CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-922

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

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Clinical Pharmacology/Biopharmaceutics Review

NDA: 20-922 SUBMISSION DATE: 12/30/97

PRODUCT: 2% 4-Hydroxyanisole/0.01%All-Trans Retinoic acid Topical Solution

SPONSOR: Bristol-Myers Squibb Pharmaceutical Research
Buffalo, NY 14213

REVIEWER: Veneeta Tandon, Ph.D.

Review of a NDA

I. Background

2% 4-Hydroxyanisole/0.01% all-trans retinoic acid topical solution is indicated for the treatment of solar lentigines [redacted] These are localized, pigmented, macular lesions of the skin, usually on the areas of the body which have been chronically exposed to sunlight and are characterized by increased number of active melanocytes and increased melanin production. It has been indicated for twice daily application of the solution avoiding application to surrounding skin.

4-Hydroxyanisole is the monomethyl ether of hydroquinone. Hydroquinone is a known skin bleaching agent, marketed in US in OTC preparations at concentrations of 1.5% and 2% and in prescription products in 3% and 4%. 4-hydroxyanisole has been marketed in Europe and other countries at concentrations of 5-20% and has also been used as antioxidants in cosmetic products in the US at concentration up to 1.0%.

All-trans retinoic acid (tretinoin or vitamin A acid) is used in the US in topical preparations for the treatment of acne vulgaris at concentrations of 0.1%, 0.05%, 0.025% and 0.01%, and for the mitigation of fine wrinkles, mottled hyperpigmentation and tactile roughness resulting from chronic sun exposure at concentrations of 0.05%

The same concentration of each ingredient alone had little or no activity, but in combination showed moderate to complete reversal of the hyperpigmentation in Yucatan pig model.

II. Recommendation

Based on the results, an insignificant increase in the normal endogenous level of tretinoin occurs from the topically applied 2% 4-Hydroxyanisole/0.01% all-trans retinoic acid solution in healthy volunteers. Thus systemic toxicity is quite unlikely due to the

minimal percutaneous absorption of tretinoin. The total daily systemic exposure from 4-hydroxyanisole was 66.9 ng.hr/mL in the pharmacokinetics study (DE 132-008), which is about 35 times lower than that seen a 6 month dermal toxicity study in the rat with the highest dose (40/0.2 mg/kg/day, i.e. 2 ml/kg/day). At a dose of 2 ml/kg/day only a very slight increase in neutrophil count and serum potassium was observed. Other effects were treatment site related irritation. The total application of 2% 4-Hydroxyanisole/0.01% all-trans retinoic acid solution was about 3 times more of that used in the clinical studies for efficacy and safety.

The sponsor has not conducted a pharmacokinetic study in patients with solar lentigo. However, considering the fact that the barrier function of the skin would not be altered in this diseased state and hence the permeation of the active ingredients would not be highly affected under diseased condition, it would be reasonable to accept approval of the submission without a pharmacokinetic study in patients. This is a deviation from our general requirements of conducting pharmacokinetic studies in patients. Moreover, the applicant has also used a higher average amount of the medication in this study as compared to the average amount used in the clinical trials.

The pharmacokinetic information provided is satisfactory for approval of the application.

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III. Formulation

2% 4-Hydroxyanisole/0.01% All-trans retinoic acid topical solution is an ethyl alcohol based solution. The composition of dosage form proposed for marketing (formulation number W1133-M-06-A) is provided in the following table.

Ingredient	% Label	Function
4-Hydroxyanisole (BMS-181158)	2.0 (w/v)	Active
Tretinoin (BMS-181159)	0.01 (w/v)	Active
Ethyl Alcohol, USP	77.8 (v/v)	
PEG-8, NF		
Butylated hydroxy toluene, NF		
Ascorbic Acid, USP		
Citric acid, USP		
Ascorbyl Palmitate, NF		
Disodium EDTA, USP		
Purified Water, USP		

IV. Analytical Validation

Quantitative determination of 4-hydroxyanisole (4HA) and tretinoin (RA) in human plasma was done by Because of instability of RA to full all analyses were done under The standard curve concentrations ranged from ng/ml for 4HA and ng/ml for RA. Quality control (QC) samples containing 4HA and tretinoin were prepared in control human plasma. QC samples containing 4HA in human plasma were prepared at nominal concentrations of 0.154, 5.136, 25.68 and 41.09 ng/mL. QC samples containing tretinoin in human plasma were prepared at nominal concentrations of 4.944, 24.72, 39.44 ng/mL.

