

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-212

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review Addendum

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 22-212

Submission receipt date: December 26, 2007

Drug: Difluprednate Ophthalmic Emulsion 0.05%

Sponsor: Sirion Therapeutics, Inc.

Indication: Treatment of pain and inflammation following ocular surgery

Reviewing Division: Division of Anti-Infective and Ophthalmology Products

Comments: The pharm/tox tertiary review of this NDA concluded with the following sentence:

"I concur with the division pharm/tox recommendation that this NDA is approvable from a pharm/tox perspective."

This addendum is to clarify that no additional nonclinical studies or information is needed to support approval of NDA 22-212 and the pharm/tox information is adequate to support approval of the NDA.

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/s/

Paul Brown
6/9/2008 09:41:15 AM
PHARMACOLOGIST

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 22-212

Submission receipt date: December 26, 2007

Drug: Difluprednate Ophthalmic Emulsion 0.05%

Sponsor: Sirion Therapeutics, Inc.

Indication: Treatment of pain and inflammation following ocular surgery

Reviewing Division: Division of Anti-Infective and Ophthalmology Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for difluprednate to be sufficient to support its use in the treatment of pain and inflammation following ocular surgery.

The reviewer proposes pregnancy category C for the labeling. Embryofetal studies showed that difluprednate produced teratogenic effects when administered subcutaneously in rabbits. This is consistent with other corticosteroids. Systemic exposure to difluprednate and its metabolites in humans appear to be minimal after ocular administration. Therefore, I concur that pregnancy category C is acceptable and I agree with the pregnancy labeling as proposed by the division.

Other effects described in the toxicology information are consistent with the effects of corticosteroids. No carcinogenicity information has been provided for difluprednate. However, the recommended clinical use is for two weeks duration, so no carcinogenicity evaluation is necessary for this clinical use.

The proposed labeling includes section 13.2 Animal Toxicology and/or Pharmacology. While the information included in this section is interesting, some of it is included in other sections of the labeling and some of it is of a general nature and describes studies conducted by routes other than ocular. Therefore, it could probably be deleted without impairing the safe use of the product.

I concur with the division pharm/tox recommendation that this NDA is approvable from a pharm/tox perspective.

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/s/

Paul Brown
6/6/2008 03:19:56 PM
PHARMACOLOGIST

Memo to the Division File

To: NDA 22-212, Durezol (difluprednate ophthalmic emulsion, 0.05%)

Date: June 4, 2008

From: Wendelyn Schmidt, Ph.D., Acting Pharmacology/Toxicology Team Leader

Subject: Secondary Review of NDA 22-212 for difluprednate ophthalmic emulsion

I have assessed the pharmacology/toxicology review for NDA 22-212 and found no outstanding pharmacology toxicology issues. I concur with the conclusions of Dr. Conrad Chen and agree that the application can be approved from the pharmacology/toxicology perspective.

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/s/

Wendelyn Schmidt
6/4/2008 03:21:53 PM
PHARMACOLOGIST



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: 22-212
SERIAL NUMBER: 0000
DATE RECEIVED BY CENTER: 12/26/07
DRUG NAME: Difluprednate Ophthalmic Emulsion 0.05%
INDICATION: Treatment of pain and inflammation
following ocular surgery
SPONSOR: Sirion Therapeutics, Inc.
DOCUMENTS REVIEWED: Electronic submission
REVIEW DIVISION: Division of Anti-Infective and
Ophthalmology Products
PHARM/TOX REVIEWER: Conrad H. Chen, Ph.D.
PHARM/TOX TEAM LEADER: Wendelyn Schmidt, Ph.D. (Acting)
DIVISION DIRECTOR: Wiley Chambers, MD (Acting)
PROJECT MANAGER: Jane Dean
Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

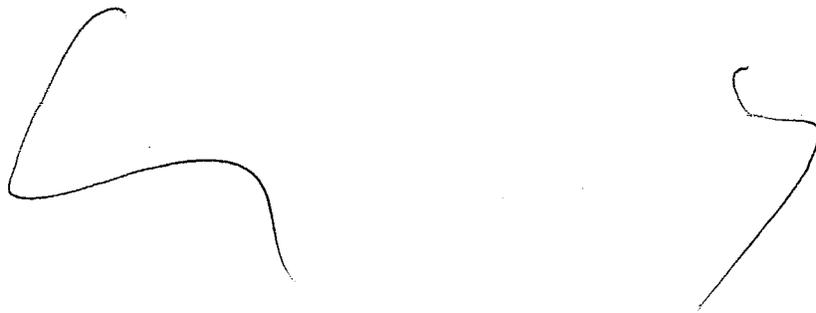
I. Recommendations

- A. Recommendation on approvability
The approval of NDA 22212 is recommended.
- B. Recommendation for non-clinical studies
None
- C. Recommendations on labeling

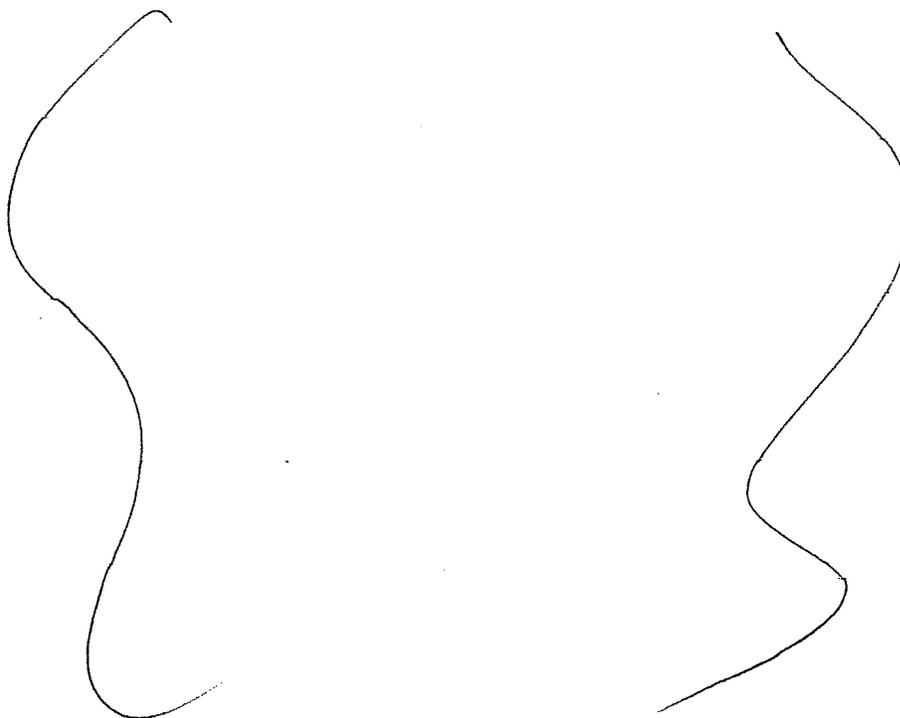
**1. PREGNANCY
Teratogenic Effects**



2. CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY



3. ANIMAL TOXICOLOGY AND/OR PHARMACOLOGY



II. Summary of non-clinical findings

A. Brief overview of non-clinical findings

The pharmacology studies revealed that difluprednate ophthalmic emulsion suppressed uveitis in animals in a dose-dependent manner. The systemic absorption of ocularly administered difluprednate is very small. During the 7 days ocular instillation studies in rabbits of 0.05% ^3H -difluprednate, the C_{max} in the plasma was not more than 10 ng/g dry wet. The conducted safety pharmacology studies did not show any significant effect. Only small effects were observed at drug levels (10^{-4} and 10^{-3} g/mL) much higher than those obtained by ocular route. Therefore, there is no safety concern.

