

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-070

PHARMACOLOGY REVIEW(S)

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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:	22-070
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	09/27/06
PRODUCT:	Tretinoin Gel, 0.05%
INTENDED CLINICAL POPULATION:	patients with acne vulgaris
SPONSOR:	Coria Laboratories, Ltd.
DOCUMENTS REVIEWED:	electronic document, module 4
REVIEW DIVISION:	Division of Dermatology and Dental Products
	(HFD-540)
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TABLE OF CONTENTS

EXECUTIVE SUMMARY 3

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW 4

2.6.1 INTRODUCTION AND DRUG HISTORY 4

2.6.2 PHARMACOLOGY 7

 2.6.2.1 Brief summary 7

 2.6.2.2 Primary pharmacodynamics 7

 2.6.2.3 Secondary pharmacodynamics 7

 2.6.2.4 Safety pharmacology 7

 2.6.2.5 Pharmacodynamic drug interactions 8

2.6.3 PHARMACOLOGY TABULATED SUMMARY 8

2.6.4 PHARMACOKINETICS/TOXICOKINETICS 8

 2.6.4.1 Brief summary 8

 2.6.4.2 Methods of Analysis 8

 2.6.4.3 Absorption 8

 2.6.4.4 Distribution 9

 2.6.4.5 Metabolism 10

 2.6.4.6 Excretion 10

 2.6.4.7 Pharmacokinetic drug interactions 10

 2.6.4.8 Other Pharmacokinetic Studies 10

 2.6.4.9 Discussion and Conclusions 10

 2.6.4.10 Tables and figures to include comparative TK summary 10

2.6.5 PHARMACOKINETICS TABULATED SUMMARY 11

2.6.6 TOXICOLOGY 11

 2.6.6.1 Overall toxicology summary 11

 2.6.6.2 Single-dose toxicity 14

 2.6.6.3 Repeat-dose toxicity 14

 2.6.6.4 Genetic toxicology 14

 2.6.6.5 Carcinogenicity 18

 2.6.6.6 Reproductive and developmental toxicology 19

 2.6.6.7 Local tolerance 19

 2.6.6.8 Special toxicology studies 19

 2.6.6.9 Discussion and Conclusions 20

 2.6.6.10 Tables and Figures 21

2.6.7 TOXICOLOGY TABULATED SUMMARY 21

OVERALL CONCLUSIONS AND RECOMMENDATIONS 21

APPENDIX/ATTACHMENTS 24

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability – Tretinoin gel (0.05%) for the treatment of acne vulgaris is approvable from a pharmacological/toxicological perspective

B. Recommendation for nonclinical studies

As discussed during the pre-NDA meeting (June 1, 2006) the sponsor intends to conduct a ——— dermal dose range-finding study in mice with appropriate toxicokinetics to support a dermal mouse carcinogenicity study with Tretinoin Gel, 0.05% as a post-marketing commitment

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C. Recommendations on labeling – A number of changes were recommended to the sponsor's proposed labeling. These changes are detailed in the Overall Conclusions and Recommendations.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

This NDA is submitted under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act, relying on the Agency's finding of safety and effectiveness of the previously approved product, Retin-A Micro (NDA 20-475, Johnson & Johnson), and including nonclinical and clinical studies to support the use of a different pharmaceutical form. As such the nonclinical studies were not designed primarily to address the active substance per se, but rather the issues of local toxicity, reproductive toxicity and potential for systemic exposure to the active and its metabolites.

B. Pharmacologic activity – Tretinoin is a retinoid.

C. Nonclinical safety issues relevant to clinical use – Tretinoin, like other retinoids, is teratogenic and embryotoxic in multiple species when administered at sufficient doses and at the vulnerable gestational time period. Doses of tretinoin that do not cause morphological changes in offspring may cause behavioral effects in the developing animals. Topical application of tretinoin appears to be less likely to result in teratogenic or other effects probably due to lower systemic and embryo exposure to tretinoin by the topical route than by the oral route.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-070

Review number: 1

Sequence number/date/type of submission: 0000/09-27-06/original NDA submission

Information to sponsor: Yes (x)

Sponsor and/or agent: Coria Laboratories, Ltd

Manufacturer for drug substance: _____

Reviewer name: Jill C Merrill

Division name: Division of Dermatology and Dental Products

HFD #: 540

Drug:

Trade name: Atralin

Generic name: tretinoin, all-*trans* retinoic acid

Code name: _____

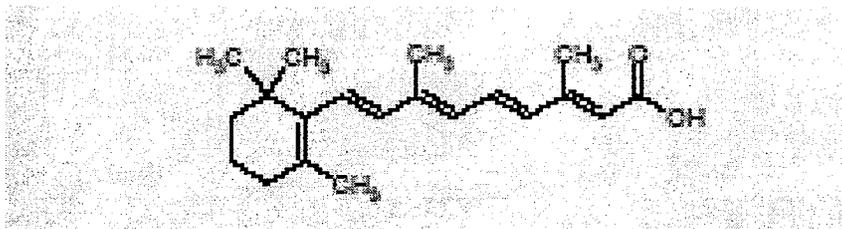
Chemical name:

3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid

CAS registry number: 302-79-4

Molecular formula/molecular weight: C₂₀H₂₈O₂ / MW=300.44

Structure:



Relevant INDs/NDAs/DMFs:

IND 63067, Tretinoin Gel, (0.05%, w/w), Healthpoint, Ltd

DMF _____

DMF _____

NDA 20-475, Retin-A Micro (Johnson & Johnson)

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Drug class: retinoid

Intended clinical population: patients with acne vulgaris

Clinical formulation:

Component	% (w/w)
Tretinoin, USP	0.05
_____ collagen, from Pancogene® Marin	_____
Sodium hyaluronate-LP	_____
Octoxynol-9	_____
Butylated hydroxytoluene, NF	_____
Methylparaben, NF	_____
Propylparaben, NF	_____
Benzyl alcohol, NF	_____
Carbopol _____ NF	_____
Trolamine, NF	_____
Glycerin, USP	_____
Purified water, USP	_____

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Three ingredients are noncompendial: Pancogene® Marin (which contains _____ collagen derived from teleost fish skin), sodium hyaluronate (which the sponsor stated was a _____ at the pre-IND meeting), and octoxynol-9. The first two are claimed to act as _____, and the third is listed as a _____. The formulation for Pancogene® Marin is not provided. Octoxynol-9 is listed in the National Formulary, but the sponsor previously stated the NF grade material is not commercially available. Sodium hyaluronate is present in the approved drug product Solaraze gel at _____

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Route of administration: topical

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-070 are owned by Coria Laboratories, Ltd. or are data for which Coria Laboratories, Ltd. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-070 that Coria Laboratories, Ltd. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Coria Laboratories, Ltd. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-070.

Studies reviewed within this submission:

Tretinoin USP bacterial mutation test (Report # 9320-003-004)

Tretinoin USP chromosome aberration test (Report # 9320-003-005)

Tretinoin USP rat micronucleus test (Report # 9320-003-006)

Tolerance tests in rabbits: primary irritation index; ocular irritation (Tox 75306)

Studies not reviewed within this submission:

Test to evaluate the acute toxicity following a single oral administration (limit test) in the rat (Report # 44994) The test article was Pancogene® Marin and the study was previously submitted to IND 63,067 and reviewed by Dr. Amy Nostrandt (review #1).

A primary skin irritation study in rabbits with ~~_____~~TM (tretinoin gel 0.05%) (Report # 3551.4). This study was previously submitted to IND 63,067 and reviewed by Dr. Amy Nostrandt (review #1). **b(4)**

A dermal sensitization in guinea pigs (maximization design) (Report # 3551.5). This study was previously submitted to IND 63,067 and reviewed by Dr. Amy Nostrandt (review #1).

Test to evaluate ocular irritation in the rabbit (Report # 44894). The test article was Pancogene® Marin and the study was previously submitted to IND 63,067 and reviewed by Dr. Amy Nostrandt (review #1).

A dermal tolerance study of ~~_____~~TM gel (tretinoin, 0.05%) in female Sprague Dawley rats (Report # 3551.10). This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3). **b(4)**

A dermal segment I/II combination study of ~~_____~~^M (tretinoin gel 0.05%) in female Sprague-Dawley rats (Report # 3551.11). This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3). **b(4)**

A 3-month dermal toxicity study of ~~_____~~^M (tretinoin gel 0.05%) in Hanford minipigs (Report # 3551.6). This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3). **b(4)**

Octoxynol-9: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (Report # 2192/13). This study was previously submitted to IND 63,067 and reviewed by Dr. Paul Brown (review #5).

Octoxynol-9: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes (Report # 2192/14). This study was previously submitted to IND 63,067 and reviewed by Dr. Paul Brown (review #5).

Octoxynol-9: Induction of micronuclei in the bone marrow of treated mice (Report # 2192/15). This study was previously submitted to IND 63,067 and reviewed by Dr. Paul Brown (review #5)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

All-*trans*-retinoic acid (tretinoin; vitamin A acid) is a naturally occurring metabolite of vitamin A. When administered by the oral route all-*trans*-retinoic acid can replace vitamin A with respect to normal growth and development. However, it differs significantly from vitamin A in certain major biological characteristics; it is not absorbed through the lymphatic system but by the portal route; it is not stored in the liver but is rapidly excreted as a glucuronide conjugate in the bile; and it does not support retinal (vitamin A aldehyde) pigment synthesis (Zbinden, 1975). The major interest in all-*trans*-retinoic acid in dermatology stems from its important actions in the skin when applied topically as opposed to systemically. It is of particular interest in the topical treatment of acne vulgaris because of its significant effects on epidermal differentiation, keratin metabolism, repair and inflammatory processes, and its comedolytic activity (Cahn et al., 1975).

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Although the exact mechanism by which retinoids are beneficial in acne is unknown, current evidence suggests the therapeutic effect of tretinoin relates to its ability to modify abnormal follicular keratinization. Comedones form in follicles with an excess of keratinized epithelial cells. Tretinoin promotes detachment of cornified cells and the enhanced shedding of corneocytes from the follicle. By increasing the mitotic activity of the follicular epithelia, tretinoin also increases the turnover rate of thin, loosely-adherent corneocytes. Through these actions, the comedo contents are extruded and the formation of the microcomedo, the precursor lesion of acne vulgaris, is reduced.