- *Performance of standard curve*: % CV within of their nominal values. Standard curves were prepared with both 4HA and RA to mimic the composition of study samples.
- *Accuracy and precision*: %CV less than for 5/6 samples and for the lowest level QC sample for 4HA. % CV less than for RA. Inter- and Intra-day %CV less than for 4HA and less than for RA.
  QC criteria for deviation from nominal was recommended for daily acceptance of analytical runs.
- *Specificity and LLQ*: ng/mL for 4HA and ng/mL for RA. For RA this was over the endogenous level of RA ng/mL) in plasma.
- *Long term stability*: 6 weeks in human plasma.
- *Freeze-thaw stability*: performed with rat and rabbit plasma, not repeated again.

was used to measure radioactivity in biological samples. Adequate measurements were also taken to validate the radioactivity in the plasma, urine and fecal samples and standards. Assessment of radioactivity due to

plasma, urine and fecal samples and standards. Assessment of radioactivity due to tritiated water resulting from the exchange of [redacted] from the [^{3}H] tretinoin or one of its metabolites was also quantitated.

According to the sponsor the accepted analytical runs should meet the folllowing criteria:

- the predicted concentrations of at least three-fourths of all calibration standards shall be within [redacted] for lowest standard) of their nominal concentrations. At least two-thirds of the quality control samples should be within [redacted] of their nominal concentrations.
- r^2 for the standard curve should be [redacted]

Comment

The permissible % CV for the assay validation ([redacted] and [redacted] for LLQ) is a bit higher than the usual acceptance criteria of [redacted] and [redacted] for the lowest concentration (LLQ). However, considering the light sensitivity for tretinoin and derivative volatility of 4-hydroxyanisole the proposed acceptance criteria for this submission should be acceptable.

V. Summary of in-vitro Studies

Study # AU-ST-91001

Effect of Tretinoin on the in-vitro skin permeation of 4-hydroxyanisole

Objectives

Using [redacted] diffusion cells (side-by-side) and excised human cadaver skin, the objectives of in-vitro skin permeation studies were :

(1) to define skin permeation characteristics of 4-hydroxyanisole using saturated solution in the donor compartment
(2) to compare permeation profiles of 4-hydroxyanisole in the presence of tretinoin ranging from 0.005% to 0.05%
(3) to determine the effect of tretinoin pretreatment on skin permeation profile of 4-hydroxyanisole

Saturated solution of 4-hydroxyanisole was prepared by adding excess amount of drug in phosphate buffer (pH 7.0). The equilibrium solubility of 4-hydroxyanisole in buffer was about 4% (w/w). For other skin studies the actives were dissolved in ethyl alcohol (71.5 mL), PEG-8 ([redacted] gm) and water ([redacted] mL. For the pretreated skin experiment, the skin was soaked in the tretinoin containing formulation for 16 hours.

Conclusions

- 4-hydroxyanisole permeated through the skin rapidly, with the permeation rate decreasing over time, which could be due to the rapid increase in receptor concentration over time.
- The flux of 4-hydroxyanisole increased in the presence of tretinoin, the flux values are shown in the table below. The increase in flux was more pronounced at tretinoin concentration of 0.05%. Increasing concentration to 0.1% caused precipitation of the tretinoin. The reason for skin permeation enhancement for 4-hydroxyanisole is not understood.

