CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-583

PHARMACOLOGY REVIEW(S)

Review and Evaluation of Pharmacology and Toxicology Data
Division of Topical Drug Products (HFD-540)

NDA#:	20-583 (Original and Amendments 002, 003 & 004)
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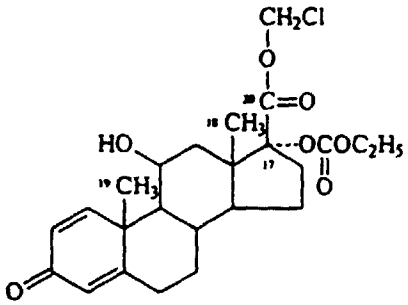
Name of Drug:	Lotemax®; loteprednol etabonate (LE); Chloromethyl-17α-ethoxycarbonyloxy-11β-hydroxyandrosta-1,4-diene-3-one-17β carboxylate, P-5604, OPC-5604

Structure:



Loteprednol etabonate (P-5604)

Formula Weight:	466.96
Empirical Formula:	$C_{24}H_{31}O_7Cl$

Solubility:

Solvent	% Solubility
DMF	34 (340 mg/ml)
DMSO	31 (310 mg/ml)
EtOH	0.836 (8.36 mg/ml)
PEG	0.22 (2.2 mg/ml)
Water	0.0008 (8μg/ml)

Pharmacological Category: A glucocorticoid corticosteroid with no detectable mineralocorticoid activity

Related Submissions:

Review Objectives: Review the preclinical animal safety data for LE to evaluate and characterize the local and systemic toxicity of LE, and determine if the proposed labeling adequately describes the potential risks associated with use of this compound.

Background: Anti-inflammatory therapy for the eye is an important pharmacologic tool for treatment of inflammation. While potent and effective agents exist they are not without significant side effect potential; therefore, they are most often administered topically to the eye and even this localization does not eliminate untoward systemic and ocular effect of these drugs. Loteprednol was synthesized i.e., a compound that would be intrinsically active at the site of administration, but be rapidly metabolized to an inactive compound following absorption. The hoped for advantage is that they exert their effect locally and are inactivated before they are exposed to other organs.

Prednisolone

Expected toxicology of class: The ocular complications associated with corticosteroid use include posterior subcapsular cataract formation, elevation of intraocular pressure (IOP) and resultant steroid-induce glaucoma, secondary ocular infection, delayed wound healing, uveitis, mydriasis, transient ocular discomfort, and ptosis. Additionally, topical ophthalmic steroids may cause systemic effects such as adrenal insufficiency, osteoporosis, hypertension, muscular weakness or atrophy, Cushing's Syndrome, peptic ulcers inhibition of growth, diabetes, and mood changes.

Ocular adverse of adrenocorticosteroid include glaucoma with optic nerve damage, visual acuity and field defects, posterior subcapsular cataract formation, secondary to ocular infection form pathogens including herpes simplex, perforation of the globe.

Systemic adverse effects of adrenocorticosteroid can occur as a result withdrawal or from continual use. In humans the withdrawal syndrome consists of fever, myalgia, arthralgia, and malaise.

The use of corticosteroids for days or a few weeks does not lead to adrenal insufficiency upon cessation of treatment, but prolonged therapy may result in suppression of pituitary-adrenal function that may be slow in returning to normal.

In addition to obtunding the pituitary-adrenal axis, the principal complications resulting from prolonged therapy are fluid and electrolyte disturbances; hypertension; hyperglycemia; and glucosuria; increased susceptibility to infections; peptic ulcer; osteoporosis; a characteristic myopathy; behavioral disturbances; arrested growth and Cushing's habitus, consisting of "moon face," "buffalo hump," enlargement of supraclavicular fat pads, "central obesity," striae, ecchymoses, acne and hirsutism.

Indication: Treatment of inflammatory conditions of the anterior segment of the eye.
Treatment of allergic conditions of the conjunctiva and cornea.

Route of Administration: Topically to the eye

Dose: One to two drops of 0.5% suspension qid. During the initial 24 to 48 hours the dosage may be safely increased to two drops every two hours.

Formulation:

Ingredient	%
Loteprednol etabonate	
Povidone , USP	
Benzalkonium Chloride	
Glycerin, USP	
Tyloxapol, USP*	
Purified Water, USP	QS to 100%

* 2-(2-Isopropyl-5-methylphenoxymethlyl)-2-imidazoline - surfactant, detergent

Preclinical Efficacy Studies:

The preclinical animal efficacy data are not being reviewed for the NDA because they were previously reviewed
and also efficacy will be measured by the clinical studies.

Review perspective:

are compounds synthesized so that when administered locally, have their therapeutic effect, but when absorbed they are rapidly metabolized to an inactive moiety. In this way the body burden of a potent and active compound is substantially reduced. So the expectation for LE is that local application to the eye would achieve a local anti-inflammatory effect without achieving significant plasma levels.

In normal human subjects given eye drops 8 times-a-day for two days and then 4 times-a-day for 41 days, for detecting LE and PJ-91 showed that all samples collected over a two hour period following the 1st and 5th doses on Day 0 and then once on Days 7, 14, 21, 28, 35 and 42 (samples collected over a two hour period following dose 4) were below the limits of quantitation. These data confirm that LE that behaves as a (ref. NDA 20-583, 1:166).

In a pilot study using method[2] oral administration of 40 mg of LE resulted in detection of both in human plasma. Levels were and no pharmacokinetic parameters could be calculated.

Since this compound is intended to be administered by the topical route to the eye it is important to estimate the maximum potential daily dose that could be absorbed if all the administered drug substance were totally absorbed. The maximum human dose is 2 drops Q2H during the initial 24 to 48 hours. This makes the total maximum exposure for the first two days of therapy a maximum possible of 6 mg (5 mg/ml x 0.05 ml/drop x 2 drop/dose x 12 dose/day) and for the duration of the therapy the dose will be a maximum of 2 mg. If these doses are converted to mg/m^2 the for a 60 kg ($1.6m^2$) patient the maximum possible dose could be 33 $\mu g/kg$ or 1.25 mg/m^2. If the body surface for a 150 g rat is 0.025 m^2 and for a 1.5 kg rabbit is 0.127 m^2 then the doses in mg/ m^2 are:

Dose (mg/kg)	Dose (mg/m^2)			
	Rat	Rat	Rabbit	Human
	0.150kg	0.200kg	1.5kg	60kg
	0.025 m^2	0.035 m^2	0.127 m^2	1.6 m^2
0.1*	0.6	0.89	1.2	3.8
0.5	3	2.8	5.9	18.8
3	18	17.1	35.4	112.5
5	30	28.6	59	188
50	300	285	590	1875
100	600	571	1181	3750

* The maximum human dose (6 mg divided by 60 kg)

Animal Safety Studies:

The following studies have been performed in support of this NDA:

Animal Safety Studies:

Study Title	Species	Route	Study Duration (Day)	Study Number (GLP)	Vol:pg	Study Site
P-5604 Acute Oral Toxicity in the Mouse	Mouse	p.o.	1	PTC/2 (yes)	6:33	1
P-5604 - Acute Oral Toxicity in the Rat	Rat	p.o.	1	PTC/1/88 (yes)	6:2	1
Acute Subcutaneous Toxicity in the Mouse	Mouse	s.c.	1	PTC/4 (yes)	6:154	1
Acute Subcutaneous Toxicity in the Rat	Rat	s.c.	1	PTC/3/88 (yes)	6:115	1
P-5604 Eye Irritation Study 0.1%	Rabbit	Ocular	1	PTC/5/A (yes)	6:205	1

