CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-400

BIOEQUIVALENCE REVIEW(S)

APR 18 1994

NDA:	20,400 and 20,404
SUBMISSION DATE:	OCT. 28, 1993
PRODUCT:	Acticin Cream and Gel.
SPONSOR:	Penederm 320 Lakeside Drive, Suite A Foster City, CA 94404
TYPE OF SUBMISSION:	NDA amendment
REVIEWER:	HE SUN, Ph.D.

BIOPHARMACEUTICS REVIEW

NDA 20,400 and 20,404

1. BACKGROUND

Tretinoin is a metabolite formed from all-trans-retinol, vitamin A, via conversion to all-trans-retinaldehyde. The sponsor developed two topical formulations, cream and gel, for acne treatment. The original NDAs (20,400 and 20,404) were submitted to the Agency on Oct. 24, 1993. This amendment includes two Research Protocols of two ongoing studies (as of Oct. 28, 1994) for tretinoin gel formulation. As learned from the sponsor, both studies were completed and study reports are awaiting to be submitted to the Agency.

The original NDAs (20,400 and 20,404) were refused to be filed by the Agency (RTF) after initial review. The RTF letters were sent to the sponsor on Nov. 23, 1993.

2. RECOMMENDATION

One of these two studies, entitled "A 91-day dermal toxicity study in mice with PDT 004-006 and PDT 004-002" was designed as a in-life phase of a 91 day dermal toxicity study in mice exposed to daily doses of Acticin Gel 0.025% and vehicle. The study has been completed before December 15, 1993.

The other study entitled "A single center, double-blind, parallel study to determine the effect of multiple applications of tretinoin-containing formulations on plasma levels of tretinoin in normal volunteers" was scheduled to be completed on October 31, 1993.

In the light of this information, no review of these protocols is necessary. Please convey the Recommendation, as appropriate, and the following comments #1-4 to the sponsor.

3. COMMENTS:

1. The sponsor mentions that up to 18 subjects would be enrolled in the volunteer study. It is requested that all data be submitted for all subjects who participated in the study irrespective of whether (or not) they completed the study.

2. Detailed assay method and assay validation features (sensitivity, specificity, linearity, accuracy and precision within and between runs) for the parent compound (as well as the active metabolite if possible) should be submitted in the final study report. In addition, stability data during the collection and processing of plasma samples, during storage and assay procedures should be provided for tretinoin.

3. The firm should submit all individual (as well as mean±SD) plasma concentration/time data for tretinoin.

4. The sponsor is encouraged to submit the results of the study as an electronic submission (i.e., text and raw data via the ASCII file) to help facilitate review of the submission.

/S/ 2/14/94

He Sun, Ph.D.

Pharmacokinetics Evaluation Branch II

RD/FT Initialed by Frank Pelsor, Pharm. D. F. Pelsor 4/14/94

cc: NDA 20,400 and 20,404, HFD-520 (Clinical, Fogarty), HFD-426(Fleischer, Pelsor), Chron, Drug, Reviewer, HFD-19(FOI), HFD-340(Viswanathan).

MAR 1 1995

NDA: 20,400 and 20,404 **SUBMISSION DATE:** March 28, 1994
June 03, 1994
June 08, 1994

PRODUCT: Acticin Gel 0.025% (NDA 20,400)
Acticin Cream 0.025%, 0.05% and 0.1% (NDA 20,404)

SPONSOR: Penederm
320 Lakeside Drive, Suite A
Foster City, CA 94404

TYPE OF SUBMISSION: Resubmission **REVIEWER:** HE SUN, Ph.D.

BIOPHARMACEUTICS REVIEW

NDA 20,400 and 20,404

I. SYNOPSIS

The sponsor re-submitted these two New Drug Application (NDA 20,400 and 20,404) to support two new topical formulations-Acticin Gel and Cream. Two types of studies are included: *in vitro* percutaneous absorption studies and *in vivo* pharmacokinetic studies. The *in vitro* percutaneous absorption studies include 6 major studies to determine the absorption of tritiated tretinoin from Acticin and Retin-A Gel or Cream formulations, absorption of polyolprepolymer-2 (PPP-2) from neat material, Gel vehicle and Cream vehicle; and 6 supportive studies. The *in vivo* absorption studies include the absorption of PPP-2 from neat material, from Cream vehicle and a bioavailability study of Acticin Gel and Retin-A Gel formulation.

Based on these studies, the sponsor concluded the following:

(1) Acticin Gel and Cream offers lower or similar low systemic exposure to tretinoin (%) when compared to the commercial Retin-A Gel and Cream product.

(2) A very small amount (% of the applied dose) of PPP-2 penetrates excised human cadaver skin *in vitro* and the predominant component that penetrates is the lower molecular weight polyol, PPG-725. The higher molecular weight oligomers, comprising at least % (GPC peak area) of PPP-2, are retained in the upper layers of the stratum corneum and readily be removed from the skin surface by washing and/or tape stripping after topical application.

(3) There were no statistical differences between Retin-A 0.025% Gel and Acticin 0.025% Gel in the plasma pharmacokinetic parameters for tretinoin and isotretinoin.

II. RECOMMENDATIONS

II.1. For the Gel 0.025% formulation (NDA 20,400),

A. Due to problems found (see specific comments), the *in vitro* percutaneous absorption studies #PD168-60, #PD34-21, #PD24-77, #PD37-21, #PD37-25 (Gel, PDT004-002) are only of informative value. The *in vivo* plasma pharmacokinetic study of tretinoin and isotretinoin for Retin-A 0.025% Gel (Gel, PDT004-002) and Acticin 0.025% Gel is acceptable. Therefore, the Biopharmaceutics Section of the Gel 0.025% formulation (NDA 20,400) is acceptable.

B. However, the higher resistance of Acticin Gel formulation to stripping suggests deeper skin penetration which may results in higher local skin irritation rate of Acticin Gel 0.025% compared to Retin-A 0.025% (although washing with alcohol, soap and water is able to remove all drug residual). The significance of such higher resistance to stripping should be evaluated with other clinical observations

C. The sponsor should evaluate gender effect in tretinoin and isotretinoin absorption for Retin-A 0.025% Gel (Gel, PDT004-002) and Acticin 0.025% Gel.

II.2. For the Cream 0.025%, 0.05% and 0.1% formulations (NDA 20,404),

The *in vitro* data alone is unable to support the formulation. The sponsor should test, at least for the 0.1% strength, *in vivo* pharmacokinetics profiles and local skin reactions of the Cream formulation. Therefore, the Cream formulation (NDA 20,404) is not fully supported by studies submitted and is not acceptable to the Division of Biopharmaceutics.

II.3. For PPP-2 polymer

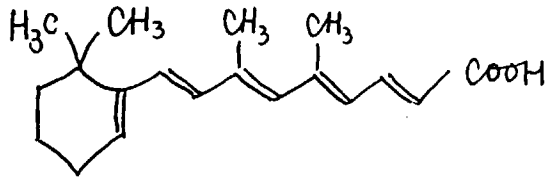
The *in vitro* studies of PPP-2 (PDT002-002) used for supporting both NDAs, #PD168-33, #PD168-21 and #PD-168-27, and the *in vivo* studies #PD112-18 (PDT002-002) and #TOX002-020 (Cream vehicle, PDT004-054) are acceptable.

TABLE OF CONTENTS:

III. BACKGROUND:

III.1. THE DRUG

Tretinoin, also known as retinoic acid or all-trans-retinoic acid, is a metabolite formed from all-trans-retinol, vitamin A, via conversion to all-trans-retinaldehyde.



Tretinoin was reported by Stuttgen in 1962 to be effective topically in disorders of keratinization; and by Kligman, et. al. in 1969 to be effective topically in acne. This early

work led to the development of a family of marketed products, Retin-A Cream, Gel, and liquid (Ortho Pharmaceutical Co.).

Retinoic acids and their derivatives exert substantial effects on epithelial growth and differentiation. In high oral doses, retinoic acids and some retinoic acid deriavatives are known to be human teratogens. Topical formulations of retinoic acid have not been shown to be human teratogens, however, evaluation of the potential risk associated with retinoic acid includes analysis of the results of percutaneous absorption of this drug on endogenous blood levels in order to identify any potential systemic effects.

Previous studies in humans with radioactive retinoic acid in both Gel and Cream formulations indicated minimal systemic absorption of the drug following topical administration.

III.2. THE ANDA/NDA APPLICATION HISTORY

Acticin was originally submitted as an ANDA for a generic equivalent to Ortho Pharmaceutical Corporation's Retin-A Gel. Since the limited systemic absorption of topical tretinoin does not lend itself to development of ANDA bioequivalence data by the simple measurement of blood drug levels, a protocol (#PDC 004-003) was developed to evaluate the therapeutic equivalence of the Acticin formulation versus vehicle and the innovator product, Retin-A Gel, in the treatment of acne vulgaris over a twelve-week period. In addition, a second twelve-week study (#PDC 004-015) was conducted to evaluate the Acticin formulation in comparison to vehicle only. The sponsor obtained the Agency's concurrence on the general design of bioequivalence protocol #PDC 004-003 on September 26, 1990. The subsequent study protocol, #PDC 004-015, was developed based on this prior concurrence and was submitted to the Agency on September 14, 1992.

The bioequivalence data were compiled and submitted in an ANDA application on May 29, 1991 to the Office of Generic Drugs. The ANDA was accepted for filing on August 9, 1991. Subsequently, at a meeting held on August 13, 1992 with representatives of the Office of Generic Drugs and the Division of Anti-Infective Drug Products, CDER. The Agency refused to accept the application for continuing review as an ANDA, due to the inclusion of an excipient in the Acticin formulation which is not present in the innovator's product. This ingredient is the sponsor's proprietary excipient, polyolprepolymer-2, which has not been previously approved for use in pharmaceutical products.

A non-approvable letter was sent by the Office of Generic Drugs because of the presence of polyolprepolymer-2 (PPP-2) on February 4, 1993. ANDA application was officially withdrawn by the sponsor and acknowledged by the Agency (Office of Generic Drugs) on April 8, 1993.

On February 11, 1993, a letter from the Division of Anti-Infective was sent defining additional requirements to allow substantive review of the tretinoin Gel application as an NDA. A determination was sent out from the Agency on April 26, 1993 that the ANDA application would require reformatting as an NDA submission.

The original NDAs (20,400 and 20,404) were submitted to the Agency on Oct. 24, 1993 and were refused to be filed by the Agency (RTF) after initial review. The RTF letters were sent to the sponsor on Nov. 23, 1993.

The sponsor then resubmitted NDA 20,400 and NDA 20,404 in March and June, 1994 to the Agency.

IV. DRUG FORMULATION

The Acticin Gel and Cream formulation are listed on the following pages.