Difluprednate (DFBA) is rapidly metabolized by deacetylation (at 21-position) in the rabbit eye tissues to the metabolite DFB (active metabolite), which is in turn converted to DF. No quantifiable difluprednate or DFB reaches the blood following a single ocular instillation (50 μl /eye) of difluprednate 0.05% in rabbits. The C_{max} in the eye was observed within 1 hour of ^3H -difluprednate instillation. The assay method using ^3H -difluprednate is sensitive enough to measure difluprednate and its metabolites at the levels of 0.3 ng equivalent/gm tissue. By autoradiography, difluprednate was cleared from the ocular tissues after a single instillation within 24 hours. Over 99% of radioactivity was excreted within 7 days. These results indicated that difluprednate and its metabolites did not remain

in the body and were mainly excreted in the feces. After repeated instillation, radiolabelled difluprednate and its metabolites did not tend to accumulate in ocular tissues.

Difluprednate has been marketed as a topical dermatological ointment in Japan. The toxicity data from the completed dermatological studies in animals were for a longer duration than the ophthalmic studies. The NOELs in the 6-month dermatological studies in rats and dogs were 1.0 µg/kg/day and 1.25 µg/kg/day, respectively. Neither deaths nor serious toxicologic findings were noted in the studies. Many changes at higher doses were those generally observed in glucocorticoid-treated animals.

Ocular administration of 0.05% difluprednate ophthalmic emulsion (0.1 ml/eye) QID for up to 4 weeks in dogs and in rabbits did not cause any ocular toxicity. The recommended clinical dose to the affected eye is one drop (0.03-0.05 ml) BID for two weeks. Therefore, it appears that there is a sufficient margin of safety. Instillation of heat-degraded difluprednate 0.05% in rabbits was tolerated as well as the normal difluprednate 0.05%. The instillation of polysorbate 80 excipient for 7 days was tolerated at concentrations up to 4% in rabbit eyes.

Mutagenesis and chromosomal aberration tests of difluprednate and difluprednate metabolites were negative. In the bacterial reverse mutation tests and the *in vitro* mammalian cell clastogenicity tests, difluprednate, metabolites, degradants, and impurities (DF17C, DF21B, and DFB) were all negative. An *in vivo* micronucleus test of difluprednate in mice was also negative. No carcinogenicity studies of difluprednate have been performed.

During the IND and pre-NDA submission, no reproductive toxicity studies for difluprednate were submitted. At that time this reviewer recommended that the class labeling of glucocorticoid may be acceptable for difluprednate when approved. However, reproductive toxicity studies conducted in Japan (in 1981-1984) during the development of dermatologic formulation of difluprednate were submitted in this NDA.

Reproductive toxicity tests were performed with difluprednate in rats and rabbits. Fetal death and malformations such as cleft palate (commonly associated with high-dose administration of GCs) were observed during the organogenic period in rabbits. The effects of difluprednate on rat fetuses were weak; fetal death and/or malformed fetuses were not found.

B. Pharmacologic activity

Difluprednate is a corticosteroid that was first developed in Japan by Mitsubishi Pharma for topical dermatological use and has been classified to have a "very strong" effect based on its clinical results in dermatology. Difluprednate is a derivative of prednisolone; a trivial name for difluprednate is difluoroprednisolone butyrate acetate (DFBA). In its dermatological formulation, difluprednate 0.05% is over 4 times more potent than prednisolone valerate

acetate 0.3%, and 3200 times stronger than prednisolone 0.5%, as measured by peripheral vasoconstriction.

The pharmacology studies in animal models of uveitis have demonstrated that difluprednate ophthalmic emulsion is effective in a dose-dependent manner.

C. Non-clinical safety issues relevant to clinical use

Difluprednate, like other glucocorticoids, is a known animal teratogen.

Difluprednate should be used during pregnancy only if the potential benefit justifies the potential risk to the embryo or fetus.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-212

Review number: No.1

Sequence number/date/type of submission: SN0000/December, 21, 2007/Original Submission

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Sirion Pharmaceuticals, Inc., Tampa, Florida 33619

Manufacturer for drug substance:

Reviewer name: Conrad H. Chen, Ph.D.

Division name: Division of Anti-Infective and Ophthalmology Products

Review completion date: March 20, 2008

Drug:

Trade name: Durezol (proposed)

Generic name: Difluprednate

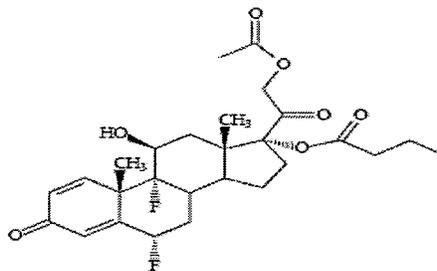
Code name: ST-601, MY-307, DFBA

Chemical name: 6 α , 9-Difluoro-11 β ,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-acetate 17-butyrate

CAS registry number: 23674-86-4

Molecular formula/molecular weight: C₂₇H₃₄F₂O₇/508.56

Structure:



Relevant INDs/NDAs/DMFs: IND 75,713

Drug class: Corticosteroid

Indication: Treatment of pain and inflammation following ocular surgery

Clinical formulation:

Table 1. Composition of the ST-601 Drug Product (per mL)

Components	Function	Weight (mg/mL)	% w/v
Difluprednate	Active ingredient		0.05%
Polysorbate 80	Emulsifier		4.0%
Glycerin	Tonicity		2.2%
Sorbic acid	Preservative		0.1%
Sodium acetate, anhydrous	Buffer		0.05%
Boric acid	Buffer		0.1%
Sodium EDTA	Stabilizer		0.02%
Castor oil	Oil phase		5.0%
Water for injection	Water phase		-
Sodium hydroxide	pH adjustment		As needed

qs, sufficient quantity; w/v, weight/volume

Route of administration: Apply 1 drop into the conjunctival sac of the affected eye(s)-2 times daily, beginning 24 hours after surgery and continuing for up to 2 weeks after surgery.

Proposed use: Difluprednate Ophthalmic Emulsion 0.05% is a topical corticosteroid that is indicated for the treatment of inflammation and pain associated with ocular surgery.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Difluprednate is a corticosteroid that was first developed in Japan by Mitsubishi Pharma for topical dermatological use and has been classified to have a “very strong” effect based on its clinical results in dermatology. Difluprednate is a derivative of prednisolone; a trivial name for difluprednate is difluoroprednisolone butyrate acetate (DFBA). In its dermatological formulation, difluprednate 0.05% is over 4 times more potent than prednisolone valerate acetate 0.3%, and 3200 times stronger than prednisolone 0.5%, as measured by peripheral vasoconstriction. The potency of difluprednate is approximately the same as that of betamethasone.

All the following pharmacodynamic studies were conducted by ocular administration to animals. The study in 5) was conducted by the incubation rat liver homogenate with the drug.

2.6.2.2 Primary pharmacodynamics

1) Rat model of uveitis: In a model of acute uveitis induced by endotoxin administered to the foot pad, difluprednate ophthalmic emulsion (0.01% and 0.05%) significantly inhibited inflammation in a dose-dependent manner. The effect of 0.05% difluprednate was superior to 0.1% betamethasone in this test.

2) Melanin uveitis model in rats: In an autoimmune model of chronic anterior uveitis induced by footpad injection of melanin, difluprednate 0.01% and 0.05% significantly reduced anterior ocular inflammation. Equivalent efficacy was observed between difluprednate 0.01% and betamethasone 0.1% in this test.