P-5604 Eye Irritation Study 0.5%	Rabbit	Ocular	1	PTC/57 (yes)	6:219	1
P-5604 - 7 Day Ocular Dose Range finding Study in the Rabbit	Rabbit	Ocular	7	PTC/6 (yes)	7:185	1
28 Day Ocular Range finding Study in the Rabbit	Rabbit	Ocular	28	PTC/7/88 (yes)	7:228	1
P-5604 - 28 Day Oral (Gavage) toxicity study in the Rat	Rat	p.o.	28	PTC/9 (yes)	7:1	1
26 -Week Ocular Dose Study in the Rabbit	Rabbit	Ocular	182	PTC/89/94 (yes)	9:1	1
52 Week Ocular Toxicity Study in the Dog	Dog	Ocular	367	PTC/74/91 (yes)	8:1	1

Reproductive Studies:

Study Title	Species (Strain)	Route	Study Number (GLP)	Vol:pg	Study Site
P-5604 Rat general reproductive performance dose ranging study	Rat	p.o.	PTC/48/89 (yes)	12:339	1
Fertility and General Reproductive Study	Rat	p.o.	PTC/50 (yes)	13:155	1
P-5604 Rat Teratology Study	Rat	p.o.	PTC/49/89 (yes)	13:1	1
P-5604 Peri and Post Natal Study	Rat (Crl:CD-1(ICR)BR)	p.o.	PTC/51 (yes)	14:1	1
Rabbit Teratology Range Finding Study	Rabbit	p.o.	PTC/46/89 (yes)	12:113	1
Loteprednol Etabonate Rabbit Teratology Study	Rabbit	p.o.	PTC/67/90 (yes)	12:208	1

Pharmacokinetic Studies:

Study Title	Species	Route	Study Duration (Day)	Study Number (GLP)	Vol:pg	Study Site
Pharmacokinetics, Metabolism and Excretion of a Soft Corticosteroid, Loteprednol Etabonate	Rat	i.v.	1	PHA-34 (yes)	15:176	2
Pharmacokinetics of Loteprednol Etabonate in Dogs	Dog	i.v. & p.o.	1	PHA-27 (yes)	15:94	2
Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug loteprednol etabonate in dog blood and plasma - Part 1 Stability of Loteprednol Etabonate in Dog Blood and Plasma	Dog	In Vitro	1	PHA-27A (no)	15:223	2
Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug loteprednol etabonate in dog blood and plasma Part 2 Protein binding and Erythrocyte Partition	Dog	In Vitro	1	PHA-27B (no)	15:241	2
Hydrolysis of loteprednol etabonate in plasma samples from rats, rabbits, beagles and humans and human liver In Vitro	Rat, Dog, Rabbit, Human	In Vitro	1	PHA-5A (yes)	15:209	3
Preliminary evaluation of Oral Absorption and Distribution of the Steroidal anti-inflammatory drug Loteprednol Etabonate in Rabbits	Rabbit	Ocular	1	PHA-25 (yes)	15:1	2
Preliminary Evaluation of Oral Absorption and Distribution of the Steroidal Anti-inflammatory Drug Loteprednol Etabonate in Rats	Rat	p.o.	1	PHA-26 (yes)	15:47	2

Genotoxicity Studies:

Study Title	Species	Route	Study Number (GLP)	Vol:pg	Study Site
Mutagenicity Study of OPC-5604 by the Ames Test and in E. coli	E. coli, WP2uvrA; S. typhimurium TA 1535, TA1537, TA1538, TA100 & TA98	In Vitro	PTC/1431 (yes)	14:153	3
Metaphase Analysis of human lymphocytes treated with P-5604	Human	In Vitro	PTC/10/M (yes)	14:132	1

Mouse lymphoma L5178Y	Mouse	In Vitro	PTC 24596 (yes)	14:210	1
Mutagenicity Study of OPC-5604 by the	S. typhimurium	In Vitro	PTC/1430 (yes)	14:116	3
Mouse Micronucleus Test	Mouse	In Vivo	PTC 24595 (yes)	14:178	1

Studies with PJ-90-(Secondary Metabolite):

Study Title	Species	Route	Study Duration (Day)	Study Number (GLP)	Vol:pg	Study Site
PJ-90 Primary Eye Irritation Test in the Rabbit	Rabbit	Ocular	1	A/E40367 (yes)	6:257	1
PJ-90 Acute Subcutaneous Toxicity in the Rat	Rat	s.c.	1	A/MISC/40368 (yes)	6:190	1
PJ-90 (0.5%) 28 Day Ocular Tolerance Study in Rabbits	Rabbit	Ocular	28	PTC/92/A (yes)	12:53	1

The above studies were conducted at the following study sites:

Number	Study Site
1	
2	
3	

Animal Safety Studies:

Acute and Subchronic Studies:

The presentation of the information conveyed in this review of NDA 20-583 will be presented as summaries and details of the individual studies will be appended at the end followed by copies of the previous reviews.

LE has been evaluated for toxicity in mice and rat following single dose (table 1) and 4 week (table 2) parental administration.

Table 1 Effects of Single Systemic Dose Administration of LE or PJ-90 on 14 day Survival in Rats and Mice

Compound	Species	Route	Maximally Tolerated Dose	Study Number (Vol.:Pg.)
Loteprednol etabonate	Rat	p.o.	>4 g/kg	PTC/1/88 (6:2)
		s.c.	>1.3 g/kg	PTC/3/88 (6:115)
	Mouse	p.o.	4 g/kg	PTC/2 (6:33)
		s.c.	>1.3 g/kg	PTC/4 (6:154)
Secondary Metabolite (PJ-90)*	Rat	s.c.	>100 mg/kg	A/MISC/40358 (6:190)

* see metabolic scheme above

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Table 2 Effects of Multiple Systemic Doses of LE Administration in Rats

Species	Route	Study Duration	Comments	Study Number (Vol.:Pg.)
Rat	Oral	28 Days	A daily dose of 0.5, 5.0 & 50 mg/kg of LE caused a dose dependent reduction in body weight gain (9 and 10% for males and females, respectively for high dose rats) decreases (approx. 19 and 33% for spleen and thymus, respectively) in organ to body weight ratios and histological and blood count changes that are consistent with immune suppression. Oral administration of daily doses of 5.0 and 50 mg/kg has adverse effects in rats.	PTC/9/88 (7:1)

Ocular Administration:

Effects of ocular administration to rabbits and dogs are presented in tables 3 and 4.

Table 3 Effects of Ocular Administration of LE or PJ-90 to Rabbits

Compound [Study Number (Vol.:Pg.)	Study Duration	Vehicle	Maximally Tolerated Dose
LE [PTC/5/A (6:209) & PTC/57/A (6:219)]	Single	20% HPCD*	Single daily dose 0.1 ml of 0.1 or 0.5% suspension caused slight conjunctival redness at 1 hr after administration
[PTC/6/88 7:185]	7 days	50% HPCD	Daily doses of 0.1 ml of 0.5, 0.7 or 5.0%w/v suspensions caused a decrease in the organ weights of the thymus, adrenals and gonads.

[PTC/7/88 (7:228)]	28 days	50% HPCD	Test article was administered once-a-day. Concentrations of active in 0.1 and 0.7% formulations could not be reliably delivered, but the 5.0% formulation was within 10% of expected concentration for the last 3 weeks of the experiment. The decreased thymus, adrenal and gonad weights observed in the 7 day range finding study were not repeated and the results suggest that administration of a concentration that was 10 times the clinical concentration was well tolerated.
[PTC/89/94 (9:1)]	26 weeks	Clinical Formulation	Intraocular administration of one drop ($-30\mu l$) of a 0.5% suspension qid caused no biologically meaningful differences in the rate of body weight gain or food consumption. There were some differences in the ratio of organ weight to body weight (namely decreased adrenals, lungs (males) and ovaries); but adverse histologic effects did not accompany these weight changes. A low incidence of slight conjunctival erythema was seen at 13 weeks, but was not observed at 26 weeks and often a clear watery conjunctival discharge was noted in about half the rabbits in the treated group (no data is presented for the vehicle treated control treated rabbits). In two treated males this discharge was noted as bilateral.
Secondary Metabolite (PJ-90) [A/E 4036 (6:257)]	Single	Not specified	Formulation is not specified. After administration of 0.1 ml of a 0.1% suspension slight conjunctival redness was observed after 1 hr which resolved by 24 hrs.
[PTC/92/A (12:92)]	28 days	50% HPCD	Macroscopic and histologic examination of 0.5% PJ-90 treated rabbit eyes showed no differences from the contralateral vehicle treated eyes, however, no data were provided to support that the formulation could reliably deliver the specified dose of test compound.