APPEARS THIS WAY
ON ORIGINAL

3. Composition

1. Statement of Composition

A complete description of the quantitative composition of the drug product including any applicable range of inactive ingredients follows:

Acticin (tretinoin) Gel, 0.025%
Penederm formulation PDT 004-002

mg/g	Ingredient	% w/w
0.275	Tretinoin, USP	0.0275*
	Ethanol, 95%, denatured	
	Polyolprepolymer-2	
	Hydroxypropyl cellulose, NF	
	Butylated hydroxytoluene, NF or F.C.C.	

* The sponsor will manufacture the drug product with a % overage of tretinoin.

** The concentration range is %.

† Ethanol concentration to be adjusted based on concentration of butylated hydroxytoluene decided prior to manufacture.

Composition

1. Statement of Composition

A complete description of the quantitative composition of the drug products including any applicable range of inactive ingredients follows:

Acticin (tretinoin) Cream, 0.025%
Penederm formulation PDT 004-044

mg/g	Ingredient	% w/w
	Tretinoin, USP	
	Purified water, USP	
	Stearic acid, NF	
	Polyolprepolymer-2	
	Isopropyl myristate, NF	
	Polyoxyl 40 stearate, NF	
	Propylene glycol, USP	
	Stearyl alcohol, NF	
	Xanthan gum, NF, Food Grade	
	Sorbic acid, NF	
	Butylated hydroxytoluene, NF or F.C.C.	

* The sponsor will manufacture the drug product with a % overage.

1. Statement of Composition (continued)

Acticin (tretinoin) Cream, 0.05%
Penederm formulation PDT 004-045

mg/g	Ingredient	% w/w
	Tretinoin, USP	
*	Purified water, USP	
	Stearic acid, NF	
	Polyolprepolymer-2	
	Isopropyl myristate, NF	
	Polyoxyl 40 stearate, NF	
	Propylene glycol, USP	
	Stearyl alcohol, NF	
	Xanthan gum, NF, Food Grade	
	Sorbic acid, NF	
	Butylated hydroxytoluene, NF or F.C.C.	

* The sponsor will manufacture the drug product with a % overage.

1. Statement of Composition (continued)

Acticin (tretinoin) Cream, 0.1%
Penederm formulation PDT 004-046

mg/g	Ingredient	% w/w
	Tretinoin, USP	
	Purified water, USP	
	Stearic acid, NF	
	Polyolprepolymer-2	
	Isopropyl myristate, NF	
	Polyoxyl 40 stearate, NF	
	Propylene glycol, USP	
	Stearyl alcohol, NF	
	Xanthan gum, NF, Food Grade	
	Sorbic acid, NF	
	Butylated hydroxytoluene, NF or F.C.C.	

* The sponsor will manufacture the drug product with a % overage.

V. GENERAL SUMMARY OF STUDIES.

V. 1. Study list

1, Studies in #PD94-71 are *in vitro* percutaneous absorption studies of tritiated tretinoin from Acticin (test) and Retin-A (reference) Cream formulations at tretinoin concentrations of 0.025%, 0.05% and 0.1% using dermatomed human skin.

2, Studies in #PD168-60 are *in vitro* percutaneous absorption studies of tritiated tretinoin from Acticin (test) and Retin-A (reference) Gel formulations at tretinoin concentrations of 0.025% using dermatomed human skin.

3, Studies #PD34-21, 24-77, 37-21 and 37-25 are supportive studies to assess the effect of rubbing, instead of detergent washing, on epidermal levels of tretinoin from Gel formulation.

4, Study #PD91-79 was to test the percutaneous absorption of PPP-2 from test materials and from Cream vehicle.

5, Study #PD168-33 was to test the percutaneous absorption of PPP-2 from Gel vehicle.

6, Studies #PD168-21 and #PD168-27 were to develop methods to evaluate the localization of PPP-2 and its higher molecular weight polyol component in human skin.

7, Study #PD11-01 was to evaluate the localization of PPP-2 *in vivo*.

8, Study #TOX002-020 was to evaluate the localization of PPP-2 from Cream vehicle

9, Study #PDC004-017 was an *in vivo* clinical study to determine the effect of multiple applications of tretinoin-containing formulation on plasma levels of tretinoin in normal volunteers.

V. 2. The sponsor made following conclusions:

(1) The *in vitro* percutaneous absorption studies indicate that penetration of radiolabeled drug from two formulations never exceeded 0.3%. Acticin Gel and Cream offers similar low systemic exposure to tretinoin when compared to the commercial Retin-A Gel and Cream product, which have been used for many years.

(2) A very small amount (< 0.3% of the applied dose) of PPP-2 penetrates excised human cadaver skin *in vitro* and the predominant component that penetrates is the lower molecular weight polyol, PPG-725. The higher molecular weight oligomers, comprising at least 80% (GPC peak area) of PPP-2, are retained in the upper layers of the stratum corneum and readily removed from the skin surface by washing and/or tape stripping after topical application.

(3) Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal irritation response as

assessed by erythema, peeling and dryness. Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal physiological alteration of the stratum corneum as assessed by trans-epidermal-water-loss.

(4) There were no statistical differences between Retin-A 0.025% Gel and Acticin 0.025% Gel in the plasma pharmacokinetic parameters, decrease in AUC, Cmax and Css plasma tretinoin values from Study Day 7 to Study Days 14 and 28. There were no statistical differences in the plasma pharmacokinetic parameters for isotretinoin.

(5) There was a slight but statistically significant increase in Css tretinoin from Study Day 0 to Study Day 7.

V.3. Summary of studies

V.3.1. *in vitro* Percutaneous Absorption Studies

V.3.1.1. Percutaneous Absorption of tretinoin

The *in vitro* tretinoin percutaneous absorption was determined using radiotrace method for the assessment of potential systemic toxicity following topical exposure. In these studies, test formulations (Gel and Cream, Acticin, Penederm) and reference formulations (Gel and Cream, Retin-A, Ortho) were evaluated in human skin for *in vitro* percutaneous absorption and penetration using modified Franz flow-through diffusion cells. Three concentrations (0.1%, 0.05%, and 0.025%) of tretinoin were investigated (test formulations: PDT 004-046, PDT 004-045 and PDT 004-044 and control formulations: PDT 004-031, PDT 004-030 and PDT 004-024, respectively). Dermatomed human cadaver skin was used. Each formulation was applied to the epidermal surface of the skin at a surface dose of 10.0 ± 1.1 mg over the 0.64 cm^2 test area. After the 48-hour exposure period, each skin surface was washed. The skin and washing were saved for analysis of radiolabeled drug content.

Results of Cream:

The penetration of radiolabel from the Acticin formulations never exceeded 0.3%. Furthermore, receptor phase data indicate that the Acticin Creams, at concentrations of 0.025% and 0.1%, deliver statistically equivalent amounts of tretinoin compared to the corresponding Retin-A Creams. The Acticin 0.05% Cream formulation, however, delivered statistically less tretinoin to the receptor phase compared to the Retin-A 0.05% Cream.

Tretinoin skin levels, although generally greater from the Acticin Cream formulations than from the Retin-A formulations, were not statistically different at any of the corresponding tretinoin concentrations.

Results of Gel:

The absolute epidermal levels of radiolabeled tretinoin varied in magnitude among these studies, especially for Acticin Gel, whereas Retin-A Gel was relatively constant across studies. When the wipe and tape strip procedures were used, higher epidermal levels of the

radiolabel were observed following topical application of Acticin Gel compared to Retin-A Gel. This suggests a greater resistance to the rub off of tretinoin following topical application of Acticin Gel than Retin-A Gel. In contrast, when a detergent washing procedure was employed, lower epidermal levels of tretinoin are observed following topical application of Acticin Gel compared to Retin-A. This suggests that washing with detergent is more efficacious in the removal of tretinoin from the skin following topical application of Acticin Gel when compared to Retin-A Gel.

V.3.1.2.Percutaneous Absorption of PPP-2

Method

The test materials were applied (3-6 mg/cm^2) to the epidermal surface of dermatomed human skin mounted on Franz static diffusion cells. The dermal surface of the skin was perfused with phosphate buffered saline containing 0.01% sodium azide and 1.5% Oleth 20 equilibrated at 37 °C. At 48 hours, the skin surface was washed with one soap:water (50:50, v/v) cotton swab, 3 consecutive ethanol swabs and one dry swab. Along with each individual wash sample, skin samples were solubilized and assayed for radioactivity.

The individual polyol components of PPP-2, tritiated higher molecular weight oligomers and tritiated PPG-725, were incorporated separately into neat PPP-2 and into Acticin Gel (PDT 004-006) to characterize the percutaneous absorption of each component into and through human skin.

Acticin Cream vehicle (PDT 004-054), a research Gel vehicle, and an ethanol vehicle, each containing 10% PPP-2, were tested for its effect on the *in vitro* percutaneous absorption of tritiated PPP-2. The penetration of the polyol components, oligomers and PPG-725, were measured simultaneously.

In order to characterize the localization of PPP-2 in skin *in vivo,* the higher molecular weight polyol component of PPP-2 was radiolabeled and then incorporated into neat PPP-2. The radiolabeled polymer was applied to the dorsal forearm of two subjects (3-5 mg/cm2) under either occluded or semi-occluded, protected conditions. At 24 hours post-dosing, the chamber was removed and the skin surface was washed. The upper layers of the stratum corneum were removed with 10 tape-strips and each tape-strip was analyzed for radioactivity.

The *in vivo* localization of PPP-2 in human skin was characterized by FTIR-ATR spectrophotometric method. Cotton pads were saturated with a test solution of 10% PPP-2 in ethanol:water (60:40 v/v) and applied to the dorsal forearm of two subjects under occluded conditions. At 3 hours post-dosing, the pads were removed and the test area was lightly wiped with two cotton swabs. The skin was tape-stripped eight times and after each tape-strip, analyzed by FTIR-ATR for the presence of PPP-2.

Results

The results indicate that the higher molecular weight oligomers of PPP-2 do not penetrate

the skin. The lower molecular weight PPG-725 penetrates the skin from both vehicles, but levels are very low (% of the applied dose). Skin levels of each component, from both vehicles, are very low (%), with the majority of the polyols localized in the epidermis. In addition, the soap/water and ethanol wash employed readily removes both components of PPP-2 from the skin. The majority of the radiolabeled PPP-2 in the test materials was readily removed from the skin surface by washing with soap/water and ethanol (%). Receptor fluid data indicated that only a very small amount, less than % of the applied dose of PPP-2, penetrated through the skin from all three vehicles. In addition, PPP-2 skin levels were very low from all three vehicles (%).

Approximately 95% of the applied radiolabeled dose was readily removed from the skin surface by washing with soap and water. In addition, all of the radiolabeled oligomers were removed from the skin surface after the sixth tape-strip, suggesting that minimal amounts of the higher molecular weight oligomers of PPP-2 were localized in the upper layers of the stratum corneum (% of the applied dose).