Drug activity related to proposed indication: Topical application of all-*trans*-retinoic acid increases epidermal mitotic activity, reduces the formation of keratins, induces cell differentiation and tissue repair, and is comedolytic in both laboratory animals and man.

2.6.2.3 Secondary pharmacodynamics

Electron microscopic evaluations of human skin treated with all-*trans*-retinoic acid have shown a reduction in the keratin precursors, notably the tonofilaments, a reduction in the numbers and size of the desmosomes and an increase in the number of keratinosomes.

2.6.2.4 Safety pharmacology

No specific safety pharmacology studies have been undertaken with Tretinoin Gel, 0.05%. Topical tretinoin has been in extensive clinical usage for many years and no toxicologically important safety pharmacology issues have been identified. The absence of specific safety pharmacology data for this formulation is considered to be fully justified since preclinical and clinical pharmacokinetic exposure at large multiples of the therapeutic dose does not result in alteration of circulating levels of endogenous tretinoin

or its metabolites. It should be noted that in the dermal toxicity study conducted with minipigs (study # 3551.6) ECG examinations were performed for all animals prior to dosing and on days 44 and 89. Lead II traces were evaluated. All minipigs remained in sinus rhythm throughout the study and ECG's were within normal limits on all sampling occasions.

2.6.2.5 Pharmacodynamic drug interactions

No pharmacodynamic drug interactions with tretinoin have been identified from review of the published literature. Preclinical and clinical pharmacokinetic studies with Tretinoin Gel, 0.05%, at large multiples of the anticipated maximum human dose do not result in alteration to circulating levels of endogenous tretinoin or its metabolites (study 3551.6). In view of the extremely poor absorption of topically applied tretinoin, pharmacodynamic drug interactions are unlikely.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

This section is not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No specific pharmacokinetic studies in laboratory animals have been undertaken with Tretinoin Gel, 0.05%, but the database includes toxicokinetic studies at large multiples of the anticipated maximum human dose in both the rat (study number 3551.10, submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3) and the minipig (study number 3551.6, submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3).

2.6.4.2 Methods of Analysis

Not applicable.

2.6.4.3 Absorption

Single topical applications of ^3H all-*trans*-retinoic acid to the rat have been reported by Brode *et al.*. The plateau of percutaneous absorption occurred between 10 and 24 hours. The quantity of absorbed all-*trans*-retinoic acid was shown to be dependent upon the surface area of treatment, the concentration and quantity of all-*trans*-retinoic acid applied and the formulation tested. After 24 hours, approximately 5% to 6% of the applied dose had been absorbed.

In humans, the percutaneous absorption of ^{14}C all-*trans*-retinoic acid was measured in 8 male subjects with mild to moderate facial acne (Franz and Lehman, 1989). For comparative purposes, application was also made to the normal leg skin of two volunteers. Facial skin was found to be an order of magnitude more permeable than

normal leg skin (1.1% versus 0.15% of the applied dose). Although repeated application induced clinical signs of irritation, it did not significantly increase percutaneous absorption (1.5% versus 1.1% of the applied dose).

Blood concentrations of all-*trans*-retinoic acid, following a single topical application in humans have been reported (Lucek and Colburn) to be essentially zero from 2 to 48 hours after application. The urinary excretion was on average 1.82% of the applied dose; however, this was increased to 4.5% in patients pre-treated for 7 days with cutaneously applied all-*trans*-retinoic acid.

The percutaneous absorption of tretinoin following repeated topical administration in humans has been studied by Buchan *et al.* Four healthy volunteers applied 2 g of a commercially available 0.025% tretinoin gel (Aberel®) to the face, neck, and upper part of the chest, each evening between 20:00 and 21:00 h for 14 days. The application site was not washed until the next morning. Blood samples were collected 7 and 5 days before the beginning of treatment, then at days 0 (the first day of application), 6, 13, and 16 (2 days post-treatment), at different collection times. Topical administration of tretinoin (all-*trans*-retinoic acid) did not significantly increase the plasma levels of all-*trans*-RA, 13-*cis*-RA, and 4-oxo-13-*cis*-RA. No quantifiable levels of 4-oxo-all-*trans*-RA were detected in any samples analyzed. However, significant decreases in the level of these retinoic acids were observed during the night, before, during and after the treatment period. This may reflect diurnal variations of retinoid metabolism or lowered absorption of dietary vitamin A. The authors concluded that diurnal and nutritional factors influence plasma levels of endogenous retinoids to a greater extent than topical administration of tretinoin at doses used for acne therapy.

2.6.4.4 Distribution

All-*trans*-retinoic acid administered orally to pregnant animals has been shown to be teratogenic and in addition, as reported in the hamster (Sharma *et al.*, 1993) and mouse (Kochhar DM, 1976), to cross the placenta. A published study in humans (Jick *et al.*, Lancet, 1993) suggests that exposure of mothers to topical all-*trans*-retinoic acid during the first trimester of pregnancy was not associated with an increased risk for major congenital disorders. This has been challenged (Caron, Lancet, 1993) and there have been reports (Camera and Pregliasco, Lancet, 1992) associating human teratogenic effects (ear malformation) with the topical use of all-*trans*-retinoic acid. It has been suggested that the irritation associated with topical application of all-*trans*-retinoic acid preparations in human skin limits the amount of retinoid that can be applied and subsequently reach the systemic circulation and consequently the developing embryo. However, safe and effective contraception is typically recommended during the use of orally active retinoids (Ceyrac *et al.*, 1992).