* 2-hydroxypropyl-β-cyclodextrin (HPCD)

Table 4 Effects of Multiple Ocular Doses of LE Administration in Dogs

Study Duration [Study Number (Vol.:Pg.)]	Comments
52 weeks [PTC/74/91 (8:1)]	Ocular administration of 0.05, 0.1, and 0.5% w/v suspensions of LE* to beagle dogs for 52 weeks caused a dose and time-dependent increase incidence of stromal deposits in the corneas of both genders. Elevation of IOP was also observed following loteprednol administration at 52 weeks for all doses evaluated, however, no clear dose dependency was established.
	The positive control, 0.1% dexamethasone, caused both an increase in cornea stromal deposits and IOP which increased over time. Dexamethasone also caused other signs of glucocorticoid toxicity, such as reduction in body weight gain, involution of the thymus, and decreased adrenal weights and microscopic changes typical of corticosteroids.

* Preservative: 0.01% benzalkonium chloride
Inactive: gelatin, hydroxypropyl methylcellulose, tetronic 1107, boric acid, sodium borate, edetate disodium, sodium chloride, purified water

Reproductive Review: (Study Numbers - PTC/46/89, PTC/48/89, PTC/49/89, PTC/50/90, PTC/51/90 and PTC/67/90):

Segment I:

Treatment in rats with 5.0 mg/kg (intermediate) and 25 mg/kg (high) for females and 50 mg/kg (high) for males had adverse effects in F0 males and females and F1 offspring. These adverse effects include decreased body weight gain for males and females in the F0 generation and lower fetus and pup body weight. The F1 generation failed to regain the lost body weight but were able to mate and produce offspring without any adverse effect. In these latter animals retardation or absence of ossification was noted and for the highest dose group umbical hernias were noted. Even at the low dose, 0.5 mg/kg, there was a nonstatistically different decrease in fetal and pup body weight and this continued throughout the period of observation. No adverse effects were observed in the F2 generation.

In a preliminary study parturition was delayed at the high dose.

Segment II:

In a rat teratology study oral administration of 50 and 100 mg/kg per day adversely affected both maternal and fetal weight and caused fetal toxicities. Doses of 0.5 and 5.0 mg/kg per day cause retarded ossification in the fetuses while having no deleterious effects on weight gain in the dams.

In this experiment the suspensions at the lower concentrations were not within 10% of expected, and it was difficult to predict accurately the dose administered on any one day.

In a preliminary dose-range finding rabbit teratology study, oral administration of a suspension of LE to female rabbits was toxic to both the does and their fetuses. Because of the inability to formulate reliable concentrations of LE suspensions for doses of 1.5 mg/kg and lower this study shows that doses of 3.0 mg/kg administered orally from day 6 to day 18 of pregnancy are fetotoxic. These results were not seen when the study was repeated.

Segment III:

Maternal treatment with orally administered LE during late pregnancy and lactation caused a dose dependent decrease in body weight gain with only slight decreases observed at 0.5 mg/kg and marked effects on body weight, food consumption and clinical condition at 50 mg/kg. In spite of these dramatic effects there were no effects on the onset or progress of parturition.

In the offspring, LE treatment elicited toxic changes at both 5.0 and 50 mg/kg treatments. In comparison to control pups, the high dose pups exhibited body weight and developmental retardation, poor survival, diminished clinical condition and the occurrence of umbical hernia. At the 5.0 mg/kg dose adverse effects were limited to retarded body weight at birth only and the observance of an umbical hernia in one litter.

No adverse effects were observed for either the dams or the pups in the 0.5 mg/kg dose group.

Pharmacokinetic Review (Study Numbers - PHA-25, PHA-25, PHA-26, PHA-27, PHA-27A, PHA-27B, PHA-34, PHA-35, PHA-5A and PHA-5A)

In Vitro evaluation of LE demonstrates that it is not hydrolyzed in human, dog or rabbit plasma, but is rapidly and completely hydrolyzed when incubated in rat plasma. Also incubation with human liver homogenates for 20 min caused a 27% hydrolysis and the appearance of two peaks, presumably the primary (PJ-90) and secondary metabolite (PJ-91). In dog blood studies the half-life of LE in blood and plasma is 18 and 22 hr, respectively, and the erythrocyte partition coefficient is approximately 7.8 which is about 30 times greater than the PJ-91.

In Vivo studies following intravenous administration in rats and dogs (table 5) show that loteprednol was rapidly eliminated from the systemic circulation, and that no parent compound was present in bile but significant levels of both the PJ-91 and PJ-90 were present. In dogs following intravenous administration extensive conversion to PJ-91 occurred.

Table 5 Comparison of Some Pharmacokinetic Parameters in Rats and Dogs Following Intravenous Administration of 5 mg/kg

Parameter	Rat	Dog
Mean Resident Time	0.53 hr	2.0 ± 0.3 hr
Volume of Distribution	1.44 L	43.6 ± 10.1 L
Terminal $t_{½}$	0.49 hr	2.8 ± 0.3 hr

Ocularly applied ^{14}C-LE results in distribution of parent compound into the conjunctiva, cornea, iris/ciliary body, and aqueous humor, and no detectable levels in blood. The peak concentrations were achieved within the first 0.5 to 1 hr after administration and diminished to the lowest concentration by 6 to 8 hr.

Concentrations of metabolites were highest in the cornea and peak concentrations were observed at 0.5 hr after administration.

Genotoxicity:

LE has been evaluated in a number of In Vitro and In Vivo tests for it potential for genotoxicity (table 6).

Table 6 Genotoxicity Results

Study	Test species	Results	Study Number (Vol.:Pg.)
Mutagenicity Study of OPC-5604 by Ames Test and E. coli	S. typhimurium, - and - E. coli,	LE at the limit of solubility (between 10 and 50 μg/plate) did not induce gene mutations in the bacterial strains evaluated with and without activation.	PTC/1431 (14:153)

this assay not in label

Mouse Micronucleus Test -LE	Mouse/Charles River CD-1 outbred	In previous studies in mice the maximally tolerated dose of LE was determined to be 4 g/kg and this dose had no effect on the induction of chromosomal or spindle damage in developing erythrocytes in bone marrow.	PTC 24595 (14:178)
Mutation in L5178Y Mouse Lymphoma Cells	Mouse/L5178Y lymphoma cells	In this system loteprednol was not found to induce a point mutations at the TK locus of L5178Y TK +/- cells with or without metabolic activation.	PTC 24596
Metaphase Analysis of Human Lymphocytes Treated with P-5604 (LE)	human lymphocytes	In the presence and absence of rat liver microsomal faction concentrations of 6.25, 12.5, 25 and 50μg/ml. of LE caused no statistically significant increases in chromosome aberration in human lymphocytes.*	PTC/10/M (14:132)

* It should be noted that by inspection the data appear to shows no differences from control; the statistical analysis does not appear to be appropriate for the comparisons that the sponsor has made.

Pharmacokinetic Review (Study Numbers - PHA-25, PHA-25, PHA-26, PHA-27, PHA-27A, PHA-27B, PHA-34, PHA-35, PHA-5A and PHA-5A)

In Vitro evaluation of LE demonstrates that it is not hydrolyzed in human, dog or rabbit plasma, but is rapidly and completely hydrolyzed when incubation in rat plasma. Also incubation with human liver homogenates for 20 min caused a 27% hydrolysis and the appearance of two peaks, presumably the primary (PJ-91) and secondary metabolite (PJ-90). In dogs, pharmacokinetic studies demonstrated the half-life of LE in blood and plasma is 18 and 22 hr, respectively, and the erythrocyte partition coefficient is approximately 7.8 which is about 30 times greater than the PJ-91.

