogs were dosed with 1.0 mg/kg ^{14}C-timolol po in aqueous solution. Rats were dosed with 10 mg ^{14}C-timolol/kg po or iv, and two received 100 mg/kg po.

RESULTS

- peak plasma levels occurred at 1-2 hr following po administration-
- plasma $t_{1/2}$ = 28 min (rat), 48 min (dog), 5.5 hr in humans-
- human: 66% ^{14}C in urine (20% unchanged) - 6% in feces at 48 hr-
  10% excreted as the lactic acid metabolite-
  principal urine metabolites (2) derived from morpholine ring cleavage-
- rat: ~ 58% of ^{14}C po dose excreted in urine-
  - 26% in feces over 48 hr-
  - 2% excreted unchanged-
  - 6% of po dose excreted as the lactic acid metabolite-
- dog: ~ 68% of po ^{14}C dose excreted in urine-
  - 19% in feces over 48 hr-
  - 50% of ^{14}C in urine as lactic acid metabolite-
- highest concentrations of ^{14}C observed in small intestine, kidney, and liver-

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P. Vareilles, D. Silverstone, B. Plazonnet, J. C. Le Douarec, M. L. Sears and C. A. Stone, Comparison of the Effects of Timolol and Other Adrenergic agents on Intraocular Pressure in the Rabbit, Invest. Opthalm. Vis. Sci., 1977, 16, 987-996.

Timolol maleate, propranolol HCl, epinephrine bitartrate, and isoproterenol sulfate were evaluated in rabbits, following topical administration. The drugs were evaluated in animals with and without IOP. Compounds were prepared daily and adjusted to pH 6.5-7.0. Ocular hypertension was induced by injecting the posterior chamber of the right eye with α-chymotrypsin one year prior to the start of the study. Control readings, measured by tonometry, were taken 90 and 60 minutes prior to drug application of 50 μL in the lower conjunctival cul-de-sac of each eye. IOP was again measured at 30, 90, 150, 210, and 270 minutes after drug administration. Multiple eye dosings with timolol in both eyes three times a day were also evaluated.

RESULTS

IOP effect in Rabbits With α-Chymotrypsin Induced Ocular Hypertension
(From Table III of reprint)

DRUG	Maximum Decrease In IOP (mm Hg±SD)	Time to ≥90% of Peak Effect (min)
Epinephrine (1.0%)	8.4 ± 3.6	270
Norepinephrine (2.0%)	11.7 ± 5.7	150
Isoproterenol (2%)	10.3 ± 2.9	150
Propranolol (2.0%)	4.8 ± 2.8	150
Timolol (1.5%)	11.8 ± 3.0	210

Mean IOP was significantly reduced with 0.1%, 0.5%, 1.0%, and 1.5%, but no significant change occured with 0.25%. The effect of timolol on normal IOP was inconsistent. The maximum mean timolol aqueous humor and plasma concentrations were 2.47 μg/mL and 0.188 μg/mL, respectively, at 30 minutes.

M. B. Affrime, D. T. Lowenthal, J. A. Tobert, J. Shirk, B. Eidelson, T. Cook and G. Onesti., Dinamics and Kinetics of Ophthalmic Timolol, Clin. Pharmacol. Ther., 1980, 27, 471-477.

Plasma and urinary concentrations were reported in this paper, following administration of single and multiple dosing of 0.5% timolol maleate ophthalmic eyedrops to healthy males. Timolol and placebo were administered at 2 drops to each eye. Intraocular pressure, pulse, systolic and diastolic BP, exercise heart rate, FEV_1 and incidence of eye-related adverse reactions were evaluated.

RESULTS

- timolol urine: Day 1 (21.5 ± 4.8 μg from 0 to 12 hr)-
  Day 5 (22.0 ± 6.3 from 0 to 8 hr)-
- timolol detected in plasma samples of 8/11 subjects at 3.1 to 9.6 ng/mL)-
- exercise heart rate (beats/min) was lower when counted at 70 and 255 min-
- IOP was significantly lower ($p<0.01$) at 3 and 8 hr after dosing-
- no change in post-exercise FEV_1-
- dynamic effects were said to be no greater after the 9th dose-
- no increase in urinary excretion after 9th dose-

LABELING:

The proposed labeling for the Carcinogenesis, Mutagenesis, Impairment of Fertility section is identical to Merck's labeling for

Timoptic-XE. Likewise, the labeling for the Pregnancy-Teratogenic effects section is identical to Merck's labeling, with the exception that Timolol Maleate Ophthalmic Gel Forming Solution replaces Timoptic-XE. The last three lines of this section requires a period after pregnant women, followed by "Timolol maleate ophthalmic gel forming solution should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus."