Tretinoin Conc (%w/v)	4-hydroxyanisole flux (μg/hr-cm²)± S.D.
0.00	72.58 ± 9.42
0.005	103.40 ± 5.95
0.01	96.00 ± 29.53
0.05	160.12 ± 48.55

- The flux of 4-hydroxyanisole increased two folds when the donor concentration was raised from 2% to 4% which is in accordance with the Fick's first law of diffusion. With the addition of 0.01% tretinoin skin permeation was enhanced with both 2% and 4% solutions with an enhance factor of 1.32 and 1.45, respectively. However, the skin permeation of 4-hydroxyanisole, expressed as the percent of applied dose was less with the hydroalcoholic solution. The solubility of 4-hydroxyanisole in 95% ethanol is about 40% (w/w).

4-hydoxyanisole conc. (%w/v)	Tretinoin conc (%w/v)	4-hydroxyanisole flux (μg/hr-cm²)± S.D.
2	0	72.58 ± 9.42
2	0.01	96.00 ± 29.53
4	0	160.86 ± 15.67
4	0.01	232.91 ± 17.52

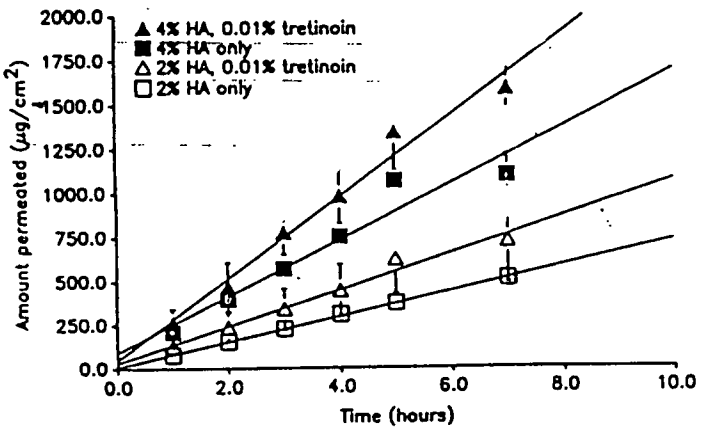

Figure: Skin permeation of 4HA from hydroalcoholic solutions using [redacted] diffusion cells

- Skin permeation of 4-hydroxyanisole was enhanced with tretinoin pretreated skin regardless of the presence of tretinoin in the donor compartment.

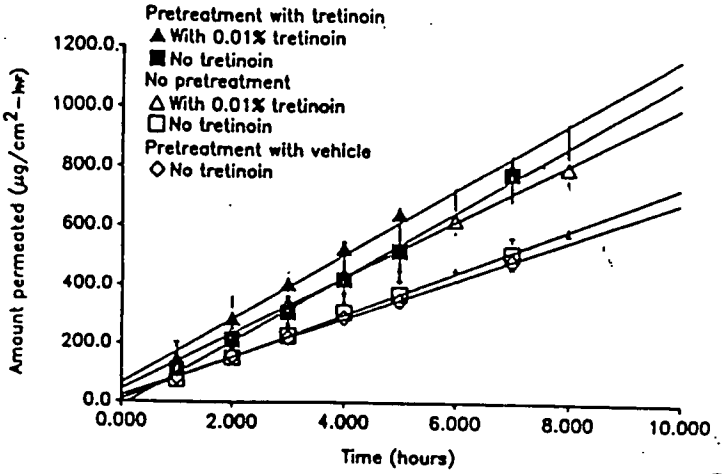

Figure: Skin permeation of 4HA from a 2% hydroalcoholic solution using [redacted] diffusion cells

Study # PARAB-92005

In-vitro human skin permeation of 4-hydroxyanisole after finite dose application of hydroalcoholic solution formulations of 2% 4-hydroxyanisole with and without 0.01% tretinoin.

Objectives

(1) To determine the permeation of 4-hydroxyanisole across the skin
(2) to determine the retention of 4-hydroxyanisole in the epidermis and dermis of the skin after finite dose application (28 µl/cm^2) of hydroalcoholic solution formulations of 2% 4-hydroxyanisole with and without 0.01% tretinoin

Two hydroalcoholic solution formulations FN1-31816-20 (2% 4-hydroxyanisole + 0.01% tretinoin) and FN1-31816-58 (2% 4-hydroxyanisole) were used in this skin permeation study. Both formulations were quantitatively similar, except for the presence and absence of tretinoin. Excised human cadaver skin and [redacted] diffusion cells were used to study the permeability for 72 hours.