In Vivo studies following intravenous administration in rats and dogs show that LE is rapidly eliminated from the systemic circulation, and that no parent compound was present in bile but significant levels of both the PJ-91 and PJ-90 were present. In dogs following intravenous administration (table 7) extensive conversion to PJ-91 occurred.

Table 7 Comparison of Some Pharmacokinetic Parameters in Rats and Dogs Following Intravenous Administration of 5 mg/kg

Parameter	Rat	Dog
Mean Resident Time	0.53 hr	2.0 ± 0.3 hr
Volume of Distribution	1.44 L	43.6 ± 10.1 L
Terminal $t_{1/2}$,	0.49 hr	2.8 ± 0.3 hr

Ocularly applied ^{14}C-LE results in distribution of parent compound into the conjunctiva, cornea, iris/ciliary body, and aqueous humor, and no detectable levels in blood. The peak concentrations were achieved within the first 0.5 to 1 hr after administration and diminished to the lowest concentration by 6 to 8 hr.

Concentration of metabolites were highest in the cornea and peak concentration were observed at 0.5 hr after administration.

Problems of Formulation:

With few exceptions the sponsor has not ensured that the dosage forms that were prepared for subsequent dosing of animals could reliable deliver the expected amount of test substance (Table 8). In the case of formulations made with they have not provided data showing that the test material was actually released or if it was released how much and at what rate it was released. In another incidence, in the rat Fertility and General Reproductive Study, PTC/50, they prepared the dosing formulation

Most often the lower concentrations of the suspensions were the ones with significantly less than expected concentration, and because systemic administration of the compound resulted in adverse reaction typical of the corticoid steroids it can be concluded that the sponsor has dosed the animals with doses that were adequate to show that the test material does have toxicity. The problem is that it is difficult to determine the relationship.

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Table 8 The Accuracy of Formulations Used to Support NDA 20-583

Animal Safety Studies:

Vehicle	Formulation Accuracy	Study Title - Study Number (Vol:pg)	Species	Route	Study Duration (Days)
50 % HPCD[1]	Not specified	P-5604 Acute Oral Toxicity in the Mouse - PTC/2 (6:33)	Mouse	p.o.	1
50 % HPCD	Not specified	Acute Subcutaneous Toxicity in the Mouse - PTC/4 (6:154)	Mouse	s.c.	1
20 % HPCD	Not specified	P-5604 Eye Irritation Study 0.1% - PTC/5/A (6:205)	Rabbit	Ocular	1
NS[2]	Not submitted	PJ-90 Primary Eye Irritation Test in the Rabbit - A/E40367 (6:257)	Rabbit	Ocular	1
20% HPCD	Not specified	P-5604 Eye Irritation Study 0.5% - PTC/57 (6:219)	Rabbit	Ocular	1
50% HPCD	Not specified	P-5604 - Acute Oral Toxicity in the Rat - PTC/1/88 (6:2)	Rat	p.o.	1
50% HPCD	Not specified	Acute Subcutaneous Toxicity in the Rat - PTC/3/88 (6:115)	Rat	s.c.	1
50% HPCD	Not specified	PJ-90 Acute Subcutaneous Toxicity in the Rat - A/MISC/40368 (6:190)	Rat	s.c.	1
50% HPCD	Not specified	P-5604 - 7 Day Ocular Dose Range finding Study in the Rabbit - PTC/6 (7:185)	Rabbit	Ocular	7

50% HPCD	Not specified	PJ-90 (0.5%) 28 Day Ocular Tolerance Study in Rabbits - PTC/92/A (12:53)	Rabbit	Ocular	28
50% HPCD	The concentration varied between of expected, however, on one occasion it was as low as 43% of expected.	28 Day Ocular Range finding Study in the Rabbit - PTC/7/88 (7:228)	Rabbit	Ocular	28
CMC[3]	Concentration varied of expected.	P-5604 - 28 Day Oral (Gavage) toxicity study in the Rat - PTC/9/88 (7:1)	Rat	p.o.	28
Clinical[4]	Not specified	26 -Week Ocular Dose Study in the Rabbit - PTC/89/94 (9:1)	Rabbit	Ocular	182
Formula[4]	Preserved formulation	52 Week Ocular Toxicity Study in the Dog - PTC/74/91 (8:1)	Dog	Ocular	367

1 2- Hydroxypropyl-β-cyclodextrin
2 Not specified
3 carboxymethylcellulose
4 See study

Reproductive Studies:

Vehicle	Formulation Accuracy	Study Title - Study Number (Vol:pg)	Species	Route
1% CMC[1]	On one occasion the range was of Theoretical values.	P-5604 Rat general reproductive performance dose ranging study - PTC/48/89 (12:339)	Rat	p.o.
1% CMC	The concentration varied from of theoretical.	Fertility and General Reproductive Study - PTC/50 (13:155)	Rat	p.o.

1% CMC	On the first occasion the range of concentrations found as a percentage of theoretical was , and on the second occasion the range was	P-5604 Rat Teratology Study - PTC/49/89 (13:1)	Rat	p.o.
1% CMC	The formulations were tested on two occasions and found to be within of theoretical.	P-5604 Peri and Post Natal Studies PTC/51 (14:1)	Rat	p.o.
1% CMC	Can only use 3 mg/kg or higher	Rabbit Teratology Range Finding Study - PTC/46/89 (12:113)	Rabbit	p.o.
1% CMC	Within 10% of expected	Loteprednol Etabonate Rabbit Teratology Study - PTC/67/90 (12:208)	Rabbit	p.o.

1 Carboxymethylcellulose

Pharmacokinetic Studies:

Vehicle	Formulation Accuracy	Study Title - Study Number (Vol:pg)	Species	Route	Study Duration (Days)
NS[1]	Not specified	Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug LE in dog blood and plasma - Part 1 Stability of LE in Dog Blood and Plasma - PHA-27A (15:223)	Dog	In Vitro	1
NS	Not specified	Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug LE in dog blood and plasma Part 2 Protein binding and Erythrocyte Partition -PHA-27B (15:241)	Dog	In Vitro	1
PEG[2] (i.v.) & CMC[3] (p.o.)	Not specified	Pharmacokinetics of Loteprednol Etabonate in Dogs - PHA-27 (15:94)	Dog	i.v. & p.o.	1

Clinical[4]	Not specified	Preliminary evaluation of Oral Absorption and Distribution of the Steroidal anti-inflammatory drug Loteprednol Etabonate in Rabbits - PHA-25 (15:1)	Rabbit	Ocular	1
1 % CMC	Not specified	Preliminary Evaluation of Oral Absorption and Distribution of the Steroidal Anti-inflammatory Drug Loteprednol Etabonate in Rats - PHA-26 (15:47)	Rat	p.o.	1
50% HPCD[4]	Not specified	Pharmacokinetics, Metabolism and Excretion of a Soft Corticosteroid, Loteprednol Etabonate - PHA-34 (15:176)	Rat	i.v.	1
NS	Not specified	Hydrolysis of loteprednol etabonate in plasma samples from rats, rabbits, beagles and humans and human liver In Vitro - PHA-5A (15:209)	Rat, Dog, Rabbit, Human	In Vitro	1

1 Not specified
2 Polyethylene glycol
3 Carboxymethylcellulose
4 2- Hydroxypropyl-β-cyclodextrin

Genotoxicity Studies:

Vehicle	Formulation Accuracy	Study Title - Study Number (Vol:pg)	Species
DMSO[1]	Not specified	Mutagenicity Study of OPC-5604 by the Ames Test - PTC/1431 (14:153)	E. coli S. typhimurium
DMSO	Not specified	Metaphase Analysis of human lymphocytes treated with P-5604 - PTC/10/M (14:132)	Human
DMSO	Not specified	Mouse lymphoma L5178Y - PTC 24596 (14:210)	Mouse
0.5% CMC[2]	Not specified	Mouse Micronucleus Test - PTC-24595 (14:178)	Mouse
DMSO	Not specified	Mutagenicity Study of OPC-5604 - PTC/1430 (14:116)	