SUMMARY AND EVALUATION:

Timolol maleate is in a solution form which gels when instilled into the eye. The formulation is similar to Merck's Timoptic-XE, a solution containing an anionic heteropolysaccharide (GELRITE, a gellan gum) that forms a gel when the ionic strength increases upon topical addition to the eye. Timolol Maleate Ophthalmic Gel Forming Solution (TGFS), on the other hand, contains xanthan gum as the geling agent which gels in the presence of protein in tears. Xanthan gum has been approved for use in the US since 1969. It is present in forty-nine NDAs (Inactive Ingredient Guide, January 1996) in capsule, drop, granule, powder, suspension, tablet, enema, and emulsion dosage forms for oral, rectal, and topical administration. There are no ophthalmic drugs containing xanthan gum currently listed.

Of the many studies submitted, two were directed specifically to the toxicity of TGFS administered to eyes. They were the three-month topical ocular irritation study in rabbits and the one year topical ocular irritation and systemic toxicity study in primates, with an interim six-month report. The one year study was submitted as an amendment dated July 2, 1998. The interim study came in with the NDA.

In the three-month topical irritation study in rabbits, the animals were administered one drop, approximately 80 μL, of 0.5% TGFS to both eyes twice a day for three months. This study also included one group of rabbits treated with 80 μL of Timoptic XE 0.5% (Merck). The results of the study did not produce any reported changes in the eyes, adnexa, or nasal lacrimal tissues in the group treated with TGFS. Ocular discharge was reported in the Timoptic XE treated group.

In the six-month interim report and the one year primate study, monkeys were administered 40 μL of 0.5% TGFS bid to the right eye for one year; the left eye served as the contralateral untreated control. A control group was administered the vehicle containing the basic formula (all excepients), minus timolol maleate. Three animals per sex per group were evaluated in this interim report. The results indicated no ocular eye irritations or lesions that were treatment related. All other reported data appeared to be of a random occurrence and not related to the treatment.

Other studies in this submission were related to the potential

toxicity of the diffusible substances from the DROP-TAINERs or the acute toxicity of extracts from the DROP-TAINER closures. These studies were conducted to meet the requirements of the USP XXII or XXII. There were no adverse results from these studies.

Pharmacokinetic and metabolism data of timolol were submitted as literature reprints. In healthy volunteers (2♂, 3♀), peak plasma concentrations were ~26 ng/mL, the elimination half-live was ~2.5 hr, and the apparent volume of distribution was 1.3 to 1.7 L/kg. Administration of ^{14}C-timolol to rats resulted in 58% excretion of the radioactivity in the urine and 26% excretion in the feces over 72 hours. High concentrations of radioactivity were found in the small intestine, kidney, and liver. The drug is extensively metabolized in rats and dogs, with only 2% of the dose excreted unchanged. In humans, however, about 20% of the dose in excreted unchanged. Ocular dosing in rabbits with 50 µL of 50% timolol maleate solution produced mean aqueous humor concentrations of 2.47 µg/mL and plasma concentrations of 0.19 µg/mL at 30 minutes.

In summary, all of the submitted studies did not indicate any ocular toxicity was produce by Timolol Maleate Ophthalmic Gel Forming Solution, or from extracts of the containers or closures.

RECOMMENDATIONS:

There are no objections to the approval of Timolol Maleate Ophthalmic Gel Forming Solution (0.25% and 0.5%), based on the submitted preclinical studies.

Almon W. Coulter, Ph.D.

Andrea B Weir 7-17-98

Team Leader: Andrea Weir, Ph.D., D.A.B.T.

cc:
NDA 20-963
HFD-550/Division File
/ACoulter
/WChambers
/RUppoor
/LGorski
HFD-345
F/T by AWC: 7/17/98