The results reveal that PPP-2 is localized in the upper layers of the stratum corneum under the conditions employed. In addition, all detectable PPP-2 is completely removed from the skin surface by five repetitive tape-strips, *in vivo.*

IV.3.1.3 In vivo absorption studies

The objectives of the study were to answer two primary questions: (1) does the topical application of either 0.025% tretinoin Gels alter endogenous plasma concentration of tretinoin and/or isotretinon; and (2) is there any difference in plasma concentration between the Retin-A Gel formulation and the Acticin Gel formulation? The irritation parameters, trans-epidermal water loss (TEWL) and plasma concentration provide various measures to compare the two test formulations.

Pharmacokinetic studies

This is a double blind comparison study. Eighteen subjects (9 males and 9 females), free of any skin disease, were enrolled. The subjects were carefully advised to avoid Vitamin A supplements. 20 gm tube of either Retin-A 0.025% Gel or Acticin 0.025% Gel were provided. Application was to the forehead and both cheeks (125-175 cm^2), excluding the nose, around the eyes and chin. Applications commenced on study day 1 and thereafter on each evening 30-40 minutes prior to bed. At each study visit day, the tubes were collected and tube weights recorded. Target application was to be 2 mg/cm^2 Gel over 150 cm^2. Tube weights demonstrated that mean daily usage over 28 days was 0.307±0.066 gms (Mean±SD) for Retin-A Gel and 0.312±0.057 gms for Acticin Gel. On the morning of study days 7, 14, and 28, the subjects washed their face with soap and water (Purpose Soap, Johnson and Johnson, Skillman, NJ). Thirty minutes after the face wash a weighed application was performed by the investigator to each subject. Subjects remained in a darkened room lighted only by low wattage yellow tungsten lamps for four hours after Gel application. Blood samples were collected at 15 minutes prior to and at 2, 4, 8, 10, 12, and 24 hours after Gel application. After-the 24 hour blood sample the tubes of medication were returned to the subject for subsequent evening applications until the next study day. Tretinoin and

isotretinoin were assayed by a sensitive beam/mass spectrometry method.

Irritation and TEWL

On day 0, 7, 14, and 28, prior to the face wash, subject's forehead and both cheeks were first evaluated for signs of cutaneous irritation defined as erythema, peeling, and dryness. Each factor was graded on a 3 point scale (O = none, 1= light, 2 = moderate, 3 = severe) with 0.5 unit increments. In addition, trans-epidermal water loss (TEWL) was measured from the center of the forehead and both cheeks simultaneously using a multi-probe Courage+Khazaka Tewameter (Germany). No adverse events occurred during this study.

Data were collated by subject, sample hour and day, and by formulation. For continuous data (AUC, Css, Cmax, TEWL), a repeated measures analysis was used. For scaled data (erythema, dryness and peeling), nonparametric analyses were used (Kruskal-Wallis test and Wilcoxon Signed Rank test).

Study results

(1) There were no statistically significant changes in the plasma levels of tretinoin or isotretinoin relative to baseline for either treatment as measured by AUC, C_{max} and C_{ss}, except for a statistically significant increase in tretinoin C_{ss} on Study Day 7 (1.75±0.27 vs. 1.49±0.39 ng/ml) for both treatments.

(2) AUC, C_{max} and C_{ss} values on days 14 and 28 were significantly lower than values on day 7 regardless of formulation.

(3) There were no statistical differences observed in AUC, C_{max} and C_{ss} for the isotretinoin data.

(4) In addition, there was no statistically relevant correlation between these three parameters and the clinical observation data.

(5) Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal irritation response as assessed by erythema, peeling and dryness. Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal physiological alteration of the stratum corneum as assessed by trans-epidermal-water-loss.

VI. SPECIFIC COMMENTS

Need not to be sent to the sponsor:

1. Many experimental problems are noticed in the *in vitro* percutaneous absorption studies submitted for both NDAs. Therefore, *in vitro* percutaneous absorption studies submitted are unable to support these NDAs.

2. In vivo pharmacokinetics study of Gel formulation was performed and is acceptable, which resolved some questions raised in review of percutaneous absorption studies. Therefore, the

Gel 0.025% formulation was supported by submitted studies.

3. The higher resistance of Acticin Gel formulation to skin stripping suggests deeper skin penetration which resulted in higher local skin irritation rate of Acticin Gel 0.025% over Retin-A 0.025% (although washing with alcohol, soap and water was able to remove all drug residuals), which should be considered with other clinical observations for its clinical significance. This negative influence due to, most probably, PPP-2 should be evaluated and analyzed in combination with clinical studies in which same formulations were used.

Need to be sent to the sponsor

4. Some deficiencies were noticed in the *in vitro* percutaneous absorption studies submitted. The dose per unit area should be equivalent to that normally applied in a single application (~ 5 mg of formulation/cm^2). The exact nature of the skin preparation used for these studies should be carefully documented (the manner of preparation of the membranes from tissue, for example). Any treatment of the cadaver skin prior to harvesting should be recorded. In comparing drug absorption from two formulations using human skin, twelve experiments for each formulation should be run.

5. To ensure the safety of application of the drug, *in vivo* studies are needed for determining the acceptance of these NDAs. An *in vivo* pharmacokinetic study of Gel formulation was performed and is acceptable which resolved some questions raised in review of percutaneous absorption studies. The low systemic absorption (%) of the Acticin from the Gel 0.025% formulation and the similarity with Retin-A were supported by study #PDC004-017. However, the gender difference of tretinoin absorption from Acticin 0.025% Gel and Retin-A 0.025% Gel in clinical study #PDC004-017 should be analyzed.

6. With similar considerations stated in comments 4 and 5, the characteristics of systemic absorption and potential skin reaction of 0.025%, 0.05% and 0.1% Cream formulation can not be determined without *in vivo* studies. The sponsor should, at least for the 0.1% strength, perform *in vivo* pharmacokinetics studies for the Cream formulation.

Comments on Label:

7. The outcome of study #PDC004-017 should be described in the 0.025% Gel formulation labeling. Such information is needed for clinical situations in which co-administration of vitamin A is implemented. Suggested addition: "In a single center, double-blind, parallel pharmacokinetics study to determine the effect of multiple applications of Acticin Gel 0.025% on plasma levels of tretinoin in 18 normal volunteers, the average steady-state concentration (C_{ss}) of tretinoin and isotretinoin ranged between ng/ml (baseline) to ng/ml and ng/ml (baseline) to ng/ml, respectively".

NDA 20,400
NDA 20,404

/S/ 3/1/95

He Sun, Ph.D.
Pharmacokinetics Evaluation Branch II

Biopharm-Day Mar.1, 1995. Attendees: Drs. Ludden, Malinowski, ChenM, Fleischer, Hepp, Gillespie, Hussian, Pelsor and Sun.

RD/FT Initialed by Frank Pelsor, Pharm. D. 3/1/95

cc: NDA 20,400 and 20,404, HFD-540 (Clinical), HFD-427(ChenML, Pelsor), HFD-426(Fleischer), Chron, Drug, HFD-19(FOI), HFD-340(Viswanathan), Reviewer.

APPENDIX I

in vitro percutaneous absorption studies

Performing Laboratory:
Penederm Incorporated
320 Lakeside Drive
Forter City, CA 94404

BIOPHARMACEUTICS REVIEW

NDA 20,400 and 20,404

A.1. REVIEW OF THE PERCUTANEOUS ABSORPTION STUDIES

***in vitro* Percutaneous Absorption from Cream**

Acticin Cream: 0.025% (PDT 004-044), 0.05% (PDT 004-045), 0.1% (PDT 004-046)
Study No. #PD94:71

Methods: The *in vitro* percutaneous absorption of tritiated tretinoin was evaluated from Acticin (test) and Retin-A (control) Cream formulations at tretinoin concentrations of 0.025%, 0.05% and 0.1%. Dermatomed human skin was mounted into Bronaugh flow-through diffusion cells. Each formulation was applied to the epidermal surface of the skin at a dose of 10 mg over the 0.64 cm^2 test area. The dermal surface of the skin was perfused with phosphate-buffered saline and the cells were maintained at 37 °C. The receptor phase was collected at 6-hour intervals, for 48 hours, and assayed for radioactivity to assess tretinoin percutaneous penetration from the test and control formulations. At 48 hours post-dose application, the test and control materials were removed from the skin surface by washing with 95% ethanol. The washes were pooled and assayed for radioactivity. Finally, each skin sample was solubilized and assayed for radioactivity to assess retention of tritiated tretinoin in the skin.

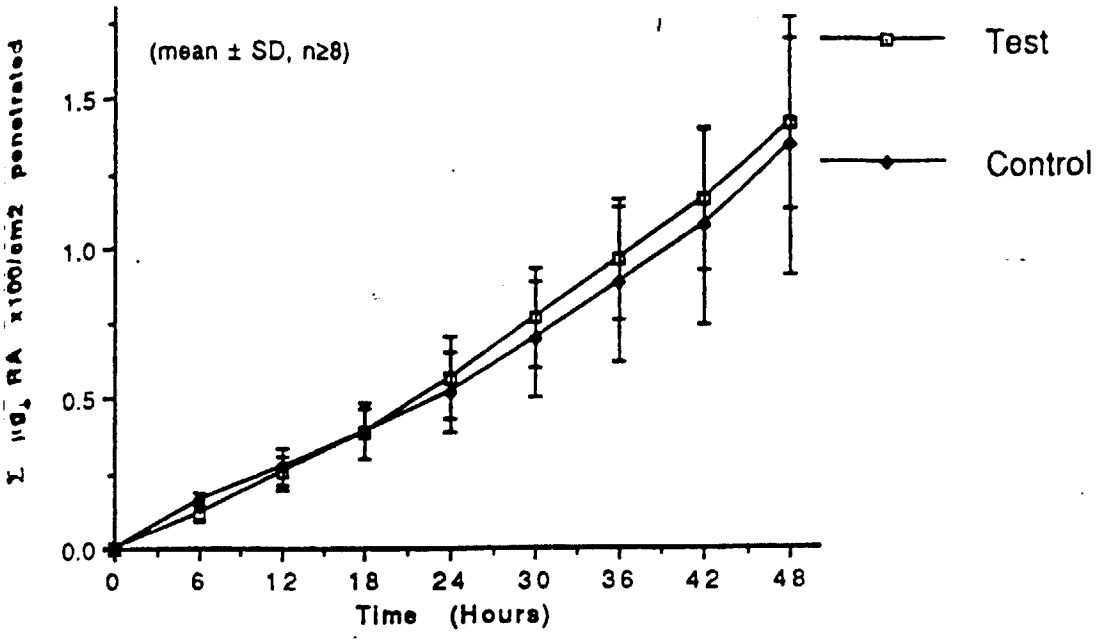
Results: The percutaneous absorption of tritiated tretinoin from Acticin Cream and Retin-A Cream formulations, after a 48-hour exposure period is given at Table 1. Drug penetration -time profile are given at page 19 and 20.