1 Dimethylsulfoxide
2 Carboxymethylcellulose

Studies with PJ-90 (Secondary Metabolite):

Vehicle	Formulation Accuracy	Study Title - Study Number (Vol:pg)	Species	Route	Study Duration (Days)
NS[1]	Not submitted	PJ-90 Primary Eye Irritation Test in the Rabbit - A/E40367 (6:257)	Rabbit	Ocular	1
50% HPCD	Not specified	PJ-90 Acute Subcutaneous Toxicity in the Rat - A/MISC/40368 (6:190)	Rat	s.c.	1
50% HPCD	Not specified	PJ-90 (0.5%) 28 Day Ocular Tolerance Study in Rabbits - PTC/92/A (12:53)	Rabbit	Ocular	28

1 Not specified
2 2- Hydroxypropyl-β-cyclodextrin

Summary: Lotoprednol etabonate behaves as a , i.e., it rapidly degrades following administration. Administration of ^{14}C labeled LE to the eyes of rabbits results in detectable levels conjunctiva, cornea, iris/ciliary body, and aqueous humor, but little detectable levels in blood.

Lotoprednol etabonate has not been shown to have genotoxic potential.

In rats and rabbits LE is both fetotoxic and toxic to the gravid female. Like other glucocorticoids it appears to cross the placental and has effect on the rate of weight gain in the offspring. In the rabbit, the most sensitive species, a dose of 0.5 mg/kg appears to be the highest dose tolerated without either fetotoxicity or toxicity to the female. This dose is estimated to be 4 or 5 times the maximum possible total daily dose for a patient when used as directed and if only 5% is absorbed systemically, the dose could be as much as 100 times the human exposure.

Regulatory Conclusion: One of the major problems with this study is that the sponsor did not provide adequate formulation to ensure homogeneity of test material in the dosage forms administered and therefore, lower concentrations of the suspensions were often significantly less potent than expected. They also did not demonstrate that the test material was released from within the hydroxypropyl-β-cyclodextrin formulation. However, since the blood level following

following ocular application in rabbits is negligible, the risk of potential untoward effects is minimal.

From a nonclinical stand point there is no reason not to approve this drug.

Label considerations:

Systemic administration of the compound results in adverse reaction typical of the corticoid steroids, and labeling considerations related to Pregnancy Category are adequate, however, the sponsor must rewrite the sections listed under **Precautions/General** and Carcinogenesis, **mutagenesis, impairment of fertility** to:

1. List mutagenesis under the proper heading
2. Address the effect of test material on F1 and F2 fetuses

The sponsors have done no studies to investigate the carcinogenicity and have so noted in the label and there is no need to conduct such studies.

Appendix:

I. Detailed reviews of individual studies
II. Previous reviews

David A. Shriver, Ph.D.
Pharmacologist

cc:

NDA 20-583 file
HFD-340
HFD-540
HFD-540/PHARM/SHRIVER
HFD-540/MO/CARRERAS
HFD-540-CHEM/GILMAN
HFD-540/PMS/CHAPMAN
HFD-540/SPHARM/AJACOBS

Correspondence Only:
HFD-540/DD/JWILKIN
HFD-540/SPHARM/AJACOBS 10/16/95

Appendix I

APPEARS THIS WAY
ON ORIGINAL

Table of Studies

Animal Safety Studies:

Study	Acute Oral Toxicity in the Mouse	
Study Number:	PTC/2/88	
Study Date:	September 1988	
Test Article:	Batch 92-001	
Species/strain:	Rat/Crl:CD-1(ICR)BR	
Vehicle:	Test article was suspended in 50% w/v aqueous solution of 2-Hydroxypropyl-β-cyclodextrin prepared on the day of dosing and mixed thoroughly before and during dosing.	
Accuracy of test article formulation:	Not specified and no determination that the compound released from the cyclodextrin inclusion particle.	
Route:	p.o.	
Dose volume:	20 ml/kg or 40 ml/kg for replacement mice in MTD test.	
Number of animals:	Range finding study	1 per sex per dose
	Maximal tolerated dose (MTD)	5 per sex*
Dose:	Range finding	0.5, 1.0, 2.0, 3.5, 5.0 g/kg to overnight fasted mice
	MTD	4.0 g/kg to overnight fasted mice
Observation times:	Both studies on day 1	Continuously for first 30 minutes and then at 1, 2, and 4 hr after dosing.
	Range finding	At least once daily for 7 days following single oral dose
	MTD	At least once daily for 14 days following single oral dose. Animals were necropsied.
Weights:	Range finding	Males; 21 to 27g
		Females, 20 to 23g
	MTD	Males; 19 to 24g
		Females, 18 to 21g

* 1 male and 2 females were added to the study following the early death of treated animals (deaths presumed to have resulted as a result of intubation error).

Results:

No deaths were observed in the 7 day range finding study (doses of 0.5, 1.0, 2.0, 3.5, 5.0 g/kg). In the 14 day MTD study (4.0 g/kg) one male and 2 females died within two hrs. All 3 mice had fluid in their abdominal cavities, a finding consistent with administering large dose volumes, 40 ml/kg. Because of the assertion that these early deaths were not drug related an additional male and 2 females were added to the study. On day 10 one female mouse in this additional group died.

There were no macroscopic abnormalities observed at necropsy of surviving mice and body weight gains were within expected parameters. However, the sponsor did not determine proof of absorption, although piloerection was observed in both males and females.

Conclusion:

The maximum tolerated oral acute dose was 4 g/kg.

Study:	Acute Oral Toxicity in the Rat	
Study Number:	PTC/1/88	
Study Date:	September 1988	
Test Article:	Batch 92-001	
Species/strain:	Rat/Crl:CD(SD)BR (VAF plus)	
Vehicle:	Test article was suspended in 50% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin prepared on the day of dosing and mixed thoroughly before and during dosing.	
Route:	p.o.	
Dose volume:	20 ml/kg	
Number of animals:	Range finding study	1 per sex per dose
	Maximal tolerated dose (MTD)	5 per sex
Dose:	Range finding	0.5, 1.0, 2.0, 3.5, 5.0 g/kg to overnight fasted rats
	MTD	4.0 g/kg to overnight fasted rats
Observation times:	Both studies on day 1	Continuously for first 30 minutes and then at 1, 2, and 4 hr after dosing.

	Range finding	At least once daily for 7 days following single oral dose
	MTD	At least once daily for 14 days following single oral dose. Animals were necropsied.
Weights:	Range finding	Males; 234 to 248g Females, 151 to 181g
	MTD	Males; 130 to 144g Females, 117 to 128g

Results:

No deaths were observed in the 7 day range finding or the 14 day MTD studies.

There were no macroscopic abnormalities observed at necropsy and body weight gains were within expected parameters. However, the sponsor did not determination proof of absorption, although piloerection was observed in both males and females.

Conclusion:

There were no untoward effect following a single oral dose of 4 g/kg.

Study:	Acute Subcutaneous Toxicity in the Mouse	
Study Number:	PTC/4/88	
Study Date:	September 1988	
Test Article:	Batch 92-001 and unidentified batch	
Species/strain:	Mouse/Crl:CD-1(ICR)BR	
Vehicle:	Test article was suspended in 50% w/v aqueous solution of 2-Hydroxypropyl-β-cyclodextrin prepared on the day of dosing and mixed thoroughly before and during dosing.	
Route:	Subcutaneous	
Dose volume:	5 ml/kg or 10 ml/kg for the range finding study and 20 ml/kg for the maximally tolerated dose (MTD) study	
Number of animals:	Range finding study	1 per sex per dose
	Maximal tolerated dose (MTD)	5 per sex

Dose:	Range finding	0.05, 0.05, 1.0, 1.5 or 2.5* g/kg to overnight fasted mice
	MTD	4.0 g/kg to overnight fasted mice
Observation times:	Both studies on day 1	Continuously for first 30 minutes and then at 1, 2, and 4 hr after dosing.
	Range finding	At least once daily for 7 days following single oral dose
	MTD	At least once daily for 14 days following single oral dose. Animals were necropsied.
Weights:	Range finding	Males; 29 to 32g Females, 20 to 25g
	MTD	Males; 17 to 20g Females, 19 to 20g

* Because the vehicle was too viscous at a concentration adequate to administer a dose of 2.5 g/kg in a dose volume of 5 ml/kg the females of this group received approximately 80% of proposed dose; therefore, the dose was repeated in another group of mice, but the concentration was diluted by 1/2 and the dose volume was increased to 10 ml/kg.