3) Bovine serum albumin model in rabbits: In a model of uveitis induced by intravitreal injection of bovine serum albumin, instillation of difluprednate (at 0.002%, 0.01%, and 0.05% QID for 19 days) significantly inhibited the inflammation. Difluprednate 0.05% was more effective than 0.1% betamethasone in this study.

4) Acute post-operative inflammation model in rabbits: In an acute model of post-operative inflammation created by anterior chamber paracentesis, difluprednate 0.05% (by a single instillation of 50 μ l) was equally effective as 0.1% betamethasone in inhibiting the flare.

5) In an *in vitro* assay, difluprednate was shown to have an approximately 100- to 10000-fold greater specific affinity for the rat liver GC receptor (glucocorticoid receptor) than its metabolite, DF21C.

6) Based on an *in vivo* GC binding assay in rabbit ocular tissues, the instilled difluprednate rapidly transferred to the aqueous humor and subsequently reached the iris/ciliary body. The time to maximum binding activity (T_{max}) was 30 to 60 minutes after instillation. The difluprednate binding activity remained constant up to 120 minutes after instillation.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

The systemic absorption of ocularly administered difluprednate is very small. During the 7 days ocular instillation studies in rabbits of 0.05% 3 H-difluprednate, the C_{max} in the plasma was not more than 10 ng/g dry wet.

The conducted safety pharmacology studies did not show any significant effect. Only small effects were observed at a drug levels (10^{-4} and 10^{-3} g/mL) much higher than that obtained by ocular route. Therefore, there is no safety concern.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Difluprednate (DFBA) is rapidly metabolized by deacetylation (at 21-position) in the rabbit eye tissues to the metabolite DFB (active metabolite), which is in turn converted to DF.

The *in vitro* degradation pathways are shown in Figure 1.

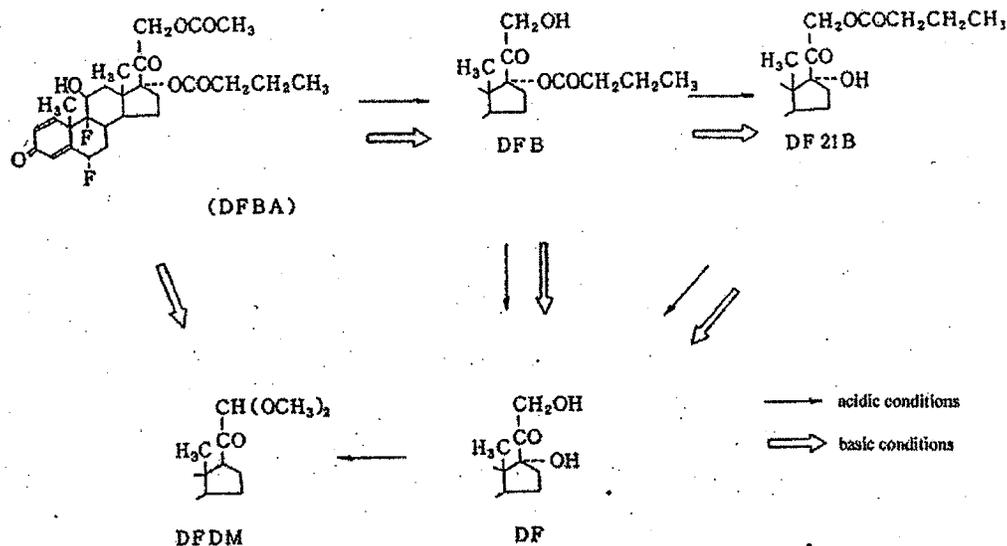


Figure 1. Degradation Pathways for Difluprednate

No quantifiable difluprednate or DFB (6 alpha-9-difluoroprednisolone 17-butyrate) reaches the blood following a single ocular instillation (50 µl/eye) of difluprednate 0.05% in rabbits. The C_{max} in the eye was observed within 1 hour of ^3H -difluprednate instillation. The assay method using ^3H -difluprednate is sensitive enough to measure difluprednate and its metabolites at the levels of 0.3 ng equivalent/g tissue. By autoradiography, difluprednate was cleared from the ocular tissues after a single instillation within 24 hours. Over 99% of radioactivity was excreted within 7 days. After repeated instillation, radiolabelled difluprednate and its metabolites did not tend to accumulate in ocular tissues. The test results showed that the emulsion formulation of difluprednate had 1.4-fold higher bioavailability than the suspension formulation of difluprednate.

- 1) Distribution of difluprednate and its metabolites to ocular tissues of rabbits: A single 50 µl dose of difluprednate 0.05% was instilled into rabbit eyes. Difluprednate concentrations in the blood, conjunctiva, aqueous humor, iris/ciliary body, lens, vitreous body, retina, and choroid were below the lower limit of quantitation at all collection times (between 15 minutes to 8 hours). Difluprednate concentrations were quantified only in cornea of a few animals (4 out of 40). The maximum concentrations of DFB were quantifiable in the cornea and conjunctiva (2.2 µg/g and 2.1 µg/g, respectively) at 15 minutes and in aqueous humor (0.04 µg/g) at 1 hour after instillation. The DFB concentrations in cornea and conjunctiva were below the lower limit of quantification (180 ng/g and 145 ng/g, respectively) at 8 hours and 2 hours, respectively. DFB concentrations in the blood, iris/ciliary body, lens, vitreous body, retina, and choroid were below the limit of quantification.

These data indicated that after single instillation in the rabbit eye, difluprednate 0.05% was cleared within 15 minute from ocular tissues *via* metabolism to DFB.

- 2) Distribution of ^3H -difluprednate after a single instillation:
After a single instillation of $50\ \mu\text{l}$ ^3H -difluprednate in rabbit eyes, the highest concentration of radioactivity was found in the cornea, followed by the iris/ciliary body, conjunctiva, anterior retina/choroid, sclera, and aqueous humor at 30 minutes or 1 hour. No radioactivity was detected at 24 hours and 168 hours after instillation in cornea and iris/ciliary body, respectively. By 168 hours after instillation, 37.1% and 62.4% of the radioactivity was excreted in the urine and feces, respectively. These results indicated that difluprednate and its metabolites did not remain in the body and were mainly excreted in the feces. The plasma levels of difluprednate were low (0-3 ng eq./g or ml) throughout the 0-168 hours observation period. The plasma C_{max} were approximately 1/700 of corneal C_{max} at 0.5 hour (T_{max}) after ocular instillation.
- 3) Distribution of ^3H -difluprednate after repeated instillation:
A study was conducted in male rabbits to determine the distribution of $50\ \mu\text{l}$ ^3H -difluprednate when instilled four times daily for 7 days (28-time instillation).
High concentrations of radioactivity were found in cornea, followed by the iris/ciliary body, conjunctiva, anterior retina, choroid, aqueous humor, and sclera.
After 28-time instillation, the T_{max} was at 0.5 hour for cornea, iris/ciliary body and conjunctiva. The C_{max} at 0.5 hour for cornea, iris/ciliary body, and conjunctiva were 278-fold, 106-fold, and 41-fold of plasma concentration (10 ng/g dry weight), respectively.
The radioactivity in the cornea on Day 28 was less than 1% of the C_{max} . On Day 28, the radioactivity in iris/ciliary body was less than 1% of the C_{max} . Due to the fact that radioactivity did not remain in specific ocular tissues and decreased with time in tissues, it appeared that repeated dosing of difluprednate would not cause accumulation in ocular tissue.
The radioactivity concentrations in ocular tissues after 28-time instillation of ^3H -difluprednate were shown in the following table:

Table Exp.2C Radioactivity concentration in applied ocular tissue after a 28 times instillation of ³H-Difluprednate to the right eye of male duoth rabbits (dose: 100µg/200µL/4times/eye/day)