Table 1.
Percutaneous Absorption of Tretinoin from Cream Formulations
(% of Applied Dose; Mean±SD)

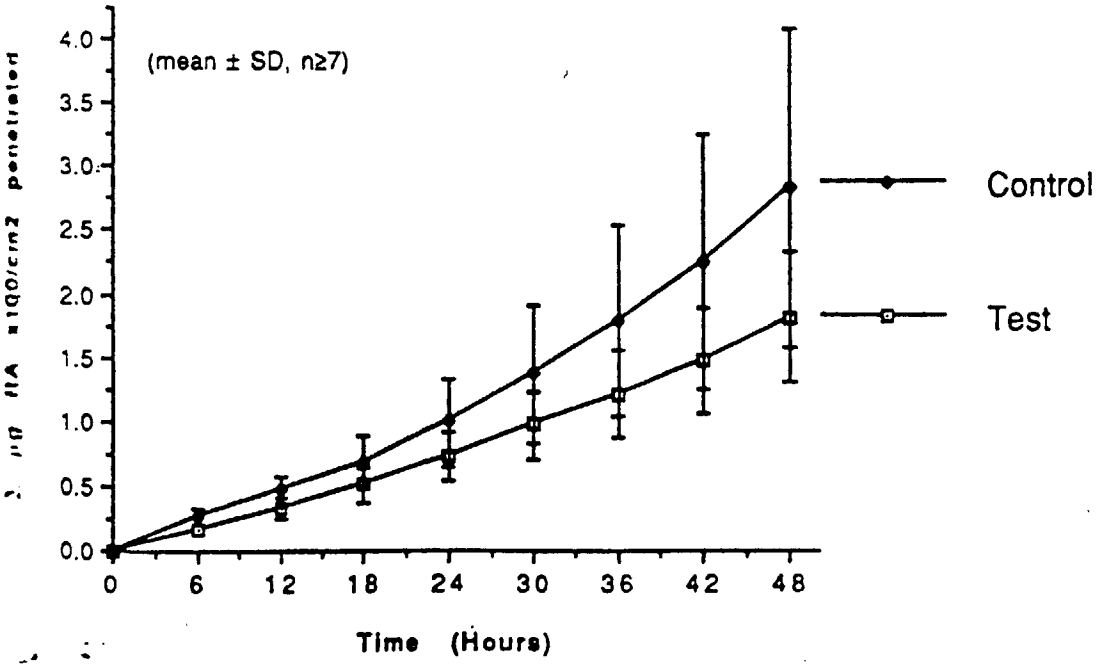
Test and Control	skin Content (%)	Receptor phase (%)	Total Recovery (%)
Acticin 0.025% (PDT 004-044) n=11	4.7 ± 2.2	0.28 ± 0.06	104 ± 4
Retin-A 0.025% (PDT 004-024) n=8	2.8 ± 0.9	0.27 ± 0.08	105 ± 3
Acticin 0.05% (PDT 004-045) n=9	6.5 ± 3.0	0.17 ± 0.04 *	10.4 ± 5
Retin-A 0.05% (PDT 004-030) n=7	3.5 ± 0.4	0.33 ± 0.15	106 ± 2
Acticin 0.1% (PDT 004-046) n=9	5.5 ± 1.7	0.21 ± 0.07	106 ± 2
Retin-A 0.1% (PDT 004-031) n=12	4.6 ± 2.3	0.32 ± 0.10	106 ± 4

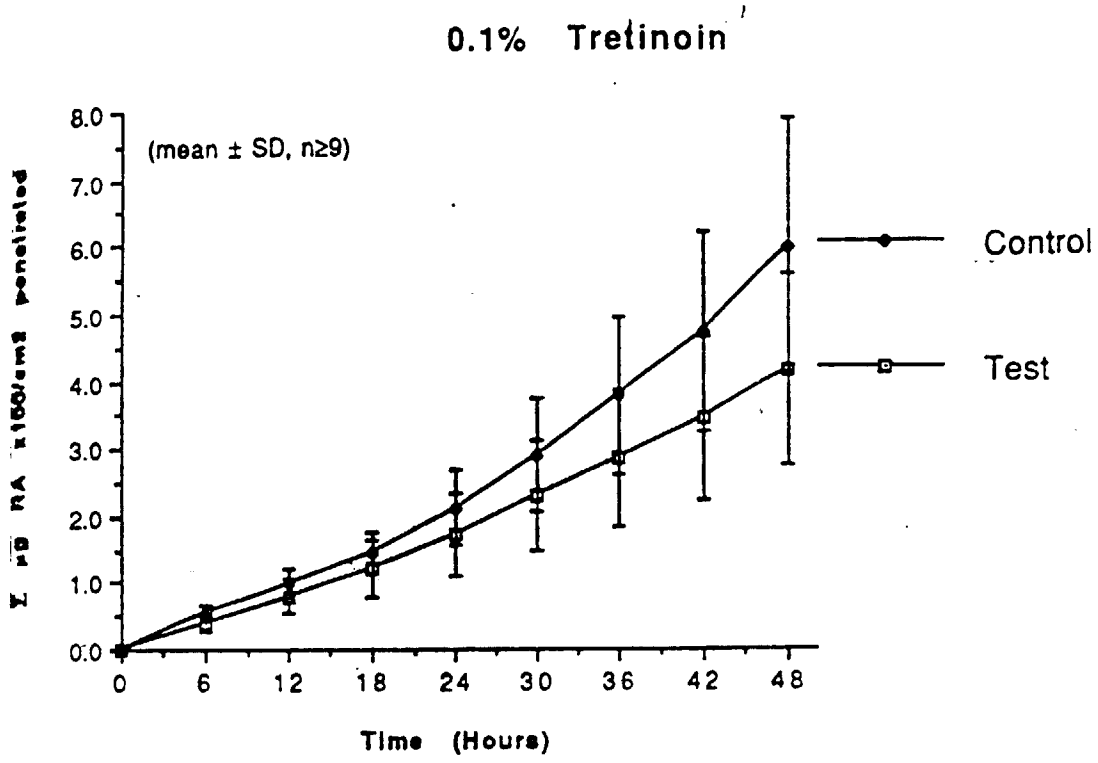
* statistically significant different.

0.025% Tretinoin



0.05% Tretinoin




0.1% Tretinoin
(mean ± SD, n≥9)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
6
12
18
24
30
36
42
48
Time (Hours)
Control
Test

The penetration of radiolabel from the Acticin formulations never exceeded 0.3%. Furthermore, receptor phase data indicate that the Acticin Creams, at concentrations of 0.025% and 0.1%, deliver statistically equivalent amounts of tretinoin compared to the corresponding Retin-A Creams. The Acticin 0.05% Cream formulation, however, delivered statistically less tretinoin to the receptor phase compared to the Retin-A 0.05% Cream.

Tretinoin skin levels, although generally greater from the Acticin Cream formulations than from the Retin-A formulations, were not statistically different at any of the corresponding tretinoin concentrations.

Summary and Conclusion: Based upon the results of this study, the Acticin Creams offer similar low tretinoin penetration as do the commercial Retin-A products.

***in vitro* Percutaneous Absorption of Tretinoin from Gel.**

Acticin Gel (PDT 004-002)
Study No. #PD168-60

Method: the *in vitro* percutaneous absorption of tretinoin was evaluated from Acticin (test) and Ortho Retin-A (control) 0.025% tretinoin Gels. Both formulations were spiked with tritiated tretinoin. Dermatomed human skin was mounted into Bronaugh flow-through diffusion cells. Each formulation was applied to the epidermal surface of the skin at a dose of approximately 10 mg over the 0.64 cm^2 test area. The dermal surface of the skin was perfused with phosphate-buffered saline and the cells were maintained at 37 °C. The receptor phase was collected at 6-hour intervals, for 48 hours, and assayed for radioactivity to assess tretinoin percutaneous penetration from the test and control formulations. At 48 hours post-dose application, test and control materials were removed from the skin surface by detergent and water washing. The washes were assayed for radioactivity. Finally, each skin sample was separated into epidermis and dermis. Then, each skin section was solubilized and assayed for radioactivity to assess retention of tritiated tretinoin in the skin.

Results: The percutaneous absorption of tritiated tretinoin from Acticin Gel and Retin-A Gel formulations, after a 48-hour exposure period, is given at below:

Table 2.
Percutaneous Absorption of Tretinoin from Gel Formulations
(% of Applied Dose; Mean±SD) n=7-9

Test/Control Formulations	Receptor (%)	Epidermis* (%)	Dermis (%)	Total Recovery (%)
Acticin Gel (PDT 004-002)	0.22(0.04)	0.58(0.19)	0.26(0.10)	93.5(3.7)
Retin-A Gel (PDT 004-003)	0.28 (0.06)	1.76(0.82)	0.28(0.16)	101.9(5.6)

* Statistical difference ($p<0.05$) between test and control formulations

Discussion: There are some inconsistencies between these results and the results of pilot studies

given below, using different techniques for removal of test materials. The absolute receptor levels of radiolabeled tretinoin from Acticin Gel and Retin-A Gel are similar and consistent with the preliminary studies described below, although receptor levels are statistically less from Acticin Gel. The epidermal content of radiolabeled tretinoin following detergent and water washing is statistically greater from Retin-A Gel than from Acticin Gel. Therefore, the washing procedure employed is more efficacious in the removal of Acticin Gel from the skin compared to Retin-A Gel. Dermal radiolabel deposition, however, is virtually identical between the two Gel formulations.

Acticin Gel (PDT 004-002)
Study Nos. #PD34-21, 24-77, 37-21, 37-25 (Supportive Studies)

In addition to the study summarized above, four supportive, developmental studies were conducted to investigate the *in vitro* percutaneous absorption of tritiated tretinoin from Acticin Gel and Retin-A Gel formulations. These studies were previously referenced in the Sponsor's IND Topical All-Trans-Retinoic Acid, serial #003. The study conditions used to measure *in vitro* percutaneous penetration of tritiated tretinoin from Acticin Gel and Retin-A Gel formulations in these four supportive studies were similar to those employed in the above study, #PD168-60.

Method: A major difference in the study design of these supportive studies was that methods were employed to assess the effect of rubbing, instead of detergent washing, on epidermal levels of tretinoin. These procedures were conducted after the collection of receptor fluid samples and, therefore, would have no effect on observed tretinoin penetration.