Conclusions

- the permeation of 4-hydroxyanisole across the skin is not significantly influenced by tretinoin after single finite dose application. These results are consistent with that observed in in-vitro skin permeation using mouse skin.

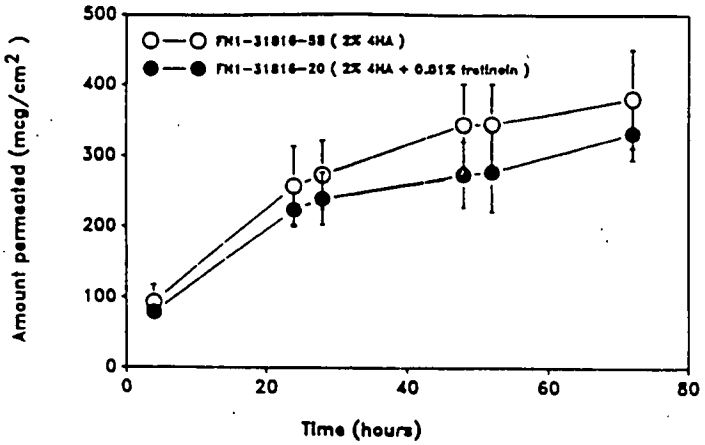

Figure: Skin permeation of 4-hydroxyanisole (mean values, N=3)

- About 45 and 70% of the applied dose of 4-hydroxyanisole permeated across the skin in 24 and 72 hours, respectively from the two formulations.
- The permeation profile was biphasic in nature, with an initial enhanced rate of permeation followed by slow permeation.
- There is no significant difference in the percentage to the applied dose of 4-hydroxyanisole retained in the epidermis and dermis after a single finite application. For the formulation containing 2% 4-hydroxyanisole and 0.01% tretinoin , the amount of 4-hydroxyanisole retained in the epidermis and dermis after 72 hours is 0.48% and 0.67% of the applied dose respectively. This is 0.78 µg and 0.60 µg per mg of dry epidermis and dermis, respectively. It is 0.61% and 0.49% respectively in the epidermis and dermis for the formulation containing 2% 4-hydroxyanisole.

Note: Increased permeability to 4-hydroxyanisole after multiple topical treatments with the combination formulation was suggested by two toxicokinetic studies in mice, where plasma and serum concentration of 4-hydroxyanisole was greater in the combination product than in the formulation containing 4-hydroxyanisole alone.

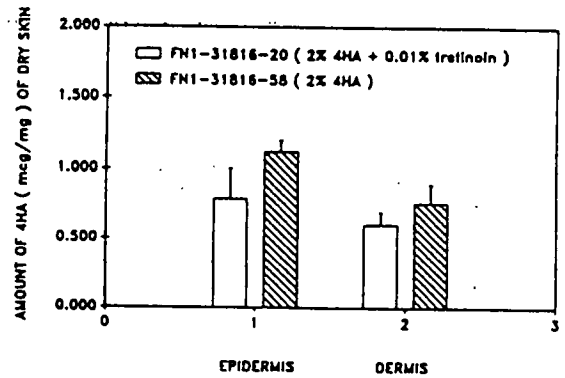

Figure : Skin retention of 4-hydroxyanisole at 72 hours (mean values, N=3)

Study #PARAB-PV-92032

In vitro human skin permeation of tretinoin after finite application of hydroalcoholic formulation of 0.01% tretinoin with or without 2.0% 4-hydroxyanisole.

Objectives

(1) To determine the permeation of tretinoin across the skin
(2) to determine the retention of tretinoin in the epidermis and dermis of the skin after finite dose application (21 µl/cm^2) of hydroalcoholic solution formulations of 0.01% tretinoin with and without 2% 4-hydroxyanisole.