Tissue	Radioactivity concentration (ng eq. of Difluprednate/g or mL)											
	0.5 h		2 h		8 h		24 h		7 day		28 day	
Plasma (dry)	10 ± 2 (1.0)		9 ± 2 (1.0)		7 ± 2 (1.0)		6 ± 1 (1.0)		2 ± 0 (1.0)		0*	
Plasma (wet)	21 ± 4 (2.1)		17 ± 4 (1.9)		16 ± 3 (2.3)		14 ± 2 (2.3)		6 ± 1 (3.0)		1 ± 1	
Blood	10 ± 3 (1.0)		7 ± 1 (0.8)		6 ± 2 (0.9)		5 ± 1 (0.8)		3 ± 1 (1.5)		0*	
Aqueous humor	217 ± 49 (21.7)		145 ± 17 (16.1)		22 ± 5 (3.1)		2 ± 1 (0.3)		0*		0*	
Conjunctiva	414 ± 79 (41.4)		202 ± 136 (22.4)		192 ± 68 (27.4)		82 ± 38 (13.7)		23 ± 17 (11.5)		6 ± 3	
Extraocular muscle	33 ± 14 (3.3)		44 ± 42 (4.9)		12 ± 6 (1.7)		7 ± 4 (1.2)		4 ± 3 (2.0)		1 ± 1	
Cornea	2781 ± 650 (278.1)		1458 ± 395 (162.0)		559 ± 310 (79.9)		148 ± 91 (24.7)		27 ± 21 (13.5)		4 ± 1	
Iris & ciliary body	1063 ± 181 (106.3)		403 ± 99 (44.8)		168 ± 54 (24.0)		55 ± 10 (9.2)		38 ± 16 (19.0)		8 ± 3	
Lens	42 ± 6 (4.2)		36 ± 10 (4.0)		51 ± 17 (7.3)		21 ± 2 (3.5)		4 ± 0 (2.0)		0*	
Vitreous body	1 ± 1 (0.1)		1 ± 0 (0.1)		1 ± 1 (0.1)		0*		0*		0*	
Anterior retina & choroid	339 ± 162 (33.9)		173 ± 105 (19.2)		106 ± 13 (15.1)		34 ± 12 (5.7)		12 ± 3 (6.0)		4 ± 2	
Posterior retina & choroid	35 ± 20 (3.5)		23 ± 7 (2.6)		13 ± 5 (1.9)		4 ± 1 (0.7)		3 ± 1 (1.5)		0*	
Sclera	198 ± 89 (19.8)		63 ± 17 (7.0)		36 ± 15 (5.1)		10 ± 3 (1.7)		5 ± 1 (2.5)		3 ± 1	
Lacrimal gland	12 ± 4 (1.2)		11 ± 5 (1.2)		14 ± 6 (2.0)		4 ± 2 (0.7)		3 ± 2 (1.5)		0 ± 1	
Sub lacrimal gland	18 ± 9 (1.8)		11 ± 3 (1.2)		11 ± 5 (1.6)		4 ± 1 (0.7)		4 ± 1 (2.0)		0 ± 1	

Data are expressed as the mean values ± S.D. of four animals.

Figures in parentheses are expressed as the ratio of concentration in tissue relative to plasma(dry).

* : Not detected

- 4) Metabolite analysis of eye tissues after instillation of ³H-difluprednate:
Difluprednate was instilled in the conjunctival sac of both eyes of rabbits. Difluprednate was not detected in cornea, aqueous humor, or iris/ciliary body. However, 2 metabolites (DFB and DF) and 3 other metabolites were observed in the ocular tissues. The DFB concentrations at 2 hours were lower than at 30 minutes after instillation. Inversely, the DF concentrations at 2 hours were higher than at 30 minutes after instillation. These results indicated that instilled difluprednate was rapidly converted to DFB, which was subsequently metabolized to DF.
- 5) Bioavailability of two formulations of difluprednate in rabbits:
The DFB concentrations in aqueous humor in rabbits were measured after single ocular instillation of 50 µl 0.05% difluprednate formulations (emulsion and suspension). The C_{max} and AUC_{0-4h} after instillation of emulsion was 1.4-fold higher than those of suspension.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Difluprednate has been marketed as a topical dermatological ointment in Japan. The toxicity data from the completed dermatological studies in animals were for a longer duration than the ophthalmic studies. The NOELs in the 6-month dermatological studies in rats and dogs were 1.0 µg/kg/day and 1.25 µg/kg/day, respectively. Neither deaths nor serious toxicologic findings were noted in the studies. Many changes at higher doses were those generally observed in glucocorticoid-treated animals.

Ocular administration of 0.05% difluprednate ophthalmic emulsion (0.1 ml/eye) QID for up to 4 weeks in dogs and in rabbits did not cause any ocular toxicity. Instillation of heat-degraded difluprednate 0.05% in rabbits was tolerated as well as the normal difluprednate 0.05%. The instillation of polysorbate 80 excipient for 7 days was tolerated at concentrations up to 4% in rabbit eyes. Mutagenesis and chromosomal aberration tests of difluprednate and difluprednate metabolites were negative.

Reproductive toxicity tests were performed with difluprednate in rats and rabbits. Fetal death and malformations such as cleft palate (commonly associated with high-dose administration of GCs) were observed during the organogenic period in rabbits. The effects of difluprednate on rat fetuses were weak; fetal death and/or malformed fetuses were not found.

2.6.6.2 Single-dose toxicity

No data are submitted.

2.6.6.3 Repeat-dose toxicity

1) Six-month subcutaneous toxicity study of MY-307 in rats: Difluprednate was initially developed as a dermatological formulation. The results from studies evaluating the toxicity of topical dermatologic preparations are briefly summarized.

MY-307 is a dermatological formulation of difluprednate 1% as a topical ointment. MY307 was administered subcutaneously to the dorsal skin of rats once daily at 0.1, 1.0, or 10.0 µg/kg/day for 6 months. The frequency of administrations was 7 times/week during the first month and 6 times/week for the rest of the duration. The vehicle was used as the control.

Suppression of body weight gain was found at 10 µg/kg/day, and the food consumption increased after drug removal during the 2-month recovery period. Increases in red blood cell count, hemoglobin, and hematocrit, and lowering of myeloid/erythroid rate owing to proliferation of erythroblasts were found at 10 µg/kg/day, suggesting enhanced hematopoiesis. These changes disappeared during the recovery period and were considered as the general glucocorticoid effects. The NOEL was 1 µg/kg/day in this study.

2) Six-month percutaneous toxicity study in beagle dogs: Difluprednate ointment 0.05% was applied to the skin of beagle dogs at 1.25, 12.5, and 125 µg/kg/day for 6 months, followed by a 2-month recovery period. The vehicle was used as the control.

At 125 µg/kg/day, decreases in lymphocytes and eosinophils, atrophy of lymphatic tissues such as thymus and adrenal gland, increase in neutrophils, increase in hepatic glycogen, and increases in water intake and urine volume, increase in sodium and decrease in potassium were found. Many of these changes were attributable to excessive

physiological effects of glucocorticoid and were reversible during the recovery period. At 12.5 µg/kg/day, slight changes in the thymus, adrenal glands were observed. The NOEL was 1.25 µg/kg/day in this study.

3) Two-week ocular irritation study of heat-degraded difluprednate: (This study was reviewed and summarized as follows).

Key study finding: One hundred µl of 0.05% difluprednate, heat-degraded 0.05% difluprednate, and sham-heat-degraded 0.05% difluprednate were administered to rabbit eyes 8 times daily for 2 weeks. There was no irritation to the anterior portion of the eyes and there were no differences in the systemic effects for the 3 products.