Results: Penetration of radiolabeled tretinoin from Acticin Gel and Retin-A Gel formulations from all five studies is summarized in Table 3 below:

Table 3
Percutaneous Penetration of Tretinoin from Gel Formulations
(% of Applied Dose in Receptor Fluid; Mean±SD)

Test/Control	Study Identification				
Formulations	#PD168-60*	#PD34-21*	#PD24-77*	#PD37-21*	#PD37-25
Acticin Gel (PDT 004-002)	0.22±0.04	0.33±0.06	0.35±0.10	0.14±0.01	0.12±0.02
Retin-A Gel (PDT 004-003)	0.28±0.06	0.43±0.05	0.43±0.07	0.28±0.07	0.20±0.08

* statistical difference ($p<0.05$) between test and control formulations

The small differences in the penetration of radiolabel among these studies can be attributed to the variation in the skin employed in each study and to differences in study conditions. Nevertheless, the levels of radiolabeled tretinoin in the receptor fluid from all five studies indicate that the penetration of radiolabel is in the same range for Acticin Gel and for Retin-A Gel. The penetration of radiolabel from Acticin Gel is consistently less than that from Retin-A Gel. Furthermore, the

penetration of radiolabel from both formulations never exceeded % of the applied radiolabel in any of the studies. The ability of Acticin Gel to resist the removal of tretinoin from the epiderm- relative to Retin-A Gel was evaluated by dry wiping the skin followed by five repetitive tape stripping of the surface of the skin. Because of the presence of formulation, the hydrated state of the skin following removal from the diffusion cells, and the vigor in which the investigator engages the wiping and tape stripping procedures, the techniques employed may be insufficient to remove all of the superficial residual test material from the epidermis. However, these techniques were designed to simulate material that would remain on the site of application if the patient did not wash and loss was due solely to rubbing and exfoliation Epidermal levels of tretinoin observed in the three most recent studies are summarized in the following table:

Table 4
Epidermal Levels of Tretinoin from Gel Formulations
(% of Dose; mean ± SD)

Test and Control Formulations	Study Identification		
	#PD168:60 detergent washing	#PD37:25 dry wipe/ tape strip	#PD37:21 dry wipe/ tape strip
Acticin Gel (PDT 004-002)	0.58 ± 0.19	6.58 ± 1.80	6.50 ± 2.62
Retin-A Gel (PDT 004-003)	1.76 ± 0.82	1.18 ± 0.94	2.81 ± 1.50

Discussion: The absolute epidermal levels of radiolabeled tretinoin varied in magnitude among these studies, especially for Acticin Gel, whereas Retin-A Gel was relatively constant across studies. When the wipe and tape strip procedure was used, higher epidermal levels of the radiolabel were observed following topical application of Acticin Gel compared to Retin-A Gel. This suggests a greater resistance to the rub off of tretinoin following topical application of Acticin Gel than Retin-A Gel. In contrast, when a detergent washing procedure was employed, lower epidermal levels of tretinoin are observed following topical application of Acticin Gel compared to Retin-A (study #PD168:60 in table above). This suggests that washing with detergent is more efficacious in the removal of tretinoin from the skin following topical application of Acticin Gel when compared to Retin-A Gel.

Summary and Conclusion: Epidermal and dermal levels of tretinoin were low following topical application of either Acticin Gel or Retin-A Gel. The presence of PPP-2 in the Acticin Gel formulation may afford greater resistance to the rub-off of tretinoin and greater ease in tretinoin removal by detergent washing when compared to Retin-A Gel.

Percutaneous Absorption of Polyolprepolymer-2 (PPP-2)

***in vitro* Percutaneous Absorption**

Introduction: PPP-2 is a liquid mixture of two polyol components with a combined average molecular weight of approximately 4000 daltons. The chemical composition of PPP-2 was reviewed in section 3.4.1 of the submission. Both polyol components comprising PPP-2, the higher molecular weight oligomers and lower molecular weight PPG-725, were radiolabeled and incorporated individually into several vehicles. Two *in vitro* studies were conducted to evaluate the extent of PPP-2 (PDT 002-002) percutaneous absorption into and through human skin. In addition, three pilot studies were conducted to characterize the percutaneous absorption of PPP-2 *in vivo*. This section summarizes the results of these studies.

Methods: The test materials were applied (3-6 mg/cm^2) to the epidermal surface of dermatomed human skin mounted on Franz static diffusion cells and then spread evenly with a glass rod. The dermal surface of the skin was perfused with phosphate buffered saline containing % sodium azide and % Oleth 20 equilibrated at 37 °C. Receptor phase samples were collected at 5, 24, 29 and 48 hours post-dose application and analyzed for radioactivity. At 48 hours, the skin surface was washed with one soap:water (50:50, v/v) cotton swab, 3 consecutive ethanol swabs and one dry swab. Along with each individual wash sample, skin samples were solubilized and assayed for radioactivity.

Results: The results of two pivotal investigations (#PD168-33, #PD91-79) characterizing PPP-2 *in vitro* percutaneous absorption are summarized in Table 5 and discussed separately in detail.

Table 5
Percutaneous Penetration of PPP-2 and its Components
(% of applied dose; mean ± SD)

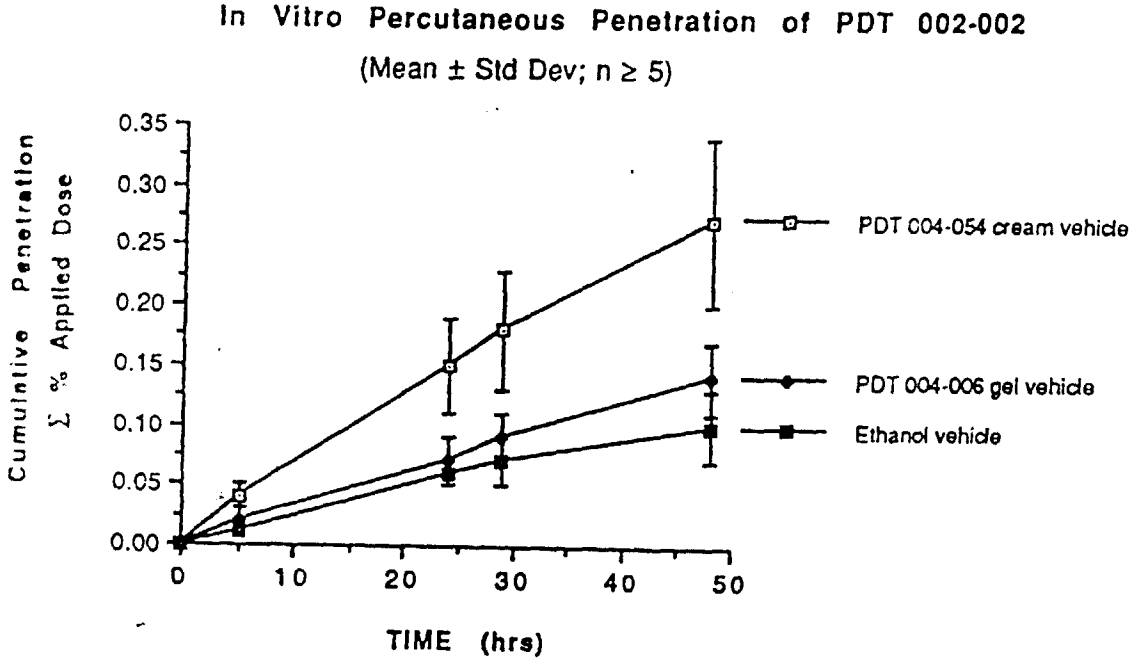
Test Materials	^{3}H-PDT 002-002 (% penetrated)	^{3}H-Oligomers (% penetrated) n=6	^{3}H-PPG-725 (% penetrated) n=6
#PD168-33			
neat (PDT 002-002)		<0.0012[b]	0.05+0.02
Gel (PDT 004-006)[a]		<0.0015[c]	0.32+0.11
#PD91-79			
Cream (PDT 004-054)[a]	0.27+0.07		
Gel (PDT 004-006)[a]	0.14+0.03		
ethanol solution	0.10+0.03		

a: vehicle contains 10% PDT 002-002
b: 0.0012% is the limit of detection
c: 0.0015% is the limit of detection
Note: PDT 004-054 is the Acticin Cream vehicle

a. **Detail of Study No. #PD168-33**
PPP-2 (PDT 002-002), research Gel vehicle (PDT 004-006)

Method: The individual polyol Components of PPP-2, tritiated higher molecular weight oligomers and tritiated PPG-725, were incorporated separately into neat PPP-2 and into Acticin Gel (PDT 004-006) to characterize the percutaneous absorption of each component into and through human skin.

The results indicate that the higher molecular weight oligomers of PPP-2 do not penetrate the skin. The lower molecular weight PPG-725 penetrates the skin from both vehicles, but levels are very low (% of the applied dose). Skin levels of each component, from both vehicles, are very low (%), with the majority of the polyols localized in the epidermis. In addition, the soap/water and ethanol wash employed readily removes both components of PPP-2 from the skin.

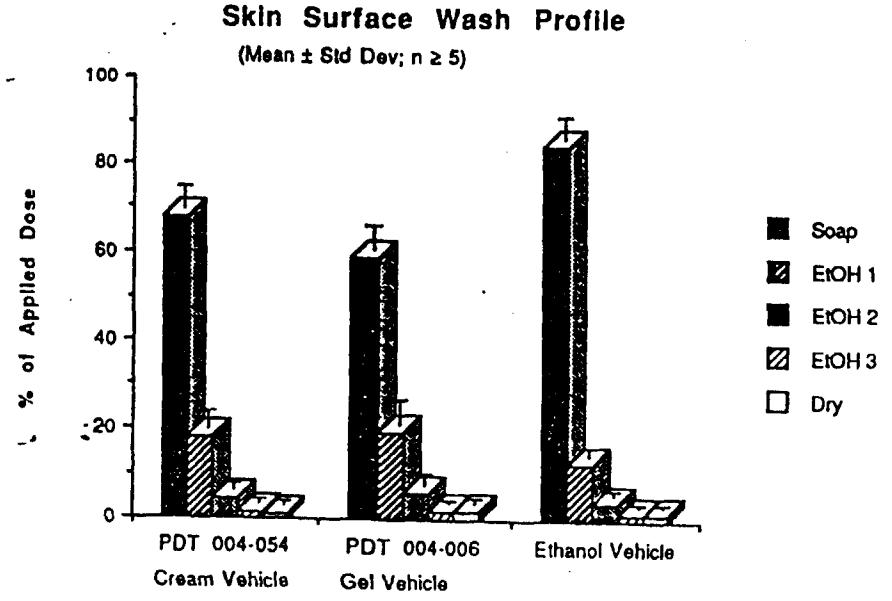


Mass Absorption of Polyolprepolymer-2 Into and Through Skin
(µg / cm^2; Mean ± Std Dev)

Test Material	Receptor (µg / cm^2/48 hrs)	Skin Content (µg / cm^2/48 hrs)
PDT 004-054 Cream vehicle	1.37 ± 0.35	1.86 ± 0.62
PDT 004-006 Gel vehicle	0.75 ± 0.16	1.81 ± 1.28
PD89:54.00 Ethanol vehicle	0.40 ± 0.10	0.79 ± 0.21

Percent Absorption of Polyolprepolymer-2 Into and Through Skin
(% of Applied Dose; Mean ± Std Dev)