2.6.4.5 Metabolism

Intravenous administration in the rat of three dose levels of all-*trans*-retinoic acid, namely 0.15, 0.25, and 5 mg/kg had three distinct phases of elimination from the plasma (Lucek and Colburn). The initial rapid decline in plasma concentrations appeared to be dose dependent in the slower secondary phase. The half-life values were 40, 60 and 120 minutes respectively. The metabolites of all-*trans*-retinoic acid were more slowly eliminated with peak plasma concentrations occurring 3 to 4 hours, respectively, after administration.

2.6.4.6 Excretion

Oral administration of all-*trans*-retinoic acid in a phase 1 evaluation of pediatric patients with cancer has been reported (Smith *et al*, 1992). Eighteen patients were studied on day 1 of therapy, and seven of these were again studied on day 28. The time to peak plasma concentration was variable, and occurred from 1 to 4 hours after dosing. Peak concentration and AUC did not seem to increase in proportion to dose with a greater than three fold increase in AUC being observed after only a 30% increase in dose (30 to 40 mg/m²). For patients studied on two occasions, the AUC on day 1 was significantly greater than the AUC on day 28 (1.49 ± 0.70 versus $0.3 \pm 0.5 \mu\text{mol}^{-1} \text{L}^{-1}\text{h}^{-1}$; ($p < 0.01$) possibly due to an upregulation of catabolic enzyme pathways, such as the cytochrome P450 system or a down-regulation in gastrointestinal absorption.

2.6.4.7 Pharmacokinetic drug interactions

No pharmacokinetic drug interactions with tretinoin have been identified from review of the published literature. In view of the lack of absorption of topically applied tretinoin, pharmacokinetic drug interactions are unlikely.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable.

2.6.4.9 Discussion and Conclusions

No specific pharmacokinetic studies in laboratory animals have been conducted with Tretinoin Gel, 0.05%, but the database includes toxicokinetic studies at large multiples of the anticipated maximum human dose in both the rat (3551.10, IND 63,067, review #3, Dr. Dave Allen) and the minipig (3551.6, IND 63,067, review #3, Dr. Dave Allen). In both species, plasma levels of tretinoin were either near or below the level of detection for the assay method used.

2.6.4.10 Tables and figures to include comparative TK summary

This section is not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

This section is not applicable.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Single-dose toxicity:

Tretinoin

The results of several single dose toxicity studies of all-*trans*-retinoic acid in the rat and mouse have been published in the scientific literature. Although there are no major marked species differences with respect to acute toxicity of tretinoin, toxicity is dependent on the route of administration (Kamm, 1982) with LD₅₀ values in mice of about 2200 mg/kg for oral and 790 mg/kg for intraperitoneal administration. In rats the LD₅₀ values were reported as 2000 mg/kg and 790 mg/kg for oral and intraperitoneal administration of tretinoin, respectively. The major toxicological symptoms found were respiratory depression, alopecia, reduced feed intake, ataxia, diarrhea and slight anemia. All of these symptoms were reversible.

Pancogene® Marin

The manufacturer of the excipient Pancogene® Marin, a soluble collagen derived from fish skin and used in Tretinoin Gel at — % (w/w), conducted an acute toxicity test in rats. This study was previously submitted to IND 63,067 and reviewed by Dr. Amy Nostrandt (review #1). The data indicate that the acute oral LD₅₀ of Pancogene® Marin in rats is >2006 mg/kg.

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Repeat-dose toxicity

A 13-week topical toxicity study was conducted in Hanford minipigs with Tretinoin Gel, 0.05%. This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review # 3). Groups of four males and four females received nominal tretinoin dose levels of 0.015, 0.045, or 0.075 mg/kg/day. Heparinized blood samples for toxicokinetic analyses were collected on day 85 (males) and day 86 (females) at 0.5, 1, 2, 4, 8, and 24 hours post-dosing. Plasma samples were analyzed by LC-MS-MS-equipped with an HPLC column for all-*trans*-retinoic acid (LLOQ = 1.00 ng/mL). The results indicate that the plasma levels of all-*trans*-retinoic acid were relatively low and not appreciably different between the treatment groups or sexes. Mild dermal irritation was observed in tretinoin-treated animals. Due to the absence of significant toxicity in any of the dosed groups, the high-dose of 0.15 mL/kg/day of Tretinoin Gel, 0.05% (i.e., 0.075 mg/kg/day tretinoin) was considered to be the NOAEL.

This 13-week test was on the drug product and therefore includes the noncompensial excipients.

Genetic toxicology:

Tretinoin

Published literature studies suggest that tretinoin is not mutagenic in the Ames test (Kamm, 1982). In some cases these studies would not meet current ICH recommendations for how such studies should be conducted (Guidance for Industry S2B Genotoxicity: A standard battery for genotoxicity testing of pharmaceuticals, July 1997). As such the sponsor has conducted the following tests with Tretinoin USP: an Ames test, a human lymphocyte chromosome aberration assay and a rat micronucleus test by subcutaneous injection. These studies are reviewed in the appropriate section below. No evidence of genotoxicity was observed in any of these studies.