Study no.: 41-75

Conducting laboratory and location: _____

Date of study initiation: July 1, 2004

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #: G038-01, G096-02, G096-01, respectively

Methods:

100 µl of 0.05% difluprednate, heat-degraded 0.05% difluprednate, and sham-heat-degraded 0.05% difluprednate were administered to rabbit eyes 8 times daily for 2 weeks. Physiologic saline was used as the negative control. The processes of heat-degradation and sham-heat-degradation were not described.

Species/strain: Japanese white rabbits

Number/sex/group or time point: 5 males/group

Results:

There were no deaths and clinical signs during the study. Neither ophthalmic abnormalities nor irritation to the anterior portion of the eyes were found for the 3 products. Changes in food consumption, body weight, hematology, blood chemistry, and organ weights were similar in all groups.

4) Seven-day ocular irritation study of polysorbate 80 in rabbit: (This study was reviewed and summarized as follows).

Key study findings: 100 µl of Polysorbate 80 solutions 0.5% and 4 % were administered to the eyes of Japanese White rabbits, 8 times/day for 7 days. No abnormalities were noted in the cornea, iris, or conjunctiva in any animal. No abnormalities were observed in the corneas stained with fluorescein sodium.

Study no.: SJ-99026

Conducting laboratory and location: _____

Date of study initiation: February 1, 1999

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #: Polysorbate 80, Lot no.24M8

Methods:

Polysorbate 80 was dissolved in physiological saline to prepare 0.5% (5mg/g) and 4% (40 mg/g) solutions. The right eyes of 3 rabbits/group were treated with a dose of 0.1 ml, 8

times daily for 7 consecutive days. The left eyes of animals were treated with physiological saline as the control.

The cornea, iris and conjunctiva were examined using a slit lamp according to a modified Draize scale on Day 1 (before the dosing) and each day (30 minutes after the last dosing). Cornea was also examined using the slit lamp and fluorescein sodium according to McDonald-Shadduck scale before the dosing on Day 1 and after the last dosing on Day 1, 4, and 7.

Results:

There were neither abnormal clinical signs nor marked body weight changes in any animals.

No abnormalities (opacity, redness, or chemosis) were observed in the cornea, iris, or conjunctiva. No fluorescein-stained areas were observed in any animal according to McDonald-Shadduck scale for scoring ocular lesions.

It was concluded that polysorbate 80 in physiological saline, instilled at 0.1 ml 8 times/day for 7 day, was not irritating to the rabbit eyes at concentrations up to 4%.

5) Four-week repeated-dose ocular toxicity study in rabbits:

Key study findings: Difluprednate 0.01% and 0.05% have no ocular toxicity when instilled to the eyes of Japanese White rabbits at 0.1 ml/right eye, 4 times daily for 4 weeks. Many systemic changes noted in the difluprednate-treated groups were considered as the general glucocorticoid effects.

Study no.: 50-48

Conducting laboratory and location: _____

Date of study initiation: August 5, 1997

GLP compliance: Yes

QA report: yes (x) no ()

Methods:

Doses: 0.1 ml of 0.01% or 0.05% difluprednate, vehicle control, and negative control (physiologic saline)

Species/strain: Japanese White rabbits

Number/sex/group or time point: (N = 20) 5 males/group

Route, formulation, volume, and infusion rate: Instilled into conjunctival sac of the right eye, 4 times daily (at 2.5 hour-intervals). The left eye was left untreated as control.

Observation times

Clinical signs: Once daily

Body weights: Twice a week

Food consumption: Once daily

Ophthalmology: Macroscopic examination, once a week; fluorescein staining, once at pre-dosing and once/week thereafter; electroretinography, once at pre-dosing and Week 4; IOP, once at pre-dosing and Week 2 and 4

Hematology, clinical chemistry, and urinalysis: Once at pre-dosing and Week 4

Gross pathology and histopathology: On the day after final dosing, the animals were sacrificed and processed for histopathology.

Results: In comparison with the control group, low body weight values were noted in the 0.01% and 0.05% difluprednate groups. This change was presumably caused by acceleration of glucocorticoid-induced protein catabolism, since no marked change in food consumption was noted. In organ weight measurement, decreased adrenal weight was noted in 0.01% and 0.05% groups. Increased liver and kidney weights and decreased splenic weight were noted in the 0.05% group.

In hematology examination at Week 4, decreases in leukocyte and lymphocyte counts were noted in the 0.05% group. The dose in this study was 200 µg/rabbit (50 µg/100 µl/right eye, QID). Based on 2 kg as the average body weight of rabbit, the dose was 100 µg/kg/day. The human equivalent dose (HED) of 100 µg/kg/day in rabbits (divided by 3) was 33.3 µg/kg/day. It appears that at dose level of 33.3 µg/kg/day (HED), difluprednate caused systemic glucocorticoid effects in this study. In the Phase III human clinical study, the proposed dose is 200 µg/person/day (25 µg/50 µl/eye to both eyes, QID). Based on the average human body weight of 60 kg, the dose is 3.3 µg/kg/day. This human clinical dose is 1/10 of the dose that caused the systemic glucocorticoid effects in the 4-week rabbit study.

No abnormalities were observed in any of the ophthalmologic examinations, whether macroscopic examination, fluorescein staining, optic media and fundus examination, ERG, or IOP.

6) Four-week repeated-dose ocular toxicity study in dogs:

Key study findings: Characteristic steroidal changes were observed in some animals in the 0.05% difluprednate- and 0.1% betamethasone-treated groups after repeated instillations of 0.1 ml/eye into both eyes of beagle dogs 4 times/day for 4 weeks. However, no ocular toxicity was noted.

Study no.: 51-95

Conducting laboratory and location: _____

Date of study initiation: March 3, 2004

GLP compliance: Yes

QA report: yes (x) no ()

Methods:

The 0.1 ml/eye of 0.05% difluprednate, vehicle control, 0.1% betamethasone, or physiological saline (negative control) was instilled into both eyes of male beagle dogs, 4 times/day for 4 weeks. The number of animals was 5/group. All animals were observed daily for clinical signs. The observations included assessment for ocular secretion, edema, conjunctiva, and eyelid abnormalities. Food consumption and body weight were monitored. Both eyes of all animals were examined macroscopically and by using slit lamp examination. Each eye was examined using indirect ophthalmoscope 10 days before dosing, and on Days 9 and 23. ERG (on Days -9, 10, and 20), pupillary diameter examination (on Days -8, 11 and 25), and IOP measurements (on Days -2, -7, -13, 5, 12, 19, and 26) were also conducted. Urinalysis, hematology, blood chemistry, serum cortisol levels, gross pathology, organ weights, and histopathology were performed.

Results:

No abnormalities were noted in ophthalmic observations, ERG, clinical signs, pupillary diameter, or IOP in any of the study animals. A decrease in plasma cortisol was similarly observed in the difluprednate and betamethasone groups.

In this study, the difluprednate- and betamethasone-treated animals showed decreased lymphocyte and eosinophil counts and their ratio, increases in neutrophils, decreased absolute and relative adrenal and thymic weights, accompanied by thymic atrophy and atrophy and vacuolation of adrenal cortex.

Many characteristic steroidal effects were observed in animals after repeated instillation with 0.05% difluprednate and 0.1% betamethasone. The changes in difluprednate group were slightly less than those observed in the betamethasone group.