Results:

No deaths were observed following s.c. administration of 0.05, 0.05, 1.0, 1.5 or 2.5* g/kg to overnight fasted mice in the 7 day range finding study. Based on the results of this study the sponsor selected 4.0 g/kg to evaluate in a 14 day MTD study. Again no deaths were observed. Proof of absorption was not determined, however, the spleens of all males and 2 females in the MTD study were observed to be small. In addition, one of the females had a markedly fluid filled and distended uterus.

Conclusion:

The acute maximum tolerated subcutaneous acute dose in mice was approximately 4.0 g/kg.

Study:	Acute Subcutaneous Toxicity in the Rat
Study Number:	PTC/3/88
Study Date:	September 1988
Test Article:	Batch 92-001
Species/strain:	Rat/Crl:CD(SD)BR (VAF plus)

Vehicle:	Test article was suspended in 50% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin prepared on the day of dosing and mixed thoroughly before and during dosing.50% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin prepared on the day of dosing and mixed thoroughly before and during dosing	
Route:	Subcutaneous	
Dose volume:	5 ml/kg for range finding study and 20 ml/kg for the Maximal Tolerated Dose (MTD) Study	
Number of animals:	Range finding	1 per sex per dose
	MTD	5 per sex
Dose:	Range finding	0.05, 0.50, 1.0, 1.5 or 2.5*g/kg to overnight fasted rats
	MTD	1333.4 mg/kg to overnight fasted rats
Observation times:	Both studies on day 1	Continuously for first 30 minutes and then at 1, 2, and 4 hr after dosing.
	Range finding	At least once daily for 7 days following single oral dose
	MTD	At least once daily for 14 days following single oral dose. Animals were necropsied.
Weights:	Range finding	Males; 170 to 183g Females, 151 to 156g
	MTD	Males; 71 to 86g Females, 77 to 88g

* Viscosity of solution precluded administration of the planned dose of 2.5 g/kg

Results:

No deaths were observed following s.c. administration of doses of 0.05, 0.50, 1.0, 1.5 and g/kg in a 7 day range finding study. Based on the results of this study the sponsor selected 1.3334 g/kg to evaluate in a 14 day MTD study. Again no deaths were observed and the weight gains were unaffected. Proof of absorption was not determined, however, the spleens of 2 males and 4 females in the MTD study were observed to be small.

Conclusion:

The acute maximum tolerated subcutaneous acute dose was greater than 1333.4 mg/kg.

Study:	P-5604 Eye Irritation Study - 0.1%
Study Number:	PTC/5/A
Study Date:	August 1988
Test Article:	Batch 819C (0.1% w/v) (an opaque white liquid)
Species/strain/sex:	Rabbits New Zealand White - female
Vehicle:	Test article was suspended in 20% w/v aqueous solution of 2-Hydroxypropyl-β-cyclodextrin, sonicated and filtered.
Route:	Ocular in right eye
Dose volume:	0.1 ml
Number of animals:	1 in preliminary screen 5 for study
Dose:	0.1 ml of a 0.1% (w/v) - The rabbits are restrained in wooden stocks and the material is instilled into the right eye by pulling away the lower lid to form a cup into which the material was placed. The lid was then held shut for a few seconds and moved about to distribute the test material around the surface of the eye and lid.
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified

Results and conclusions:

The sponsor was asked to clarify the accuracy of the formulation and they responded by submitting the following formula as the test material used in this study (PTC/5/A in NDA 20-583 correspondence 6/29/95):

Loteprednol etabonate	
Hydroxypropylmethylcellulose	
Benzalkonium chloride, 50%	

Sodium acid phosphate	0.94 g
Sodium phosphate	0.24 g
Purified water	qs to 100 ml

This is an entirely different formulation than the one contained in the protocol for this study, should be expected to have different performance characteristic and does not address the issue of accuracy of the formulation. Therefore this study is not interpretable.

Study:	P-5604 Eye Irritation Study - 0.5%
Study Number:	PTC/57/A
Study Date:	February 1989
Test Article:	Batch Number 137
Species/strain/sex:	Rabbits New Zealand White - female
Vehicle:	Test article was suspended in 20% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin, sonicated and filtered.
Route:	Ocular in right eye
Dose volume:	0.1 ml
Number of animals:	1 in preliminary screen 5 for study
Dose:	0.1 ml of a 0.5% (w/v) - The rabbits are restrained in wooded stocks and the material is instilled into the right eye by pulling away the lower lid to form a cup into which the material was placed. The lid was then held shut for a few seconds and moved about to distribute the test material around the surface of the eye and lid.
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified

Results: Slight conjunctival redness was observed after 1 hr which resolved by 24 hrs.

Conclusion: Because the accuracy of the formulation was not specified the results from this study can not be interpreted.

Study:	P-5604 7 Day Ocular Dose Range finding study in the Rabbit
Study Number:	PTC/6/88
Study Date:	April 1988
Test Article:	Not specified
Species/strain:	Rabbit/New Zealand
Weights:	Males; 2.47 to 2.57 kg Females, 2.35 to 2.53 kg
Number of animals:	1 per sex per dose
Route:	Topical in the right eye
Dose volume:	0.1 ml
Doses Evaluated:	0.1, 0.7, and 5.0%, administered once daily for 7 consecutive days
Vehicle:	50 % w/v aqueous 2 hydroxypropyl-β-cyclodextrin
Method and frequency of test article formulation:	Formulated once for the study by suspending weighted amount of material in vehicle, separately for each group.
Accuracy of test article formulation:	Not required by Sponsor
Stability of test article formulation:	Stable under test conditions
Blood Levels:	On day 7 serial blood samples were taken from the two high dose animals (detection levels <0.1 μg/ml).

Results: Conjunctival redness in the high dose female on day three and apparent dose dependent decreases in the weights of the thymus, adrenals and gonads were observed.

Conclusion: The effect on systemic absorption is unknown because the sponsor failed to characterize the release rate from the formulation. The observance of involution of the thymus, adrenals and gonads following ocular administration is consistent with known systemic effect of corticosteroid.

Study:	28 Day Ocular Dose Range finding study in the Rabbit
Study Number:	PTC/7/88

Study Date:	October-1988
Test Article:	Batch 92-001
Species/strain:	Rabbit/New Zealand
Weights:	Males; 2.32 to 2.78 kg Females, 2.32 to 2.64 kg
Number of animals:	6 per sex per dose
Route:	Ocular in the right eye
Dose volume:	0.1 ml
Doses Evaluated:	Vehicle, 0.1, 0.7, and 5.0%, administered once daily for 28 consecutive days
Vehicle:	50% w/v aqueous 2 hydroxypropyl-β-cyclodextrin (HCPD)
Method and frequency of test article formulation:	Formulated weekly for the study by suspending weighted amount of material in vehicle, separately for each group.
Accuracy of test article formulation:	The concentration of the test material in the formulation in general varied between 80 to 100% of expected, however, on specific occasions measured concentrations were as low as 43% of expected.
Stability of test article formulation:	No data presented in the pharmacology information to support the stability of the formulation.
Proof of absorption:	Blood samples were taken, but because plasma levels in rabbits could not be detected in study PTC/6/C, no analysis was attempted.

Results:

The method of preparing the dosage form was not reliable and provided variable concentration of test material (table 1). As can be seen, only for weeks 2 through 4 was the high dose administered within acceptable ranges.