Excipients

Octoxynol-9, an excipient essential to the formulation of Tretinoin Gel, is included in the formulation at a concentration of —% and has been evaluated in a series of three GLP-compliant genotoxicity tests: an Ames test, a chromosome aberration assay in human peripheral lymphocytes and a mouse micronucleus test. These tests were designed to meet current ICH guidelines and have been previously submitted to IND 63,067 and reviewed by Dr. Paul Brown (review #5). No genotoxic activity was seen in any of these assays.

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As a protein the collagen, Pancogene® Marin, is considered an unlikely genotoxic agent and as such has not been specifically tested for genotoxicity.

Carcinogenicity: In testing for other tretinoin containing products, carcinogenicity test results have been negative. However, tretinoin has repeatedly been shown to enhance photo co-carcinogenicity in studies of concurrent administration of tretinoin with ultraviolet radiation exposure. In the December 2, 2004 guidance meeting the sponsor was advised that to help characterize the octoxynol-9, the other noncompensial excipients and the possible unique effects this formulation may have on the skin, it is recommended that a standard 2-year carcinogenicity bioassay of the clinical formulation be conducted using the topical route of exposure. As there is some existing data on tretinoin carcinogenicity and no particular elevated cause for concern from the excipients, it was agreed at the pre-NDA meeting (June 1, 2006) that such a study can reasonably be conducted as a phase 4 commitment.

Reproductive toxicology:

All-*trans*-retinoic acid administered orally to pregnant mice, rats, hamsters, rabbits, and monkeys has been found to have potent effects, either direct or indirect, on reproductive function, particularly with respect to teratogenicity (Kochhar and Christian, 1997, Holson *et al.*, 2001, Christian *et al.*, 1997, Seegmiller *et al.*, 1997). Although Kochhar and Christian (1997) conclude that topically applied all-*trans*-retinoic acid does not appear to

induce developmental malformations in laboratory animals, Zbinden (1975) noted a slight increase in incomplete ossification of some skull bones in rats treated with the high concentration. However, 'this was not considered to be an indication of a teratogenic effect.' It is noteworthy that reduced ossification of skull (frontal and parietal) was observed in some treated rats in the topical Segment I/II study conducted by the sponsor (see further comments below for study # 3551.11).

The sponsor conducted a dermal tolerance study of Tretinoin Gel, 0.05% as a dose-range finding study for a Segment I/II combination study (# 3551.10). This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3). With the exception of mild dermal irritation, there were no retinoid-associated findings with tretinoin exposure at the only dose level employed (0.5 mg/kg/day). Thus the NOAEL in the rat (0.5 mg/kg/day tretinoin) when corrected for body surface area ($0.5 \text{ mg/kg} \times 6/37 = 81.08 \text{ } \mu\text{g/kg}$) is approximately 4 times the anticipated maximum human dose (2 g Tretinoin Gel, 0.05% for a 50 kg person = $(2 \text{ g} \times .05/100) \div 50 \text{ kg} = 20 \text{ } \mu\text{g/kg}$). However, as mentioned by Dr. Amy Nostrandt who reviewed the preclinical protocol (IND 63,067, review # 1), rats were only treated for 6 hours daily, whereas clinical exposure may be longer.

The sponsor then conducted a dermal Segment I/II combination study of Tretinoin Gel (0.05%) in female Sprague-Dawley rats (# 3551.11). This study was previously submitted to IND 63,067 and reviewed by Dr. Dave Allen (review #3). Apart from reduction in maternal gestational body weights and dermal irritation, no **statistically significant** retinoid-associated findings have been reported in this study. Therefore the NOAEL for maternal toxicity was determined to be 0.15 mg/kg/day. Based on the absence of statistically significant fertility indices and embryo-fetal findings, the NOAEL for reproductive and developmental toxicity was determined to be 0.50 mg/kg/day. However, there were low levels (not statistically significantly different from placebo) of effects commonly associated with retinoids: craniofacial abnormalities (hydrocephaly), asymmetrical thyroids, reduced ossification of skull, unossified sternbrae, and increased incidences of 7th cervical, and 14th rudimentary ribs. Despite the observation that these effects were within the range of historical controls, it is noteworthy that they occurred only in the treated groups and not in the placebo control group (with the exception of 4 incidences of 14th rudimentary ribs in the placebo control group).

Special toxicology:

The potential irritant effects of Tretinoin Gel, 0.05% were assessed using both intact and abraded skin in New Zealand White rabbits. The Primary Irritation Index was calculated to be 3.08 (non-irritating). This formulation was also tested for sensitizing potential in the guinea pig using the Maximization test and was found not to induce delayed contact hypersensitivity.

Pancogene® Marin

The manufacturer of the excipient Pancogene® Marin, a soluble collagen derived from teleost fish skin and used in Tretinoin Gel at $\text{---} \% \text{ (w/w)}$, conducted a test to evaluate ocular irritation in rabbits. This study was previously submitted to IND 63,067 and

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reviewed by Dr. Amy Nostrandt (review #1). The data indicate that Pancogene® Marin is a slight eye irritant in rabbits.