The daily topical dose of difluprednate in this study was 400 µg/dog (50 µg/100 µl/eye, QID to both eyes). Based on 10 kg as the average body weight of dog, the daily dose was 40 µg/kg/day. The human equivalent dose (HED) of 40 µg/kg/day in dogs (divided by 2) was 20 µg/kg/day. It was noted that in the 6-month percutaneous toxicity study in dogs, the excessive physiological effects of glucocorticoid was observed at dose levels of 12.5 and 125 µg/kg/day. The NOEL in that percutaneous toxicity study in dogs was 1.25 µg/kg/day. Therefore, it appears that a similarly significant glucocorticoid effects were observed in both ocular and dermatological toxicity studies in dogs at dose levels of 20 and 12.5 µg/kg/day, respectively. No changes were noted in food consumption, body weight, ophthalmology, urinalysis, or gross pathology in the treated groups in 4-week ocular toxicity study in dogs. The glucocorticoids have been known to cause cataracts after a long term use in animals and humans. However, no cataract was found in this study probably because the study period was not long enough.

In the Phase III human clinical study, the proposed dose is 200 µg/person/day (25 µg/50 µl/eye to both eyes, QID). Based on the average human body weight of 60 kg, the dose is 3.3 µg/kg/day. This human clinical dose is 1/6 and 1/4 of the doses that caused systemic glucocorticoid effects in the ocular and dermal toxicity studies in dogs.

6.6.6.4 Genetic toxicology

1). Bacterial reverse mutation test of metabolites of difluprednate:

Key study findings: The following compounds are degradants, impurities, and metabolites of difluprednate: DF17C, DF21B, and DFB. In the Ames test, DF17C, DF21B, and DFB did not induce gene mutation in the presence or absence of S9.

Study no.: 51-98, 51-91, and 51-90

Conducting laboratory and location: _____

Date of study initiation: June 15, 2004; February 9, 2004; and February 9, 2004

GLP compliance: Yes

QA report: Yes (x) no ()

Methods:

Three identical bacterial reverse mutation studies were performed for DF17C, DF21B, and DFB. Five strains of bacteria [*Salmonella typhimurium* (TA98, TA100, TA 1535, and TA 1537) and *Escherichia coli* (WP2uvrA)] were used. The dose-finding test (with and without metabolic activation) was performed at

dose levels of 5, 15, 50, 150, 500, 1500, and 5000 µg/plate. The main test was performed at dose levels of 156, 313, 625, 1250, 2500, and 5000 µg/plate. DMSO was used as the negative control. 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), sodium azide (NaN₃), 9-aminoacridine hydrochloride hydrate (9AA), and 2-aminoanthracene (2AA) were used as the positive controls.

Results:

The number of revertant colonies from the negative and positive controls was within historical controls in the conducting laboratory. Accordingly, it was judged that the reverse mutation tests were conducted satisfactorily.

When compared with the negative control, a 2-fold or greater increase in the number of revertant colonies was not observed in any of the 5 test strains used, in the presence or absence of metabolic activation. It was concluded that DF17C, DF21B, and DFB did not induce gene mutation in the Ames test.

2). Chromosomal aberration test of DF17C, DF21B, and DFB in cultured mammalian cells:

Key study findings: The following compounds are degradants, impurities, and metabolites of difluprednate: DF17C, DF21B, and DFB. DF17C, DF21B, and DFB did not induce chromosomal aberrations in CHL/IU cells, in the presence or absence of metabolic activation.

Study no.: 51-99, 51-93, and 51-92

Conducting laboratory and location: _____

Date of study initiation: June 15, 2004; February 9, 2004; and February 9, 2004

GLP compliance: Yes

QA report: Yes (x) no ()

Methods:

The clastogenicity of DF17C, DF21B, and DFB was evaluated using cultured mammalian cells CHL/IU (a fibroblastic cell line derived from the lungs of newborn female Chinese hamsters).

Five dose levels (for DF17C: 93.8, 234, 586, 1466, and 3664 µg/ml; for DF21B: 125, 250, 500, 1000, and 2000 µg/ml; for DFB: 80, 120, 180, 270, and 405 µg/ml) were set for short-term treatments (6-18 hours) with and without S9.

Five dose levels (for DF17C: 37.5, 93.8, 234, 586, and 1466 µg/ml; for DF21B: 51.2, 128, 320, 800, and 2000 µg/ml; for DFB: 35.6, 53.3, 80, 120, and 180 µg/ml) were set for continuous treatment for 24 hours with and without S9.

DMSO was used as the negative control. Mitomycin (MMC) and Benzo[a]pyrene (B[a]P) were used as the positive controls. The frequencies of cells having structural and numerical chromosomal aberrations were investigated.

Results:

The frequencies of cells having chromosomal aberrations in the negative and positive controls were within the range of historical control data in the conducting laboratory. Therefore, it was judged that the tests were performed satisfactorily. The frequencies of cells having structural and numerical aberrations, regardless of the presence and absence of S9 or length of treatment time, were below 5%.

Key study findings: Difluprednate did not induce chromosomal aberration in CHL/IU cells.

Study no.: S2007E0415

Conducting laboratory and location: Mitsubishi Yuka Pharmaceutical Co., Ltd.
500, Wakaguri, Ami-machi, Inashiki-gun, Ibaraki, Japan

Date of study initiation: July 18, 1983 to December 28, 1983

GLP compliance: Yes (as stated by the sponsor)

QA report: Yes (x) no ()

Methods:

The potential for difluprednate 1.0% (MY-307) to induce chromosomal aberrations in cultured mammalian cells was evaluated using CHL/IU cells (Study S2007E0415).

Difluprednate concentrations of 125.0 µg/mL, 62.5 µg/mL, 31.3 µg/mL, and 56.6 µg/mL were established from a preliminary cell proliferation inhibition study, which identified the 50% lethal concentration (LC50) value to be about 62.5 µg/mL. CHL/IU cells were co-incubated with the following for 24 and 48 hours: 4 difluprednate doses (noted above), vehicle DMSO (dimethyl sulfoxide; negative control), and 2.5 µg/mL of MNNG (N-methyl-N'-nitro- N-nitrosoguanidine; positive control).

One hundred well-spread cells in metaphase were randomly selected and the structural anomalies of the chromosomes found in the cells were classified into gaps, breaks, exchanges, and others. A cell with at least one anomaly was recorded as an abnormal cell.

The results of the observations from the difluprednate chromosome samples are shown in Table 6.

Results:

Table 6. Chromosomal Aberration Test of Difluprednate in CHL Cells (Study S2007E0415)

Treatment (hr)	Difluprednate µg/mL	No. of Cells	Chromosomal Aberrations (total %)
	125.0	17	0
	62.5	100	0
	31.3	100	0
	15.6	100	0
	0.0	100	1
	24	MNNG	95
	125.0	6	0
	62.5	100	0
	31.3	100	0
	15.6	100	2
	0.0	100	0
	48	MNNG	100

MNNG=N-methyl-N'-nitro-N-nitrosoguanidine

Cell proliferation inhibition was intense at the maximum concentration of 125.0 µg/mL and the number of cells in the metaphase was less than 100. However, an increase in the rate of cells with chromosomal aberration was not observed at any

Embryofetal development

Study title: Effect of difluprednate on embryo-fetal development in rats

Key study findings: MY-307 was administered to Wistar rats during the period of organogenesis. The 100 µg/kg (subcutaneous injection) group showed inhibition of body weight gain and a decrease in food consumption in dams. Inhibition of fetal growth was observed. However, MY-307 had no teratogenicity or embryo-fetal lethality at this dose level. Furthermore, MY-307 did not show any adverse effect on postnatal growth and reproductive function of offspring (F1). The above results lead to consider that MY-307, when administered subcutaneously to rats during the period of organogenesis, shows no teratogenicity even at the toxic dose for dams (100 µg/kg) and that the drug does not affect postnatal growth when administered at dose levels of ≤10 µg/kg.