Table 1 Concentration (% of Expected) of Test Material

Concentration	Week			
(%)	1	2	3	4
0.1	57.6	98.4	83.1	83.6

0.7	72.7	91.5	43.1	81.7
5.0	68.1	98.1	101.9	91.6

There were no statistically significant biologically meaningful changes in body weight, food consumption, hematological parameters, and blood chemical parameters (alpha-1-globulin exceeded expected range by 42 and 13% for males and females, respectively).

Absolute and relative to body weight organ weights were decreased for adrenals (significantly for females) and thymus (significantly for female and increased for liver (significantly for males) for the 5% treatment group (table 2).

Table 2 The effects of ocular administration to the right eye of rabbits of 5.0% LE in a 50% w/v aqueous HPCD vehicle for 28 days.

	Adrenal	Thymus
Male	-25	-24
Female	-30	-33

A dried crystalline material was present in all the rabbits at the three week ophthalmic examination. For all groups except the high dose males the tonometry values were lower at the 3 week evaluation than the pretreatment readings. The mean value for the high dose male group remained unchanged from the pretreatment period.

No compound-related histopathologic change were seen.

Conclusion:

The 28-day rabbit eye irritation study is difficult to interpret because the sponsor failed to provide test material release performance data for HPCD, and they provided data demonstrating that they were not able to reliably formulate the test material into a dosage form.

If, however, one assumes that the high dose, 0.1 ml of 5%, was delivered reliably for at least three of the four weeks there is reason to believe that this dose selected was the maximal dose practicable because all high dose animals exhibited dry crystalline material around the treated eye. At this concentration there were biological signs such as involution of the adrenals and thymic in both genders. While this study is seriously flawed it does suggest that LE does have biologically significant toxicology at a concentration that is 10 times the human clinical concentration, albeit in a radically different formulation.

Study:	28 Day Oral Toxicity Study in the Rat
Study Number:	PTC/9/88
Study Date:	October 1988
Test Article:	Two shipments of batch 92-001
Species/strain:	Rat/Crl:CD(SD)BR
Weights:	Males; 115 to 158 g Females, 103 to 142 g
Number of animals:	10 per sex per dose
Route:	p.o. by gavage
Dose volume:	10 ml/kg
Doses Evaluated:	0, 0.5, 5.0, and 50 $mgkg^{-1}day^{-1}$
Vehicle:	0.25% aqueous sodium carboxy-methylcellulose
Method and frequency of test article formulation:	Not specified
Accuracy of test article formulation:	Concentration of test material in formulation was not reliable. The concentration varied from of expected. The values were most variable for the low concentration (0.05 mg/ml) and varied between for the high concentration (5.0 mg/ml).

Results:

One low dose (0.5 mg/kg) male died of an ascending infection of the urinary tract, which was reasonably ascribed to a nontreatment related event.

There was a dose dependent decrease in body weight gain with both males and females having statistically significant reductions of 9 and 10% for males and females, respectively, in body weight gain in the high dose groups at 28 days of treatment

Glucocorticods tend to increase the hemoglobin and red-cell content of the blood. Administration also leads to a decrease in number of blood lymphocytes, eosinophils, monocytes and basophils and an increase in the number of polymorphonuclear leukocytes. In the present study the high dose caused an increase in RBCs and neutrophils and a decrease in WBCs males in both genders.
There was a trend for the absolute weights of the adrenals, spleen and thymus to decrease with increasing dose, table 3 shows the effect of drug treatment on the ratio of organ with to body

weight:

Table 3 Statistically Significant Changes in Organ Body Weights Ratios

Organ	0.5 mg/kg	5.0 mg/kg	50 mg/kg
Brain (females only)	N.D.*	N.D.	Increase (15%)
Spleen	N.D.	N.D.	Decrease (-19% - males; -19% - females)
Gonads	ND.	N.D.	Increase (12% - males only)
Thymus	N.D.	Slight Decrease (-15% - males; -10% - females)	Decrease (-33% - males; -34% - females)

* N.D. = No Difference

Microscopic examination of the adrenal cortex revealed changes characteristic of atrophy in only 1 high dose male, while microscopic changes in the spleen and thymus and reduced leukocyte counts suggest treatment related immunosuppression.

Conclusion:

A daily dose of 50 mg/kg of LE cause a dose dependent reduction in body weight gain, decreases in body weight ratios of the spleen and thymus and histological and blood count changes that are consistent with immune suppression. At the high dose there was a 15% increase in the body weight ratio of the brains in female and the gonads in males. Oral administration of daily doses of 5.0 and 50 mg/kg has adverse effects in rats.

Study:	26-Week Ocular Dose Study in the Rabbit
Study Number:	PTC/89/94
Study Date:	November 1994
Test Article:	Batch 92-001
Species/strain:	Rabbit/New Zealand
Weights:	Males 2.3-3.2kg Females 2.4 to 3.6kg
Number of animals:	10 per sex per dose

Route:	Topical in the right eye
Dose volume:	One drop from a "Droptainer," approximately 30 μl
Concentration Evaluated:	Vehicle, and 0.5% suspension of LE
Dosing Schedule	8 times a day for 7 days and then 4 times a day, 7 days a week, for an additional 25 weeks
Vehicle:	Clinical vehicle (NDA 20-583.002)
Method and frequency of test article formulation:	No data presented
Accuracy of test article formulation:	No data presented
Stability of test article formulation:	Data to show that formulation was stable for 19 months (NDA 20-583.002)
Proof of absorption:	Not determined in this study

Results:

Early in the study two female rabbits in the control group were killed and replaced. One was *in extremis* and one failed to eat. On day 9 one treated rabbit was killed and on day 15 one vehicle rabbit was killed. Both rabbits were removed from the study on day 9 for failure to eat (the vehicle control rabbit continued to be dosed through day 15 but failed to resume eating and was killed). Necropsies of both animals failed to reveal the reason for these animals to refuse to eat (ref NDA 20-583.002).

There were no meaningful differences in the rate of body weight gain or food consumption of treated rabbits when compared to controls, however, there were some differences in the ratio organ weight to body weight (table 4)

Table 4 Percentage difference of organ to body weight ratio in rabbit treated with ocular application of one drop of 0.5%(LE) qid for 26 weeks.

Gender	Adrenals	Lung	Kidneys	Ovaries
Male	-40	-20		
Female	-32		12	-29

Microscopic alterations in the these organs are not observed following histologic examination.

No treatment-related effects were observed for pupillary light reflex, intraocular pressure, hematology or blood chemistry. A low incidence in males and females of slight conjunctival erythema seen at 13 weeks was not observed at 26 weeks, a clear, watery conjunctival discharge was generally seen on a daily basis in most rabbits.

Conclusion:

LE 0.5% prepared in the clinical formulation and administered at the clinical dose for 25 of 26 weeks is well tolerated, however, involution of the adrenals was observed.

Study: 52 Week Ocular Toxicity Study in the Dog

Study Number: PTC/74/91

Study Date: April-1992

Test Article: The following Lot Numbers of test article were used in this study: 006-90, 007-90, 008-90, 009-90, 028-5-90, 026-90, 027-90, 028-90 and - 0.1% dexamethasone Lot No. 2NFAP

Species/strain: Dog/Beagle - 24 to 26 weeks of age

Weights: Males; 10.9 to 7.7 kg
Females, 7.5 to 10.0 kg

Number of animals: 4 per sex per dose

Route: Ocular in the right eye

Dose volume: Three drops which averaged about 0.03 ml or approximately 0.09 ml (ref. NDA 20-583.002)

Doses Evaluated: Vehicle, 0.05, 0.1, and 0.5% w/v LE suspension and 0.1% w/v dexanethasone ophthalmic suspension

Vehicle: Preservative: 0.01% benzalkonium chloride
Inactive: gelatin, hydroxypropyl methylcellulose, tetronic 1107, boric acid, sodium borate, edetate disodium, sodium chloride, purified water

Treatment Schedule:

Group No.	Treatment	Concentration %	Frequency
1	Vehicle control	0	b.i.d.*
2	Dexamethasone	0.1	b.i.d.
3	LE	0.05	Q.D.**
4	LE	0.1	b.i.d.