The proposed drug product appears to have some potential for skin irritation and is therefore likely to have some potential for eye irritation as well. Labeling should instruct patients to keep Tretinon Gel, 0.05% away from the eye during administration. It was not a contact sensitizer in the guinea pig.

2.6.6.2 Single-dose toxicity

No new single-dose toxicity studies were included in this NDA submission.

2.6.6.3 Repeat-dose toxicity

No new repeat-dose toxicity studies were included in this NDA submission.

2.6.6.4 Genetic toxicology

Study title: Tretinoin USP bacterial mutation test

Key findings: Tretinoin did not cause an increase in revertant colony counts with any strain of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100) or *E. coli* (WP2 *trp uvrA*) tested in the absence or presence of S9 mix.

Sponsor Study no.: 9320-003-004

Volume #, and page #: electronic document, Module 4 Section 960805

Conducting laboratory and location: _____

Date of study initiation: 08-30-05

GLP compliance: yes

QA reports: yes (x)

Drug, lot #, and % purity: tretinoin USP, lot # 50346316K0, purity 100.1%

Methods

Strains/species/cell line: *Salmonella typhimurium* TA 1535, TA1537, TA98, TA100, *E. coli* WP2 *trp uvrA*

Metabolic activation system: S9 fraction from livers of phenobarbital/5,6-benzoflavone induced male Sprague-Dawley rats. The supplier (_____) confirmed the S9's selected cytochrome P-450 enzyme activities and ability to convert known promutagens to bacterial mutagens. The S9 mix was prepared on the day of use and contained 10% v/v S9 fraction.

Doses used in definitive study: 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate in all strains (with and without S9)

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Basis of dose selection: initial toxicity-mutation assay at dose levels of 50, 158, 500, 1581, 5000 µg/plate in all strains. Toxicity to background lawn was assessed and revertant colonies counted.

Negative controls: DMSO

Positive controls:

Strain	S9 activation	Positive Control	Concentration (µg/plate)
TA1535	rat	2-aminoanthracene	5
<i>E. coli</i>			15
TA1537, TA98, TA100		benzo[a]pyrene	5
TA1535, TA100	none	sodium azide	0.5
TA1537		9-aminoacridine	50
TA98		2-nitrofluorene	1
<i>E. coli</i>		4-nitroquinoline N-oxide	1.5

Incubation and sampling times: In the rangefinder study the bacteria were mixed with the test article, with and without S9, and molten agar (standard plate incorporation assay). These plates were then incubated up to 72 hours and then counted. In the definitive assay the test article, bacteria and S9 mix were preincubated for 30 minutes at 37°C before mixing with the agar and plating, followed by counting (pre-incubation method). Each incubation condition was conducted in triplicate with all 5 strains.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was valid since the mean revertant colony counts for the vehicle controls were close to or within the historical control range and appropriate positive control compounds induced increases in revertant colonies to at least twice the concurrent vehicle control levels with appropriate bacterial strain (1.5 x for strain TA100) confirming sensitivity of the test system and the activity of the S9 mix.

Study outcome: No substantial increases in revertant colony numbers were obtained with any tester strain following exposure to Tretinoin USP at any dose level in either the presence or absence of S9 mix. Some reductions in revertant colony counts were obtained at precipitating dose levels indicating that the test article was toxic towards some bacterial strains. Precipitation of the test article on plates was observed at the highest dose levels tested. Therefore it is concluded that Tretinoin USP did not show any evidence of genotoxicity in this *in vitro* mutagenicity assay.

Study title: Tretinoin USP Chromosome aberration test

Key findings: Cultures treated with Tretinoin USP did not show any statistically significant increases in the incidence of cells with aberrant metaphases at any levels.

Sponsor Study no.: 9320-003-005

Volume #, and page #: electronic document Module 4 Section 960799

Conducting laboratory and location: _____

b(4)

Date of study initiation: 08-17-05

GLP compliance: yes

QA reports: yes (x)

Drug, lot #, and % purity: tretinoin USP, lot # 50346316K0, purity 100.1%

Methods

Strains/species: human/peripheral lymphocytes from healthy non-smoking, male donors

Doses used in definitive study: 0.40, 0.80, 1.60, 3.20, 6.40, 12.8, 25.6, 50.0, 100, 200 $\mu\text{g}/\text{mL}$

Metabolic activation system: S9 fraction from livers of phenobarbital/5,6-benzoflavone induced male Sprague-Dawley rats. The supplier (_____) confirmed the S9's selected cytochrome P-450 enzyme activities and ability to convert known promutagens to bacterial mutagens.

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Negative controls: DMSO

Positive controls: mitomycin C (0.05, 0.10, 0.20 $\mu\text{g}/\text{mL}$) was used in the absence of S9 and cyclophosphamide (8.0, 12, 16 $\mu\text{g}/\text{mL}$) was used in the presence of S9

Incubation and sampling times: the non-activated system was treated for 4 and 21 hours and the activated system was treated for 4 hours. After the 4 hour treatment, appropriate cultures were centrifuged at 1000 rpm for 5 minutes and the discarded supernatant was replaced with fresh medium. The cultures were then returned to the incubator for an additional 17 hours of incubation before metaphase harvesting. For the 21-hour -treated cultures (i.e., cultures in the confirmatory phase) no replacement medium was necessary. Colcemid (final concentration 0.1 $\mu\text{g}/\text{mL}$) was added 2 hours before harvest to arrest cells in metaphase. Cells were fixed, spread on a slide and stained with 10% (v/v) Giemsa. The mitotic index was determined.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was considered valid since the proportion of cells with structural aberrations in vehicle control cultures was within the historical range and the positive control induced a significant increase in structural aberrations.