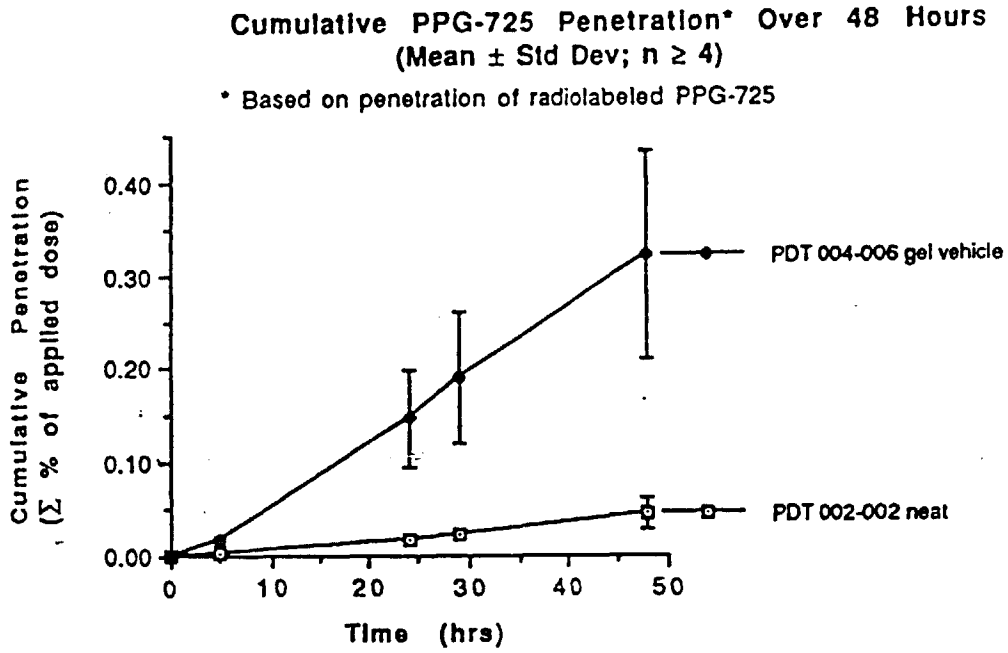
Test Material	Receptor	Skin Content	Total Recovery (including washes)
PDT 004-054 Cream Vehicle	0.27 ± 0.07	0.36 ± 0.12	94.75 ± 4.50
PDT 004-006 Gel Vehicle	0.14 ± 0.03	0.33 ± 0.24	89.12 ± 4.12
PD89:54.00 ethanol Vehicle	0.10 ± 0.03	0.20 ± 0.05	106.7 ± 6.77



b. Detail of Study No. #PD91-79
Acticin Cream vehicle (PDT 004-054), research Gel vehicle (PDT 004-006), ethanol vehicle (#PD89-54.00)

Acticin Cream vehicle (PDT 004-054), containing 10% PPP-2, was tested for its effect on the *in vitro* percutaneous absorption of tritiated PPP-2. A research Gel vehicle and an ethanol vehicle, each containing 10% PPP-2, were also tested. Both polyol components of PPP-2, oligomers and PPG-725, were radiolabeled in each vehicle, i.e., the penetration of the polyol components was measured in combination, not independently.

The majority of the radiolabeled PPP-2 in the test materials was readily removed from the skin surface by washing with soap/water and ethanol (%). Receptor fluid data indicated that only a very small amount, less than % of the applied dose of PPP-2, penetrated through the skin from all three vehicles. In addition, PPP-2 skin levels were very low from all three vehicles (%).



Mass Absorption of Polyols Comprising PDT 002-002 Into and Through Skin

(µg / cm^2; Mean ± Std Dev)

Test Material	Epidermis (µg/cm^2)	Dermis (µg/cm^2)	Receptor (µg / cm^2/48 hrs)
^{3}H-PPG-725 In:			
PDT 002-002, neat	0.86 ± 0.72	0.10 ± 0.11	0.38 ± 0.14
PDT 004-006 (gel vehicle)	0.21 ± 0.08	0.01 ± 0.01	0.24 ± 0.10
^{3}H-HMW oligomers In:			
PDT 002-002, neat	5.76 ± 6.71	0.11 ± 0.09	0.00 ± 0.00[a]
PDT 004-006 (gel vehicle)	0.71 ± 0.18	0.03 ± 0.04	0.00 ± 0.00[b]

[a] Below limit of detection: <0.050 µg/cm^2

[b] Below limit of detection: <0.006 µg/cm^2

Note: Mass of PDT 002-002 applied is 10-fold less from the gel vehicle than from neat PDT 002-002

Percent Absorption of Polyols Comprising Polyolprepolymer-2 Into and Through Skin

(% of Applied Dose; Mean ± Std Dev)

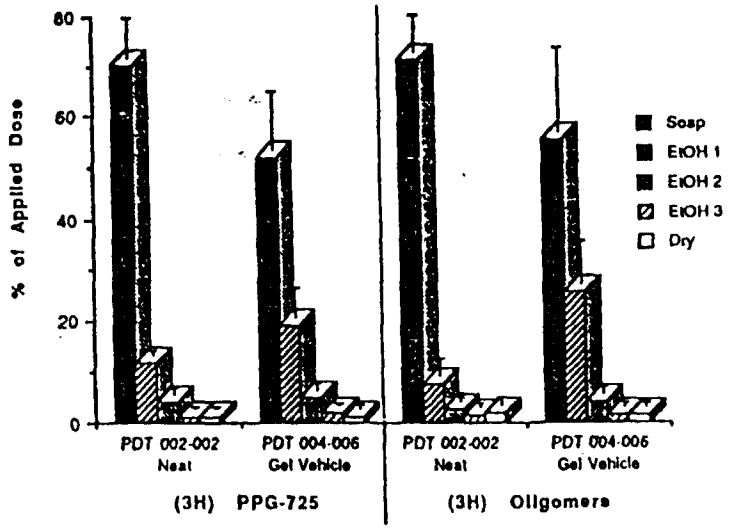
Test Material	Epidermis	Dermis	Receptor	Total Recovery (including washes)
^{3}H-PPG-725 In:				
PDT 002-002, neat	0.11 ± 0.10	0.01 ± 0.01	0.05 ± 0.02	89.50 ± 8.83
PDT 004-006 (gel vehicle)	0.29 ± 0.10	0.01 ± 0.01	0.32 ± 0.11	80.39 ± 7.89
^{3}H-HMW oligomers In:				
PDT 002-002, neat	0.14 ± 0.16	0.003 ± 0.002	0.00 ± 0.00[a]	87.34 ± 7.60
PDT 004-006 (gel vehicle)	0.20 ± 0.04	0.01 ± 0.01	0.00 ± 0.00[b]	89.03 ± 12.58

[a] Below limit of detection: <0.0012% of applied dose

[b] Below limit of detection: <0.0015% of applied dose

Note: Mass of PDT 002-002 applied is 10-fold less from the gel vehicle than from neat PDT 002-002

Skin Surface Wash Profile (Mean ± Std Dev; n ≥ 4)



Other Supportive Studies (*in vivo*)

Several *in vivo* studies were conducted to develop methods to evaluate the localization of PPP-2 and its higher molecular weight polyol component in human skin.

***PPP*-2 (PDT 002-002)**
Study No. #PD168-21, #PD168-27

In order to characterize the localization of PPP-2 in skin *in vivo*, the higher molecular weight polyol component of PPP-2 was radiolabeled and then incorporated into neat PPP-2. The radiolabeled polymer was applied to the dorsal forearm of **two** subjects (mg/cm2) under either occluded or semi-occluded, protected conditions. At 24 hours post-dosing, the chamber was removed and the skin surface was washed. The upper layers of the stratum corneum were removed with 10 tape-strips and each tape-strip was analyzed for radioactivity.

Approximately 95% of the applied radiolabeled dose was readily removed from the skin surface by washing with soap and water. In addition, all of the radiolabeled oligomers were removed from the skin surface after the sixth tape-strip, suggesting that minimal amounts of the higher molecular weight oligomers of PPP-2 were localized in the upper layers of the stratum corneum (% of the applied dose).

***in vivo* Skin Localization of PPP-2 (PDT 002-002)**
Study No. #PD112-18

The *in vivo* localization of PPP-2 in human skin was characterized by FTIR-ATR spectrophotometric methodology. Cotton pads were saturated with a test solution of % PPP-2 in ethanol:water (v/v) and applied to the dorsal forearm of **two** subjects under occluded conditions. At 3 hours post-dosing, the pads were removed and the test area was lightly wiped with two cotton swabs. The skin was tape-stripped eight times and after each tape-strip, analyzed by for the presence of PPP-2.

The results reveal that PPP-2 is localized in the upper layers of the stratum corneum under the conditions employed. In addition, all detectable PPP-2 is completely removed from the skin surface by five repetitive tape-strips, *in vivo*.

***in vivo* Research Cream vehicle (PDT 004-054)**
Study No. #TOX 002-020

An *in vivo* study with monkeys, employing topical application of tritiated PPP-2 incorporated in a research Cream vehicle containing % PPP-2, was attempted. The data from this study are not interpretable because of problems encountered during preparation of samples and operation of instrumentation. In addition, concerns were noted in the animal handling techniques employed. The potential for tritium exchange raises concerns as to whether this label accurately reflects PPP-2 absorption. A report for the study is not currently available.

APPENDIX II

In vivo Pharmacokinetic Study

#PDC 004-017

in vivo Plasma concentration measurement (#PDC004-017)

Title: A SINGLE CENTER, DOUBLE-BLIND, PARALLEL STUDY TO DETERMINE THE EFFECT OF MULTIPLE APPLICATIONS OF TRETINOIN-CONTAINING FORMULATIONS ON PLASMA LEVELS OF TRETINOIN IN NORMAL VOLUNTEERS (#PDC 004-017)

Principle Investigators:

INTRODUCTION

This study was designed to determine the effect of multiple applications of tretinoin containing formulations, Retin-A 0.025% Gel (PDT 004-003) and Acticin 0.025% Gel (PDT 004-002), on the endogenous plasma levels of tretinoin (all-trans-5-retinoic acid) and isotretinoin (1,3-cis-retinoic acid) in normal volunteers over a 28-day daily topical application regimen to their face.

Previous studies in humans with radioactive tretinoin in both Gel and Cream formulations indicate minimal systemic absorption of the drug following topical administration. With the recent advent of highly sensitive analytical techniques which allow the accurate measurement of tretinoin in plasma, non-radioactive percutaneous absorption studies are now possible.

METHODS

Human Subjects

Eighteen subjects (9 males and 9 females) were enrolled into this study. They ranged in ages from years (29 +/- 6, Mean± SD yrs), were within % of their ideal body weight using the Metropolitan Life Insurance Company standards, were in good health as assessed by medical history, physical examination and clinical laboratory results, were free of any skin disease, and had not used any topical medications or retinoid therapies within the 60 days prior to enrollment. All subjects who were enrolled completed the study. However, Subjects were unable to have Study Day 14 visit activities performed due to a scheduling conflict. No adverse events occurred during this study. The subjects were carefully advised to avoid Vitamin A supplements that would exceed its recommended daily allowance or foods with high Vitamin A content (e.g. liver) throughout the study and specifically within 48 hours prior to each blood sampling day.