Two hydroalcoholic solution formulations FN1-31816-107 (2% 4-hydroxyanisole + 0.01% tretinoin) and W1133-M-04-A (0.01% tretinoin) were used in this skin permeation study. Each formulation was spiked with [11, 12,-^{3}H]-tretinoin to result in a radioactive concentration of 60 µCi/ml of formulation. Both formulations were quantitatively similar, except for the presence and absence of 2% 4-hydroxyanisole. Excised human cadaver skin and [redacted] diffusion cells were used to study the permeability for 76 hours.

Conclusions

- About 5% and 7% of applied dose of tretinoin permeated across the skin after 76 hours from the formulation with and without 2% 4-hydroxyanisole respectively.
- The permeation profile is biphasic in nature with an initial slow input followed by a faster input during apparent steady state phase. The slopes of the initial phase (0-20 hrs) of the two treatments were not significantly different, but the slopes in the second phase (24-76 hrs) were significantly different ($p < 0.001$), indicating that in the presence of 2% 4-hydroxyanisole the apparent steady state flux for tretinoin across the skin is reduced. The apparent steady state flux of tretinoin were 2.2 and 2.7 ng/cm^2/hr in the presence and absence of 2% 4-hydroxyanisole, respectively.

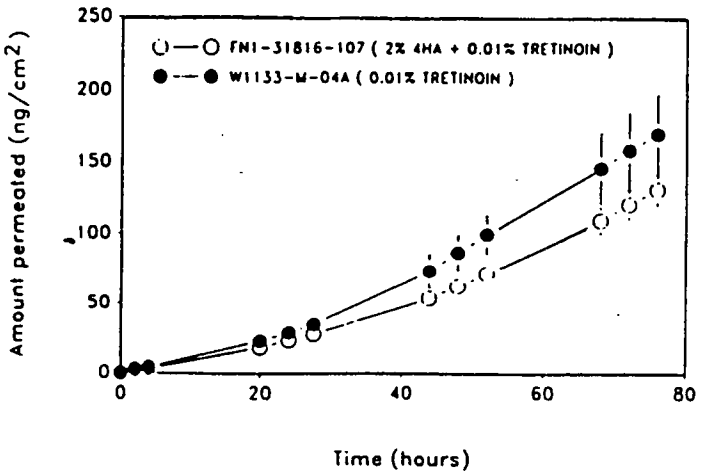

Figure: Skin permeation of treinoin (mean values, N=5)

- For the formulation containing 2% 4-hydroxyanisole + 0.01% tretinoin, the amount of tretinoin retained in the epidermis and dermis after 76 hours of application were about 22.8% and 1% of applied dose and were about 182 ng and 6.7 ng per mg of dry

epidermis and dermis, respectively. For the formulation containing 0.01% tretinoin only, the amount of tretinoin retained in the epidermis and dermis after 76 hours of application were about 26.5% and 0.7% of applied dose and were about 206 ng and 7 ng per mg of dry epidermis and dermis, respectively. This suggested that there was no significant difference in the skin retention between the two treatments, though the flux of tretinoin was reduced in the presence of 2% 4-hydroxyanisole. This may in turn suggest that after multiple treatment, the 4-hydroxyanisole may possibly reduce the permeation of tretinoin across the skin without affecting its skin retention.

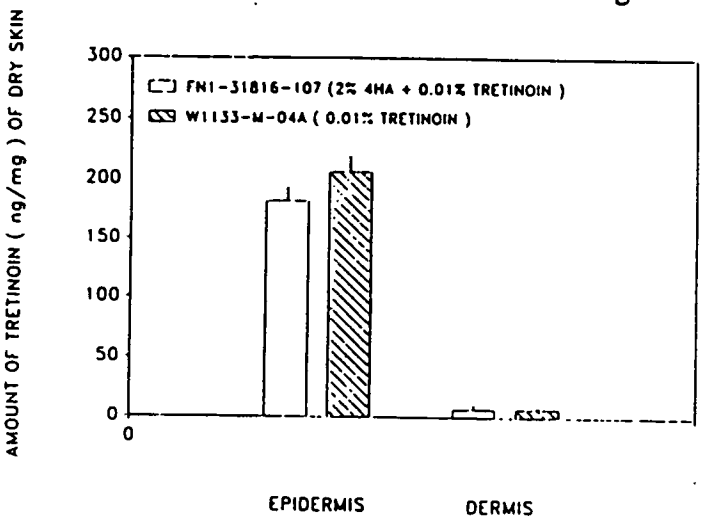

Figure: Skin retention of tretinoin at 76 hours (mean values, N=5)