Study no.: S2007E0413

Conducting laboratory and location: Research Laboratory, Mitsubishi Yuka Pharmaceutical Co., Ltd. Safety Group

Date of study initiation: February 12, 1981

GLP compliance: Yes (ascertained by the sponsor)

QA reports: yes (x) no ()

Drug, lot #, and % purity: Lot No. H800668. _____

Methods:

A study was performed during the organogenesis developmental period in rats in order to examine the toxicologic effects of difluprednate on the fetus and embryo. A total of 196 11- to 13-week-old female Wistar rats were split into 5 groups: group I (n=38), group II (n=39), group III (n=40), group IV (n=40), and group V (n=39).

Difluprednate or control was subcutaneously administered into the back of each female rat (from Day 7 to Day 17 of pregnancy) once a day at one of the following concentrations: 0 (control) or difluprednate suspended in a 1.0% aqueous solution of CMC at 0.1 µg/kg, 1.0 µg/kg, 10.0, and 100.0 µg/kg, respectively.

Results:

The 100 µg/kg group showed a trend for inhibition of body weight gain (about 6.7%) and food consumption. A significant difference was found in body weight. On the other hand, the ≤10 µg/kg groups showed no significant difference against the control group with respect to body weight gain and food consumption. With the exception of observing 1 case each of premature birth in the 0.1 and 100 µg/kg groups, all groups showed neither abnormalities in general conditions nor dead dams.

No effects on the embryos and fetuses that could be attributed to difluprednate were observed at the 0.1-µg/kg and 1.0-µg/kg doses. At 10 µg/kg, there were decreases in placental weight. At 100.0 µg/kg, decreases in the weight of the fetus and placenta and delays in fetal ossification were noted. However, there were no differences in the numbers of implantation and dead fetuses as compared with the control group.

Additionally, no fetal abnormalities in the viscera, bones, and general appearance occurred as a result to exposure to difluprednate.

Study title: Effect of difluprednate on embryo-fetal development in rabbits

Drug, lot #, and % purity: Lot No. H800668, _____

Methods:

In this study, 10- to 14-week-old female Wistar rats were split into 5 groups (28-31 per group). The test article was subcutaneously administered into the skin of each female rat (27 days from Day 17 of gestation to Day 21 after delivery) once a day at a volume of 1 mL/kg, as follows: 0.0 (vehicle control); or difluprednate (suspended in a 1.0% aqueous solution of CMC) at 0.1 µg/kg, 1.0 µg/kg, or 10.0 µg/kg.

Results:

A summary table of the results from this study is presented as follows:

Table 11. Prenatal and Postnatal Development, Including Maternal Function, in Rats (Study 1075)

Dose (µg/kg)	Vehicle (control)	Difluprednate 0.1 µg/kg	Difluprednate 1.0 µg/kg	Difluprednate 10.0 µg/kg
Females				
No. of animals	31	28	30	28
Deaths	0	0	0	0
Gestational period: Body weight, food consumption	No difference between groups			
Nursing period: Body weight, food consumption	No difference between groups			
Necropsy findings at time of weaning	No abnormalities	No abnormalities	No abnormalities	No abnormalities
No. of pregnant animals	25	27	26	24
Abnormal parturitions	0	0	0	0
No. stillborn pups	1/211	1/232	2/213	0/190
No. of litters with no live pups	0	0	1	0
Delivery Index (%)	93	92.4	92.2	93.1
No. of litters that all pups died	0	0	0	0
Litters				
No. litters evaluated	25	27	26	24
Total pups born alive	210	231	211	190
Postnatal survival D3 (after selection)	173	185	162	155
Viability Index on D3 (%)	94.8	96.1	95.3	99.5
Postnatal Day 21: No. of pups	172	185	162	155
Postnatal Day 21: Weaning Index (%)	99.4	100	100	100
Unscheduled pup necropsies	1	1	0	0
Postnatal Day 21: No. of pups	172	185	162	155

The overall results are summarized as follows:

1. During the study period, no dams (F0) died, nor were observed any changes in body weight, food consumption, and clinical signs.

2. Regarding the findings at birth and postnatal growth of neonates, any findings suggesting the effects of MY-307 were not observed.
3. Neonates showed no abnormalities at all with respect to general behavior, sensorial function, and emotional learning ability of neonates.
4. The reproductive performance of neonates (F1) showed no findings suggesting the effects of MY-307.
5. The development of F2 (next generation) showed no effects suggesting the effects of MY-307.

Based on the above results, MY-307, even when administered at the dose levels up to 10 µg/kg did not adversely affect the gestation period, delivery status, and nursing ability of dams, as well as the growth of neonates. Therefore, the no effect dose level under the conditions of this study is considered as 10 µg/kg.

2.6.6.7 Local tolerance

For ocular toxicity study, see Repeat-dose toxicity under **2.6.6.3**.

2.6.6.8 Special toxicology studies

The sponsor summarized the antigenicity and phototoxicity information for difluprednate from the studies conducted for dermatologic product Myser® marketed in Japan. Difluprednate was negative for any active systemic anaphylactic reaction, delayed-type skin reaction, and passive cutaneous anaphylaxis (PCA) and passive hemagglutination anaphylaxis (PHA) reactions. Difluprednate was also found to be negative against contact allergic and delayed-type skin reactions in the maximization test. Furthermore, the PCA reaction in rats using the sensitized plasma of mice was found to be negative. No exacerbation of the irritancy by ultraviolet irradiation was found in the phototoxicity and photosensitization tests of the formulations of difluprednate ointment and cream.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Difluprednate is a corticosteroid that was first developed in Japan by Mitsubishi Pharma for topical dermatological use and has been classified to have a “very strong” glucocorticoid effect based on its clinical results in dermatology. Difluprednate is a derivative of prednisolone; a trivial name for difluprednate is difluoroprednisolone butyrate acetate (DFBA). In its dermatological formulation, difluprednate 0.05% is over 4 times more potent than prednisolone valerate acetate 0.3%, and 3200 times stronger than prednisolone 0.5%, as measured by peripheral vasoconstriction.

The subject of the NDA is 0.05% difluprednate topical ophthalmic emulsion indicated for the treatment of inflammation and pain associated with ocular surgery. The recommended clinical dose to the affected eye is one drop BID for two weeks. The inactive ingredients in the formulation (castor oil and polysorbate 80) are used in other ophthalmic drug products or are tested in rabbit eyes. They are acceptable for ophthalmic use at the proposed concentrations.

The pharmacology studies revealed that difluprednate ophthalmic emulsion suppressed uveitis in animals in a dose-dependent manner. The systemic absorption of ocularly administered difluprednate is very small. During the 7 days ocular instillation studies in rabbits of 0.05% ^3H -difluprednate, the C_{max} in the plasma was not more than 10 ng/g dry wet. The conducted safety pharmacology studies did not show any significant effect. Only small effects were observed at a drug levels (10^{-4} and 10^{-3} g/mL) much higher than that obtained by ocular route. Therefore, there is no safety concern.

Difluprednate (DFBA) is rapidly metabolized by deacetylation (at 21-position) in the rabbit eye tissues to the metabolite DFB (active metabolite), which is in turn converted to DF. No quantifiable difluprednate or DFB reaches the blood following a single ocular instillation (50 μl /eye) of difluprednate 0.05% in rabbits. The C_{max} in the eye was observed within 1 hour of ^3H -difluprednate instillation. The assay method using ^3H -difluprednate is sensitive enough to measure difluprednate and its metabolites at the levels of 0.3 ng equivalent/gm tissue. By autoradiography, difluprednate was cleared from the ocular tissues after a single instillation within 24 hours. Over 99% of radioactivity was excreted within 7 days. . These results indicated that difluprednate and its metabolites did not remain in the body and were mainly excreted in the feces. After repeated instillation, radiolabelled difluprednate and its metabolites did not tend to accumulate in ocular tissues.