5	LE	0.5	b.i.d.

a. three drops of approximately 30 μl was applied at each dosing.
* twice-a-day
** once-a-day

Method and frequency of test article formulation: Test article was supplied twice in 5 ml "Droptainers" (supplied on 2/25/90 & 6/25/90)

Accuracy of test article formulation: Not determined

Stability of test article Formulation: No data presented in the pharmacology information to support the stability of the formulation.

Proof of absorption: Not determined

Results:

One female dog in the low dose LE(0.05%) died during the 48th day of treatment.

Both males and females in all treated groups weighed less than vehicle treated control dogs at the end of 52 weeks, however, this decrease was only significant for the dexamethasone males. With few exceptions all dogs ate all the food presented to them.

Ophthalmoscopy examination detected the corneal stromal deposits in both male and female beginning at week 26 for both dexamethasone and LE (fig. 1). As can be seen, the incidence of deposits increases with time and with dose and it is possible that females may be slightly more susceptible than males. Crystalline corneal opacities were observed at 52 weeks in both males and females treated with dexamethasone and high dose (0.5%) loteprednol and were considered related to stromal deposits.

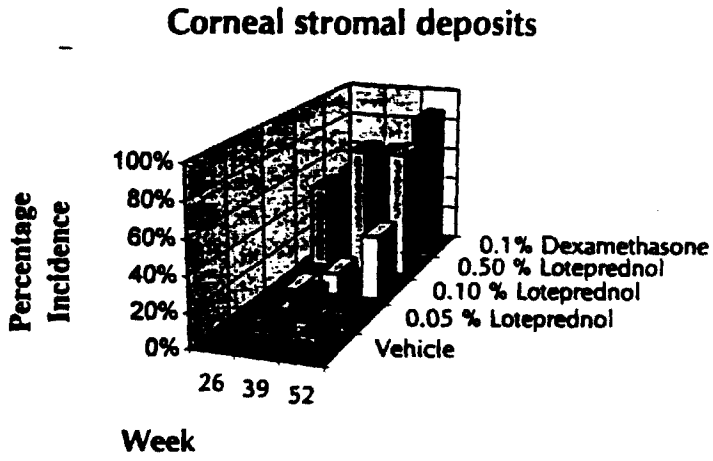

Figure 1 —The incidence of corneal stromal deposits in the right eye of male and female dog after 26,29 and 52 weeks of ocular administration.

As expected, prolonged administration (26 weeks or longer) with 1% (w/v)bid dexamethasone caused a time dependent elevation of intraocular pressure (IOP) of the treated eye (fig. 2). Likewise loteprednol caused an increase in IOP, but the time and dose-dependency was not clearly established.

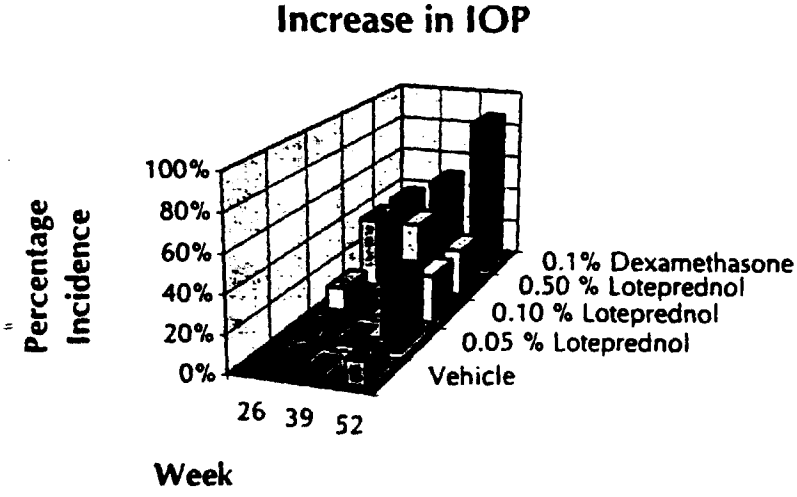

Figure 2 The incidence of male and female dogs with elevated intraocular pressure in the right after 26,29 and 52 weeks of ocular administration.

No biologically meaningful changes in hematology, blood chemistry or urinalysis were seen. As expected with a systemic body burden of dexamethasone the adrenal to body weight ratios were decreased for both males and females, however, there was no adrenal effects for any of

the animals treated with loteprednol. Also the liver to body weight ratios were increased in males receiving dexamethasone.

Histopathologic examination revealed adrenal cortex atrophy, lymphoid depletion and some involution of the thymus only in the dexamethasone treated groups.

Conclusion:

Ocular administration of LE to beagle dogs for 52 weeks caused a dose and time-dependent increase incidence of stromal deposits in the corneas of both genders. Elevation of IOP was also observed following loteprednol administration at 52 weeks for all doses evaluated, however, no clear dose dependency was established.

The positive control, 0.1% dexamethasone, caused both an increase in cornea stromal deposits and IOP which increased over time. Dexamethasone also caused other signs glucocorticoid toxicity such as, reduction in body weight gain, involution of the thymus, and decreased adrenal weights and microscopic changes typical of corticosteroids.

Reproductive Studies:

Study:	Rat General Reproductive Performance Dose Ranging Study
Study Number:	PTC/48/89
Study Date:	October 1989
Test Article:	Batches 125, 137 and 156
Species/strain:	Rat/OFA-SD(IOPS-Caw) Sprague-Dawley
Weights:	Males 300 to 319 g Females 180 to 199 g
Number of animals:	6 sex per dose
Route:	Oral
Dose volume:	10 ml/kg
Concentration Evaluated:	Vehicle, 0.5, 5.0, 50.0, and 100.0 $mgkg^{-1}day^{-1}$

Dosing Schedule:	Males: Daily for 2 weeks before mating, through the mating period and for a further 2 weeks. Females: Daily for 2 weeks prior to mating and through the mating, pregnancy and lactation periods until necropsy on day 21 post partum.
Vehicle:	1% w/v methyl cellulose
Method and frequency of test article formulation:	A suspension was prepared daily by weighing the test article and mixing with a small quantity of vehicle to form a smooth paste using a pestle and mortar. More vehicle was added slowly, while continuously mixing to make up to final volume. Concentrations for the two lower doses were prepared by dilution with vehicle.
Accuracy of test article formulation:	The formulation was sampled 4 times during the study and on three occasions the range of found concentrations as a percentage of theoretical was , however, on one occasion the range was (the failure to assay at theoretical values was particularly a problem at concentration that were used to treat the 0.05, 0.5, and 5.0 mg/kg doses). Failure to adequately resuspend the formulation is the proposed reason for this deviation.

Procedures and Observations for F0 Generation:

Mating:	At the end of the two week pre-mating period each male was paired with a female from the same dose group. Vaginal smears were taken daily until sperm was found in the smear; the stage of estrus cycle or presence of sperm was recorded. Pairing continued up to seven days.
Proof of absorption:	Blood sample were taken from all control and 5.0 mg/kg female at necropsy, 1 hr after administration of the last dose. Samples were taken by cardiac puncture into lithium heparin anticoagulant and the plasma was frozen to -20°C No LE or metabolite was detected (limit of detection was 0.5 μg/ml).

Results: There were no deaths of either males or females during the pre-mating, mating and pregnancy (females only) period. Three of 6 and 5 of 5 females were killed in the 50 and 100 mg/kg treatment groups, respectively, because they either did not deliver pups or the litter was dead (one female in the 100 mg/kg group).

The dams that delivered in the two highest doses treatment groups delivered their litter on average 1 day later than control dams. The body weight of pups delivered from females treated with 0.5, 50 or 100 mg/kg were statistically significantly lower than control or 0.05 mg/kg