VI. Summary of in-vivo study

Study # DE 132-008

Percutaneous absorption of [^{3}H] tretinoin from 2% 4-hydroxyanisole/0.01% [^{3}H] tretinoin solution in healthy volunteers

Clinical Site -

Analytical Site

Objectives

- To estimate the extent of percutaneous absorption of [^{3}H] tretinoin
- And to estimate the systemic exposure to 4-hydroxyanisole after topical application of solution of 2% 4-hydroxyanisole/0.01% [^{3}H] tretinoin to the back of healthy volunteers

- And to estimate the systemic exposure to 4-hydroxyanisole after topical application of solution of 2% 4-hydroxyanisole/0.01% [^{3}H] tretinoin to the back of healthy volunteers

Study Design

BID topical application of nonradiolabeled 2% 4HA/0.01% tretinoin solution (Formulation No.W1133-M-08-B/ Batch No. B96E002-1) on a 400 sq cm area of the back for 14 days on 8 healthy volunteers, followed by single topical application of about 0.8 mL of 2% 4HA.0.01% [^{3}H] tretinoin (Formulation/Batch No. SNB-1126-08) (about 200 μCi). The radiolabeled dose remained in contact with the skin for 12 hours, after which it was removed by washing the application site. Nonradiolabeled 2% 4HA/0.01% tretinoin solution was again applied BID for additional 7 days. For the non radiolabeled dose the subject was instructed not to shower for at least 6 hours following the application.

The mean dose applied was 0.678 gms. This corresponds to the application of 0.77 mL of the dosing solution. This is equivalent to the application rate of 1.92 μl/ cm^2, which is close to that recommended. Similar application rate was also achieved in clinical studies and same applicators were used in the clinical and pharmacokinetics study. The mean dose of active drug was 37.3 μg/cm^2 for 4HA and 0.23 μg/cm^2 for [^{3}H] tretinoin. A mean of 212 μCi of radioactivity was applied to the back of the subjects. The age of the subject ranged from 31 to 57 (mean 45 ± 8.3). The dose for each subject has been shown in the Appendix on page 16.

Note: The dose used in this study, projected to a month corresponds to 48.0 mls/month, where as in the clinical efficacy and safety studies a range of 14.8-17.6 mls/months of the formulation has been used. Therefore, it appears that the subjects used one third as much study drugs in the clinical trials as that in this percutaneous absorption study. Subjects in the clinical studies dosed 133 cm^2 (400 cm^2/3), which was an average of 1.7% of the body surface area. Therefore the dose used in this pharmacokinetics study does seem to be reasonable.

Blood sampling

At 0, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168 hours following the radiolabeled dose .

Urine sampling

Day -1, 0 to 4 hr, 4 to 8 hr, 8 to 12 hr, 12 to 16 hr, 16 to 24 hr after the administration of morning radioactive dose, and 6 collections at 24 hrs on Days 2 to 8.

Plasma concentration and pharmacokinetic parameters

Tretinoin

With the exception of two plasma samples, all samples analyzed were below the limit of assay (LLQ= ng/mL) as determined by specific Since the assay was designed to measure concentrations of tretinoin above the endogenous concentration (about 1 ng/mL), this suggests that plasma concentrations were not increased above the endogenous levels by topical application of this formulation. Regarding the two plasma samples the applicant speculates these values could be the result of variability in analytical method, normal variation in tretinoin concentrations, or dietary intake of Vitamin A. Vitamin A was contraindicated in the study, but no attempt was made to control the diet. These results are similar to other clinical trials in which the plasma concentrations of tretinoin did not significantly increase in healthy subjects after topical application[1,2] . The individual subject data is given in the Appendix on page 17.