Initials	Sex*/ID	Age (yrs)	Weight (lb)
	M	25	165
	M	37	196
	M	25	181
	M	33	187
	M	24	195
	M	26	127
	M	25	190

M	41	190
M	34	156
F	27	135
F	26	139
F	24	155
F	21	141
F	21	130
F	24	128
F	39	132
F	37	150
F	32	131

a: M male, F Female

Subjects were provided with a single 20 gm tube of either Retin-A 0.025% Gel or Acticin 0.025% Gel. Both subjects and investigators were blinded to product identification throughout the study. Each subject was carefully instructed and received a demonstration on the proper application of the Gel. Application was to the forehead and both cheeks (~125-175 cm^2), excluding the nose, around the eyes and chin. At each study visit day, the tubes were collected and tube weights recorded. Additional instructions were provided to those subjects demonstrating an over or under average daily use of product. Target application was to be 2 mg/cm^2 Gel to 150 cm^2. Tube weights demonstrated that mean daily usage over 28 days was gms ($x \pm SD$) for Retin-A Gel and gms for Acticin Gel. Applications commenced on study day 1 and there after on each evening 30-40 minutes prior to bed. On the morning of study days 7, 14, and 28, the subjects washed their face with soap and water (Purpose Soap, Johnson and Johnson, Skillman, NJ). Thirty minutes after the face wash a weighed application was performed by the investigator to each subject. Subjects remained in a darkened room lit only by low wattage yellow tungsten lamps for four hours after Gel application. Blood samples were collected at 15 minutes prior to and at 2, 4, 8, 10, 12, and 24 hours after Gel application. Ten hours post-dosing the subjects washed their face to remove any unabsorbed drug. After the 24 hour blood sample the tubes of medication were returned to the subject for subsequent evening applications until the next study day.

Clinical Observations

On each selected study day (Day 0, 7, 14, and 28), prior to the face wash, subject's forehead and both cheeks were first evaluated for signs of cutaneous irritation defined as erythema, peeling, and dryness. Each factor was graded on a 3 point scale (0= none, 1=light, 2 = moderate, 3 = severe) and 0.5 unit increments. In addition, trans-epidermal-water-loss (TEWL) was measured from the center of the forehead and both cheeks simultaneously using a multi-probe Courage+Khazaka Tewameter (Germany).

Blood Sample Collection

Blood samples were collected in 10 ml, sodium heparin under dim yellow light. Tubes were immediately covered with aluminum foil and placed in ice. Within 30 minutes of collection, the blood tubes were centrifuged, plasma isolated into 0.5 ml (for direct analysis) and 2.0 ml (for storage reserve) aliquot in amber microcentrifuge tubes and stored at -70°C protected from light. The protocol stated that duplicate 1.0 ml aliquots were to be prepared,

however, by reducing the analysis aliquot to 0.5 ml the sample was ready for direct use in the assay procedure by removing an initial pipetting step.

Retinoic Acid Assay

Tretinoin and isotretinoin were assayed by a sensitive high pressure liquid chromatography/particle beam/mass spectrometry method (detailed methodology was submitted). The mass spectrometer was operated in the negative chemical ionization mode with selected ion monitoring at 325.2 for the internal standard and 299.2 for the retinoic acids. Methane was used as the reagent gas at a source pressure of 1-2 x 10^4 torr. With each set of twenty-four samples a control spiked plasma sample and duplicate 3-point standard curve samples (1, 2 and 5 ng/ml) were analyzed. Tretinoin and isotretinoin were quantified using the internal standard normalization method to the mean standard curve generated from that batch run of samples.

Statistics

Data were collected by subject, sample hour and day, and by formulation. The data were tested across all days for statistical differences between days and between treatments. For continuous data (AUC, Css, Cmax, TEWL), a repeated measures analysis was used. For scaled data (erythema, dryness and peeling), nonparametric analyses were used (Kruskal-Wallis test and Wilcoxon Signed Rank test). Correlation analyses were performed by calculating Pearson correlation coefficients.

RESULTS

Plasma Levels of Tretinoin and isotretinoin

Plasma levels were determined at seven time points over twenty-four hours on Study Days 0, 7, 14 and 28. Study Day 0 represents baseline endogenous levels of tretinoin and isotretinoin. Days 7, 14 and 28 plasma concentrations were monitored for any change in endogenous retinoic acid levels during the topical exposure of the tretinoin containing products. The data are summarized in Tables 6 and 7.

Baseline mean retinoic acid plasma level across the entire 24 hour sampling period was found to be 1.49 $\pm$ 0.69 ng/ml (mean $\pm$ SD) for tretinoin and 1.03 $\pm$ 0.60 ng/ml for isotretinoin. These levels of endogenous retinoic acids are consistent with reported values by others. Plasma tretinoin levels on study day 7 tended to increase slightly within the 8 and 10 hour samples. Study Day 14 and 28 tretinoin plasma levels were lower, on average, when compared to Study Days 0 and 7. Isotretinoin concentrations were consistently lower throughout all study day visits.

Table 6

Summary of measured plasma tretinoin values at each sampling time on each study day. Values are the mean $\pm$ SD as ng/ml of all-trans retinoic acid.

Day	Time (hr)	Acticin Gel	Retin-A Gel
0	0	1.42 $\pm$ 0.95	1.68 $\pm$ 0.85
	2	1.88 $\pm$ 1.01	1.53 $\pm$ 0.66
	4	1.60 $\pm$ 0.72	1.63 $\pm$ 0.75
	8	1.40 $\pm$ 0.59	1.04 $\pm$ 0.87
	10	1.18 $\pm$ 0.19	1.39 $\pm$ 0.66

	12	1.46 ± 0.36	1.52 ± 0.83
	24	1.45 ± 0.51	1.50 ± 0.50
7	0	1.72 ± 0.52	1.67 ± 0,73
	2	1.51 ± 1.01	1.14 ± 0.64
	4	1.68 ± 0.96	1.74 ± 0.61
	8	2.13 ± 1.60	1.82 ± 0.45
	10	2.36 ± 0.54	2.26 ± 0.34
	12	1.54 ± 0.39	1,39 ± 0.61
	24	2.06 ± 1.01	1.44 ± 0.59
14	0	1.49 ± 0.77	1.58 ± 0.65
	2	1.27 ± 0.15	1.36 ± 0.51
	4	1.36 ± 0.28	1.53 ± 0.40
	8	1.06 ± 0.41	1.07 ± 0.39
	10	1.24 ± 0.44	1.06 ± 0.56
	12	1.20 ± 0.23	1.38 ± 0.59
	24	1.25 ± 0.35	1.27 ± 0.75
28	0	1.40 ± 0.35	1.07 ± 0.60
	2	1.33 ± 0.32	1.59 ± 0.72
	4	1.40 ± 0.12	1.57 ± 0.69
	8	1.61 ± 0.85	0.98 ± 0.23
	10	1.29 ± 0.73	1.13 ± 0.29
	12	1.23 ± 0.27	1.11 ± 0.55
	24	1.53 ± 0.45	1.06 ± 0.22

Table 7.

Summary of measured plasma isotretinoin values at each sampling time on each study day. Values are the mean ± SD as ng/ml 13-cis retinoic acid.

Day	Time (hr)	Acticin Gel	Retin-A Gel
0	0	1.10 ± 0.72	0.89 ± 0.66
	2	1.28 ± 0.96	1.07 ± 0.79
	4	1.23 ± 1.02	0.96 ± 0.43
	8	1.03 ± 0.13	0.82 ± 0.47
	10	1.02 ± 1.13	0.87 ± 0.47
	12	1.13 ± 0.42	1.13 ± 0.80
	24	0.90 ± 0.25	0.89 ± 0.38
7	0	1.16 ± 0.48	1.49 ± 0.79
	2	0.76 ± 0.56	0.69 ± 0.48
	4	0.64 ± 0.44	0.75 ± 0.59
	8	0.66 ± 0.34	0.64 ± 0.37
	10	0.75 ± 0.25	0.75 ± 0.25
	12	0.86 ± 0.25	0.83 ± 0.19
	24	0.77 ± 0.29	0.46 ± 0.26
14	0	0.87 ± 0.43	0.83 ± 0.26
	2	0.97 ± 0.67	0.91 ± 0.39
	4	0.97 ± 0.51	0.94 ± 0.26
	8	0.89 ± 0.40	0.77 ± 0.26
	10	0.93 ± 0.31	0.83 ± 0.34
	12	0.88 ± 0.47	0.73 ± 0.22
	24	1.02 ± 0.86	0.72 ± 0.25
28	0	0.80 ± 0.50	0.55 ± 0.31
	2	0.83 ± 0.50	0.73 ± 0.35
	4	0.80 ± 0.54	0.83 ± 0.42
	8	0.72 ± 0.40	0.82 ± 0.56
	10	0.79 ± 0.37	0.59 ± 0.40
	12	0.81 ± 0.51	0.69 ± 0.29
	24	0.91 ± 0.50	0.70 ± 0.33

The area-under-the-curve (AUC_{24}), Cmax (maximum concentration observed within each 24 hour period) and C_{ss} (mean concentration across the 24 hour period assuming steady-state levels) for both tretinoin and isotretinoin were calculated. The data are presented in Tables 9-10 and Figure in page 36.

Study Day 7 tretinoin values were slightly higher than the other study days, and Study Days 14 and 28 tended to be lower than Study Day 0. As can be seen in Table 9, Study Days 14 and 28 were found to be statistically different from Study Day 7 for AUC, Cmax and Css regardless of formulation. For Css, Study Day 7 was statistically greater than Study Day 0; both AUC and Cmax were not statistically different from Study Day 0 (baseline). There were no statistical differences observed in these parameters for the isotretinoin data (Table 10). In addition, there was no statistically relevant correlation between these three parameters and the clinical observation data.

Table 9:
Summary of plasma level data for tretinoin.

Product	Day	AUC	Cmax (ng/ml)	Css (ng/ml)
Acticin Gel	0	35.42 ± 8.70	2.37 ± 0.98	1.48 ± 0.42[c]
	7	42.47 ± 8.98	3.39 ± 1.01	1.83 ± 0.31
	14	29.70 ± 3.90[a]	1.83 ± 0.59[b]	1.27 ± 0.14[c]
	28	33.74 ± 9.45[a]	1.96 ± 0.75[b]	1.37 ± 0.27[c]
Retin-A Gel	0	34.63 ± 10.89	2.42 ± 0.72	1.50 ± 0.40[c]
	7	37.57 ± 4.91	2.53 ± 0.42	1.64 ± 0.18
	14	31.51 ± 9.84[a]	1.93 ± 0.60b	1.32 ± 0.37[c]
	28	27.83 ± 7.95[a]	1.67 ± 0.64b	1.19 ± 0.36[c]

a. AUC values found to be statistically different from study day 7; p = 0.0162
b. Cmax values found to be statistically different from study day 7; p = 0.0073
c. Css values found to be statistically different from study day 7; p = 0.0033
AUC = area under the plasma concentration-time curve, ng/ml-hr.
Cmax = maximum concentration observed with the 24 hour study day period, ng/ml.
Css = mean concentration over the 24 hr study day sampling period.