No. of replicates: duplicates

Study outcome: Tretinoin USP did not cause any statistically significant increases in the proportions of aberrant metaphases at any experimental point.

Study title: Tretinoin USP rat micronucleus test

Key findings: Tretinoin USP did not show any evidence of genotoxic activity in this *in vivo* test.

Sponsor Study no.: 9320-003-006

Volume #, and page #: electronic document, Module 4 Section 960800

Conducting laboratory and location: _____

Date of study initiation: 08-09-05

GLP compliance: yes

QA reports: yes (x)

Drug, lot #, and % purity: tretinoin USP, lot # 50346316K0, purity 100.1%

Methods

Strains/species: Sprague-Dawley rats (5/sex/group/timepoint)

Group	Material	Dosage (mg/kg)	Sampling Time (hours)	# of Animals per sex
1	Vehicle	-	24	5
		-	48	5
2	Tretinoin USP*	500	24	5
3	Tretinoin USP*	1000	24	5
4	Tretinoin USP*	2000	24	5
			48	5
5	CP**	20	24	3

*subcutaneous route of administration to mimic intended route of administration in humans (dermal) and to maximize absorption potential; volume of 20 mL/kg bodyweight

** oral; volume of 10 mL/kg

Doses used in definitive study: 500, 1000, or 2000 (mg/kg)

Basis of dose selection: range finder of 600 and 2000 (mg/kg) in 2 males and 2 females per dose, dosed sequentially and examined regularly for toxic signs and mortalities

b(4)

Negative controls: 1% (w/v) methylcellulose in DI water

Positive controls: cyclophosphamide (CP) in sterile water

Dosing and sampling times: single subcutaneous injection into the intra-scapular region. Rats were terminated at the time points specified by CO₂ inhalation followed by exsanguination. Bone marrow was collected from the femur. Smears were prepared, fixed in methanol and stained with acridine orange. Slides were examined by fluorescence microscopy using a blue excitation filter and a yellow barrier filter. Micronuclei were examined in a total of 2000 immature erythrocytes per animal. The proportion of immature erythrocytes was assessed by examination of a total of at least 1000 erythrocytes per animal.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was considered valid because the incidence of micronucleated immature erythrocytes in the vehicle control fell close to or within the historical vehicle control range and the positive control showed a statistically significant increase in the incidence of micronucleated immature erythrocytes compared with the vehicle control.

Study outcome: Animals treated with Tretinoin USP did not show any statistically significant increases in the incidence of micronucleated immature erythrocytes or any significant decrease in the proportion of immature erythrocytes. The positive control caused highly significant increases in the incidence of micronucleated immature erythrocytes, confirming the sensitivity of the system.

Treatment	Dose (mg/kg)	% IE/(IE+ME)	Incidence mie (M+F)	Incidence mme (M+F)
24 hour sampling time				
Vehicle control	-	47	1.3	0.0
Tretinoin USP	500	48	2.2	0.0
	1000	44	1.6	0.0
	2000	46	1.0	0.0
Cyclophosphamide	20	40	36.3	0.0
48 hour sampling time				
Vehicle control	-	48	1.3	0.0
Tretinoin USP	2000	50	1.2	0.0

%IE/(IE+ME) - Proportion of immature erythrocytes

Mie - number of micronucleated cells observed per 2000 immature erythrocytes examined

Mme - number of micronucleated mature erythrocytes observed

2.6.6.5 Carcinogenicity

No carcinogenicity studies were included in the NDA submission.

2.6.6.6 Reproductive and developmental toxicology

No new reproductive and developmental toxicology studies were included in the NDA submission.

2.6.6.7 Local tolerance

No new local tolerance studies were included in the NDA submission.

2.6.6.8 Special toxicology studies

No new special toxicology studies were included in the NDA submission; however, in the Pancogene Marin DMF () there is a study evaluating ocular irritation and skin tolerance in rabbits. The ocular irritancy test was conducted in New Zealand rabbits with approximately 0.1 mL of product applied to the left conjunctival sac of each rabbit, while the right eye served as a control. The results were read 24, 48, and 72 hours later and the sums of damage to the cornea, iris, and conjunctiva of each animal was provided as a general mean and classified according to the following scale:

b(4)

General Mean	Product Classification
0 to 10	Nonirritant
10 to 30	Somewhat Irritant
30 to 60	Irritant
60 to 110	Severely irritant

For the skin tolerance test, approximately 0.5 mL of the test substance was applied to two shaved areas of the skin (abraded and nonabraded; each approximately 2 cm²). The product was maintained in contact with the skin using a nonallergenic adhesive test patch. After 24 hours of contact, the test patches were removed and irritancy was read by evaluating the level of erythema and edema for both the abraded and nonabraded areas. This was repeated at 72 hours to evaluate the reversibility of the irritant effect. The level of total irritation (erythema and edema) was classified according to the following scale:

Total score	Classification
< 0.5	Nonirritant
0.5 - 2	Mildly irritant
2 - 5	Moderately irritant
5 - 8	Severely irritant

Under the conditions of this test, the test product was considered to be a nonirritant in both the eyes and skin of rabbits.