Difluprednate has been marketed as a topical dermatological ointment in Japan. The toxicity data from the completed dermatological studies in animals were for a longer duration than the ophthalmic studies. The NOELs in the 6-month dermatological studies in rats and dogs were 1.0 $\mu\text{g}/\text{kg}/\text{day}$ and 1.25 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Neither deaths nor serious toxicologic findings were noted in the studies. Many changes at higher doses were those generally observed in glucocorticoid-treated animals.

The followings were found at 10 $\mu\text{g}/\text{kg}/\text{day}$ in the 6-month dermatological study in rats: suppression of body weight gain (both sexes) in clinical observation, increases in erythrocyte parameters such as RBC, decrease in M/E (myeloid erythroid ratio) and proliferation of erythroblasts (males), and shortening of APTT or activated partial thromboplastin time (males in 6-month test) in clinical pathology, slight atrophy of epidermis and decreased hypodermal adipose tissue in a few cases in morphological pathology.

The followings were found at 12.5 $\mu\text{g}/\text{kg}/\text{day}$ and above in the 6-month dermatological study in dogs: decreases in lymphocytes and eosinophils in blood, atrophy of lymphatic tissues such as thymus and adrenal gland, increase in neutrophils in blood, increase in hepatic glycogen, increases in water intake and urine volume, increase in sodium and decrease in potassium in blood, slight renal disorder, elevation of alkaline phosphatase and γ -glutamyl transpeptidase in blood, slight thinning of bone and skin (abdominal region), and delayed sexual maturation. However, neither death nor serious symptoms were detected. These changes recovered by drug withdrawal and were reversible. Similar systemic glucocorticoid effects were observed in 4-week ocular instillation toxicity studies in rabbits and dogs at doses of 100 $\mu\text{g}/\text{kg}/\text{day}$ and 40 $\mu\text{g}/\text{kg}/\text{day}$, respectively.

Ocular administration of 0.05% difluprednate ophthalmic emulsion (0.1 ml/eye) QID for up to 4 weeks in dogs and in rabbits did not cause any ocular toxicity. The glucocorticoids have been known to cause cataracts after a long term use in animals and humans. However, no cataract was found in these studies probably because the study periods were not long enough. The recommended clinical dose to the affected eye is one drop (0.03-0.05 ml) BID for two weeks. Therefore, it appears that there is a sufficient margin of safety. Instillation of heat-degraded difluprednate 0.05% in rabbits was tolerated as well as the normal difluprednate 0.05%. The instillation of polysorbate 80 excipient for 7 days was tolerated at concentrations up to 4% in rabbit eyes.

Mutagenesis and chromosomal aberration tests of difluprednate and difluprednate metabolites were negative. In the bacterial reverse mutation tests and the *in vitro* mammalian cell clastogenicity tests, difluprednate, metabolites, degradants, and impurities (DF17C, DF21B,-DFB) were all negative. An *in vivo* micronucleus test of difluprednate in mice was also negative.

No carcinogenicity studies of difluprednate have been performed.

During the IND and pre-NDA submission, no reproductive toxicity studies for difluprednate were submitted. At that time this reviewer recommended that the class labeling of glucocorticoid may be acceptable for difluprednate when approved. However, reproductive toxicity studies conducted in Japan (in 1981-1984) during the development of dermatologic formulation of difluprednate were submitted in this NDA.

Reproductive toxicity tests were performed with difluprednate in rats and rabbits. Fetal death and malformations such as cleft palate (commonly associated with high-dose administration of GCs) were observed during the organogenic period in rabbits. The effects of difluprednate on rat fetuses were weak; fetal death and/or malformed fetuses were not found. Further details are given below.

When administered to Wistar rats prior to and in the early stages of pregnancy, MY-307 was confirmed not to have a negative effect on the reproductive performance of rats and on the next generation. Furthermore, MY-307 at 1 µg/kg or lower dose level showed no abnormalities in parent animals. Therefore, the maximum no effect level in the present study in rats is considered to be between 1 and 10 µg/kg.

MY-307 was administered to Wistar rats during the period of organogenesis. The 100 µg/kg (subcutaneous injection) group showed inhibition of body weight gain and a decrease in food consumption in dams. Inhibition of fetal growth was observed. However, MY-307 had no teratogenicity and embryo-fetal lethality at this dose level. Furthermore, MY-307 did not show any adverse effect on postnatal growth and reproductive function of offspring (F1). The above results lead to consider that MY-307, when administered subcutaneously to rats during the period of organogenesis, shows no teratogenicity even at the toxic dose for dams and that the drug neither affects postnatal growth when administered at dose levels of ≤ 10 µg/kg. At 100 µg/kg, delayed ossification in rat fetuses was observed.

In embryofetal development study in rabbits (at subcutaneous doses of 0.1, 1.0, and 10.0 µg/kg), some external anomalies (cleft palate, cerebral hernia, hypogenesis of the first

digit of the forelimb, club hand, etc.) occurred mostly at 10 µg/kg. Slight maternal toxicity (body weight inhibition), embryofetal lethality, and fetal growth retardation were also observed mainly at 10 µg/kg.

Subcutaneous administration of 0.1, 1.0, and 10 µg/kg of MY-307 during the lactation period to rats showed no effects at all on dams. Furthermore, this dose did not affect postnatal development and reproductive performance of offspring. The above results lead us to presume that the no observed effect level for MY-307 under the conditions of this study is 10 µg/kg.

Unresolved toxicology issues (if any):

None

Recommendations:

The approval of NDA 22212 is recommended.

Suggested labeling:

The labeling proposed by the sponsor is generally acceptable with minor changes.

1. PREGNANCY

Teratogenic Effects



2. CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY



3. ANIMAL TOXICOLOGY AND/OR PHARMACOLOGY

Information to the sponsor:

Signatures (optional):

Reviewer Signature Conrad H. Chen, Ph.D.

Acting Team Leader Signature Wendelyn Schmidt, Ph.D.

Concurrence Yes X No

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this page is the manifestation of the electronic signature.**

/s/

Conrad Chen
5/7/2008 09:46:18 AM
PHARMACOLOGIST
The approval of NDA 22212 is recommended.

Wendelyn Schmidt
5/7/2008 09:50:16 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA Number: 22-212

Applicant: Sirion Therapeutics, Inc.

StampDate:

December 26, 2007

Drug Name: Difluprednate Ophthalmic Emulsion, 0.05%

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

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?	x		
2 Is the pharmacology section of the NDA indexed and paginated in a manner to allow substantive review to begin?	x		
3 On its face, is the pharmacology section of the NDA legible so that substantive review can begin?	x		
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)?	x		
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 <u>or</u> to explain why such repetition should not be required?	x		
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?	x		
7 Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?	x		
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 <u>submitted</u> a rationale to justify the alternative route?	x		
9 Has the sponsor <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		
10 Has the sponsor <u>submitted</u> a statement(s) that the pharm/tox studies have been performed using acceptable, state-of-art protocols which also reflect agency animal welfare concerns?	x		
11 From pharmacology perspective, is this NDA fileable? If "no", please state below why it is not.	x		

Reviewing Pharmacologist: Conrad H. Chen

Date: January 15, 2008

Acting Team Leader: Wendelyn Schmidt

Date: January 16, 2008

cc:

Original NDA 22-212

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/s/

Conrad Chen
1/17/2008 01:02:20 PM
PHARMACOLOGIST
NDA 22212 is filable.

Wendelyn Schmidt
1/22/2008 03:37:44 PM
PHARMACOLOGIST