Table 10
Summary of plasma level data for isotretinoin.

Product	Day	AUC	Cmax(ng/ml)	Css(ng/ml)
Acticin Gel	0	25.72 ± 8.20	1.91 ± 0.98	1.09 ± 0.43
	7	18.08 ± 4.30	1.34 ± 0.36	0.89 ± 0.24
	14	22.49 ± 11.81	1.41 ± 0.84	0.93 ± 0.45
	28	20.49 ± 12.83	1.15 ± 0.51	0.91 ± 0.38
Retin-A Gel	0	23.04 ± 9.85	1.57 ± 0.82	0.97 ± 0.43
	7	17.12 ± 2.85	1.69 ± 0.65	0.80 ± 0.14
	14	18.85 ± 5.77	1.08 ± 0.33	0.82 ± 0.26
	28	17.61 ± 7.25	1.00 ± 0.44	0.72 ± 0.31

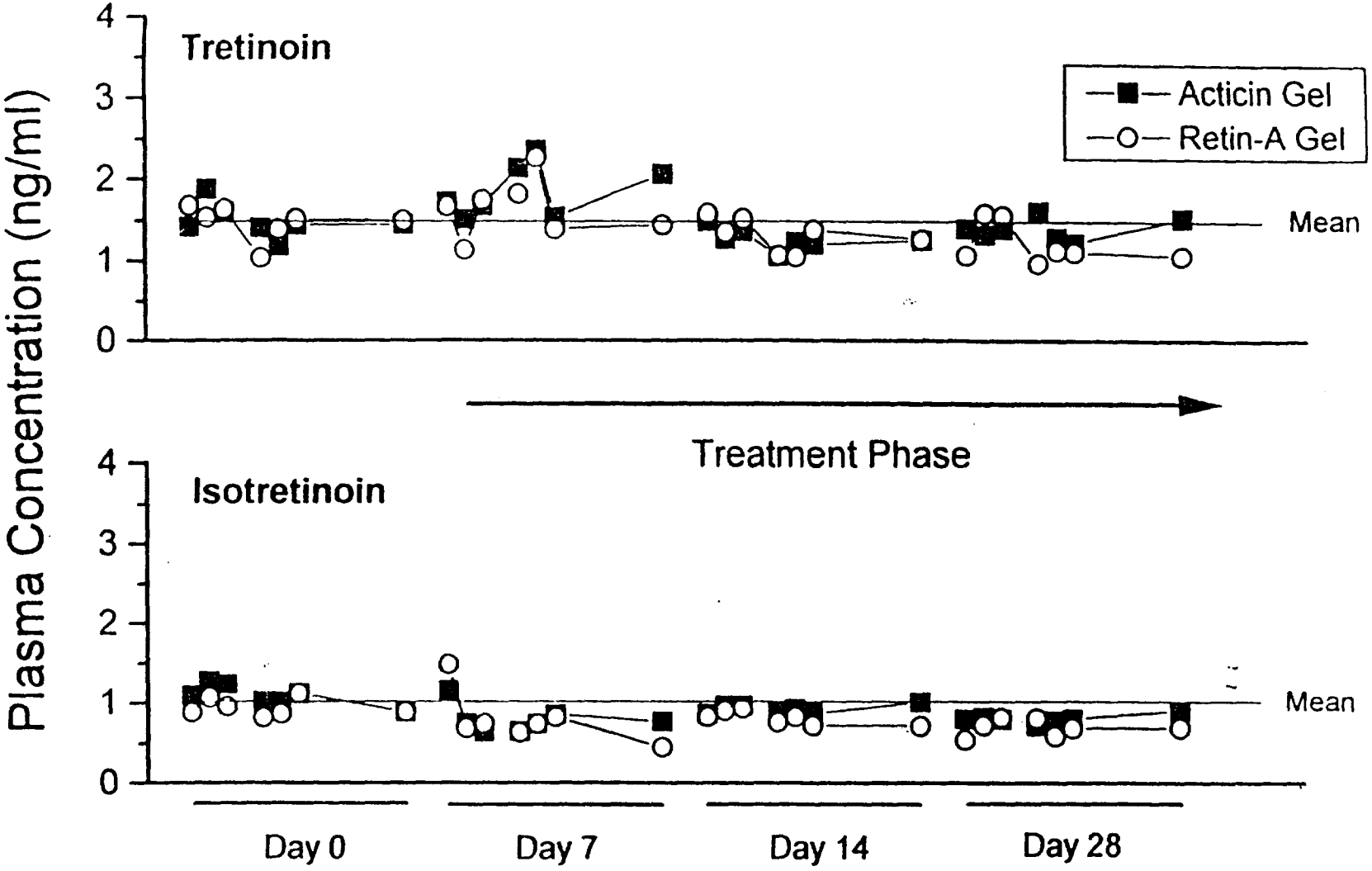


Figure 3: Mean plasma levels of tretinoin and isotretinoin grouped by formulation. Mean line derived from the baseline values across all subjects and time points on Study Day 0. Data are ng/ml from Tables 5 and 6.

Conclusion:

1. There were no statistical differences in tretinoin and isotretinoin plasma pharmacokinetic parameters between Retin-A 0.025% Gel and Acticin 0.025% Gel.

2. Decrease in AUC, Cmax and Css plasma tretinoin values from Study Day 7 to Study Days 14 and 28. Further, there was a slight but statistically significant increase in Css tretinoin from Study Day 0 to Study Day 7.

3. There were no statistical differences in the plasma pharmacokinetic parameters for isotretinoin cross study days.

APPENDIX III

Clinical observation associated with the PK study

Clinical observation associated with the PK study

In the clinical PK study, two types of clinical observation were recorded. All subjects began this study with no observable erythema, peeling or dryness. After 6 days of topical application, virtually every subject demonstrated at least two of the three observable irritation responses. These results are presented in Table 11 and 12.

Table 11

Summary of clinical observations. Number of subjects recorded per grading score for erythema, peeling and dryness for **Acticin Gel** on each Study Day visit.

Observation	Days	# of Subjects per Score				
	Score	0	0.5	1	1.5	2
	0	9	0	0	0	0
Erythema	7	4	2	3	0	0
	14	0	2	6	0	0
	28	1	3	3	2	0
	0	9	0	0	0	0
Peeling	7	3	2	4	0	0
	14	0	4	2	2	0
	28	2	4	1	2	0
	0	8	1	0	0	0
Dryness	7	1	1	7	0	0
	14	0	2	4	2	0
	28	0	4	2	0	3

Table 12

Summary of clinical observations. Number of subjects recorded per grading score for erythema, peeling and dryness for **Retin-A** on each Study Day visit.

Observation	Days	# of Subjects per Score				
	Score	0	0.5	1	1.5	2
	0	9	0	0	0	0
Erythema	7	8	0	0	0	1
	14	1	6	1	0	0
	28	0	7	1	0	1
	0	9	0	0	0	0
Peeling	7	4	2	1	1	1
	14	0	5	1	1	1
	28	0	6	2	0	1
	0	9	0	0	0	0
Dryness	7	4	1	3	1	0
	14	0	4	3	1	0
	28	1	4	0	3	1

Erythema was observable in 6 of the 18 subjects on Study Day 7, This was more evident for the Acticin Gel formulation (5 of 9 subjects > 0 score) than for the Retin-A Gel formulation (1 of 9 subjects > 0). Peeling was observable in 11 of 18 subjects (61%) and skin dryness was noted in 13 of 18 subjects (72%) on Study Day 7. Peeling and dryness were essentially equally distributed between the two formulations.

On Study Days 14 and 28, all the subjects demonstrated two or more of the three criteria for retinoic acid irritation. Consistent with the results seen on Study Day 7, the Retin-A Gel formulation gave lower scores for erythema than the Acticin Gel formulation on Study Day 14 and 28, Peeling and dryness scores were similarly distributed for both formulations on Study Days 14 and 28. Regardless of formulation, overall irritation scores lessened (indicating accommodation to the retinoic acid exposure), with daily application of the products.

No subject demonstrated an excessive irritation response, nor were any withdrawn from the study and no subject required an alteration in the dosing schedule due to the irritation. The observations were consistent with the typical irritation response seen in acne patients who have been prescribed Retin-A Gel products.

Trans-Epidermal-Water-Loss (TEWL)

All subjects demonstrated a change in normal trans-epidermal-water-loss after 6 days of topical retinoic acid exposure. The data are summarized in Table 11. To simplify the observations, the values from both cheeks and forehead were averaged for each subject on each study day to provide a mean "face" value for TEWL. On Study Day 7, mean TEWL on all three sites had significantly increased by 50% or greater over baseline values ($p = 0.0001$). Further, this increased TEWL is maintained throughout the 28 days of topical tretinoin exposure. There was no statistical difference between the two formulations for a given site on a given study day.

Table 13.

Summary of recorded (TEWL) from each site on each study day. Mean Face value is the average across the three sites per subject on each study day. Values are the mean ± SD as $gm/m^2/hr$ water.

Product	Day	Forehead	Left Cheek	Right Cheek	Mean
Acticin Gel	0	21 ± 15.6	15.9 ± 4.9	13.9 ± 3.7	17.0 ± 4.0
	7	32.6 ± 11.1	43.2 ± 14.8	43.2 ± 11.1	35.5 ± 9.7
	14	34.7 ± 7.7	32.9 ± 7.8	32.7 ± 8.6	33.4 ± 7.4
	28	37.1 ± 10.8	36.4 ± 15.9	37.5 ± 14.8	37.0 ± 12.5
Retin-A Gel	0	21.4 ± 4.3	20.0 ± 8.3	20.0 ± 7.8	20.5 ± 6.6
	7	31.6 ± 10.5	33.6 ± 11.1	33.0 ± 13.6	32.7 ± 7.5
	14	33.8 ± 8.0	41.5 ± 9.7	41.9 ± 13.9	39.1 ± 7.6
	28	34.5 ± 10.1	32.6 ± 13.9	36.6 ± 12.9	35.5 ± 12.

Conclusion:

1. Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal irritation response as assessed by erythema, peeling and dryness.

2. Retin-A 0.025% Gel and Acticin 0.025% Gel demonstrated equal physiological alteration of the stratum corneum as assessed by TEWL

APPENDIX IV

LABELING

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