2.6.6.9 Discussion and Conclusions

A dosage level of 0.15 mL/kg/day of tretinoin Gel, 0.05% (i.e., 0.075 mg/kg/day tretinoin), the highest dose tested, was considered to be the NOAEL following 13 weeks of topical application in Hanford minipigs (IND 63,067, Review 3, Dr. Dave Allen). When tested in a combined Segment I/II study in female Sprague-Dawley rats (IND 63,067, Review 3, Dr. Dave Allen), maternal toxicity, observed through reduced mean gestational body weight (~ 5%, $p < 0.05$) was seen at the highest dose tested, 1000 mg/kg/day Tretinoin gel, 0.05% (i.e., 0.5 mg/kg/day tretinoin). The NOAEL for maternal toxicity was determined to be 0.15 mg/kg/day tretinoin. The absence of statistically significant fertility indices and embryo-fetal findings was used as a justification for setting the NOAEL for reproductive and developmental toxicity at 0.5 mg/kg/day tretinoin. However, tretinoin-associated effects on offspring, although not statistically significantly different from placebo control, were noted at low instances. These included craniofacial abnormalities (hydrocephaly), asymmetrical thyroids, reduced ossification of skull, unossified sternbrae, and increased incidences of 7th cervical, and 14th rudimentary ribs. Despite the observation that effects were within the range of historical controls, it is noteworthy that they occurred only in the treated groups and not in the placebo control group (with the exception of 4 incidences of 14th rudimentary ribs in the placebo control group).

As noted by the previous reviewer (IND 63,067, review #3, Dr. Dave Allen), rats have been demonstrated to be less susceptible to the teratogenic effects of retinoids than other species (e.g., rabbits), and thus the dose employed may have resulted in insufficient exposure to see a teratogenic effect in this particular species. It is also noteworthy that in this study the concentration of tretinoin was increased by increasing the volume of the test article, and subsequently increasing the surface area of the treatment site. However, because the increases in surface area were not directly proportional to the increases in test article volume (likely due to the 'maximum practical treatment area'), there may be slight variations in the actual amount of dose absorbed through the skin relative to the anticipated dose. Such subtle variations could account for the observed low-levels of retinoid-associated effects by pre-empting dose-related and statistically significant effects. As such, the previous reviewer, Dr. Dave Allen (IND 63,067, review #3), recommended that the labeling should reflect both the historical teratogenic effects associated with tretinoin, in addition to the adverse effects seen in treated groups and not in the placebo control of this study.

The proposed drug product appears to have some potential for skin irritation and is therefore likely to have some potential for eye irritation as well. Labeling should instruct patients to keep Tretinoin Gel, 0.05% away from the eye during administration. The formulation was also tested for sensitizing potential in the guinea pig maximization test and was found not to induce delayed contact hypersensitivity.

The formulation contains three noncompensial ingredients: Pancogene® Marin (contains collagen derived from teleost fish skin), sodium hyaluronate, and octoxynol-9. Of these, sodium hyaluronate is present in the approved drug product Solaraze gel at —

b(4)

Octoxynol-9 was tested in the standard battery of tests for genetic toxicology (IND 63,067, review #5) and found to not have genotoxic potential. Although not tested for genetic toxicity, Pancogene® Marin is a protein and therefore unlikely to be genotoxic. However, the drug product contains fish-derived collagen and individuals with a sensitivity to the proteins present in teleost fish (a large group of fishes with bony skeletons, including most common fishes; distinct from cartilaginous fishes, such as sharks, rays and skates), may be susceptible to sensitization reactions following the use of this product. It is recommended that this possibility be handled by stating in the label that the product contains fish collagen and that sensitized individuals should avoid use.

2.6.6.10 Tables and Figures

Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Tretinoin Gel, 0.05%, is approvable from a pharmacological/toxicological perspective. However, in addition to the historical teratogenic effects associated with tretinoin, the label should reflect the adverse effects seen in treated animals versus the placebo controls.

Unresolved toxicology issues (if any): During the pre-NDA meeting (June 1, 2006), the sponsor agreed to conduct a $\frac{1}{10}$ dermal dose range-finding study in mice and a subsequent dermal mouse carcinogenicity study as a post-marketing commitment to provide data on long term dermal exposure of Tretinoin Gel, 0.05%.

Recommendations: The sponsor-proposed labeling has been revised and the recommended nonclinical portions are provided below.

b(4)

Comments to be conveyed to the sponsor:

Please submit a timeline for the mouse dermal carcinogenicity study which was agreed to be conducted as a phase 4 study commitment. This should include dates of submission for the $\frac{1}{10}$ dose range-finding study report, study protocol submission, study start date and date of final report submission.

2 Page(s) Withheld

 Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

APPENDIX/ATTACHMENTS**REFERENCES**

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/s/

Jill Merrill
6/28/2007 10:56:26 AM
PHARMACOLOGIST

revised as per your comments

Paul Brown
6/28/2007 11:31:55 AM
PHARMACOLOGIST