The concentrations of total radioactivity in plasma were highly variable. The mean C_{max} value for total radioactivity in plasma was 91 pg/mL (range pg/mL) and median T_{max} value was 18 hr (range hr). The pharmacokinetic parameters for total radioactivity in plasma (equivalent of [^{3}H] tretinoin) is shown in the table below:

Subject	C_{max} (pg/mL)	T_{max} (hrs)
Mean SD	91 118	18*(median) (12,48)

[1] Latriano L. et al, The percutaneous absorption of topically applied tretinoin and its effect on endogenous concentrations of its metabolites after single doses or long term use. J. Am. Acad. Dermatol, 1997, 36(3), S37-S46.

[2] Buchan P. et al., repeated topical administration of all-trans-retinoin acid and plasma levels of retinoic acids in humans. J. Am. Acad. Dermatol. 1994,30(3), 428-34.

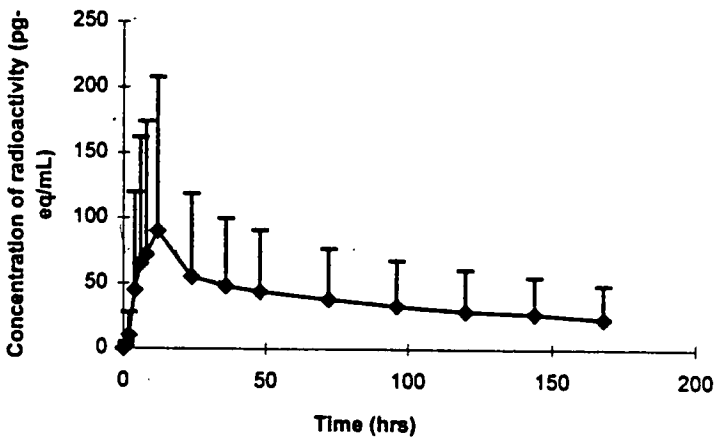

Figure: Mean (SD) Plasma concentrations of total radioactivity of (equivalent of [^{3}H] tretinoin) after topical application of 4-hydroxyanisole/[^{3}H] tretinoin to the back of healthy volunteers.

4-hydroxyanisole

The C_{max} for 4HA in plasma was 9.92 ng/mL (range [redacted] ng/mL) and the median T_{max} was 2 hr (range [redacted] hr). The mean AUC_{0-12} was 33.43 ng.hr/mL. Therefore, the daily systemic exposure will be 66.9 ng.hr/mL. The AUC was about 16.6 times higher after 13 weeks of dosing at highest dose given in 3-month range-finding study in mice, and about 34.6 times higher after 21 weeks at highest dose given in 6-month dermal toxicity study in rat. The individual plasma concentrations for 4HA are shown in the Appendix on page 17. The Pharmacokinetic parameters for 4HA are given in the table below:

Subject No	C_{max} (ng/mL)	T_{max}	$AUC_{(0-12)}$ (ng.hr/mL)
[redacted]			
Mean	9.92	2* median	33.43
SD	7.48	(1,2)	14.30

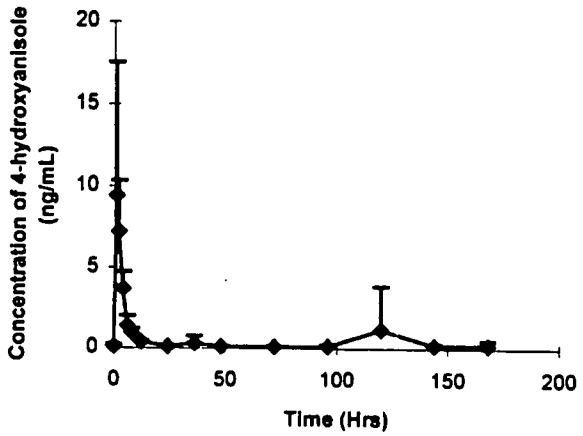

Figure: Mean (SD) plasma concentration of 4-hydroxyanisole after topical application of 4-hydroxyanisole/[^{3}H] treinoin to the backs of healthy volunteers.

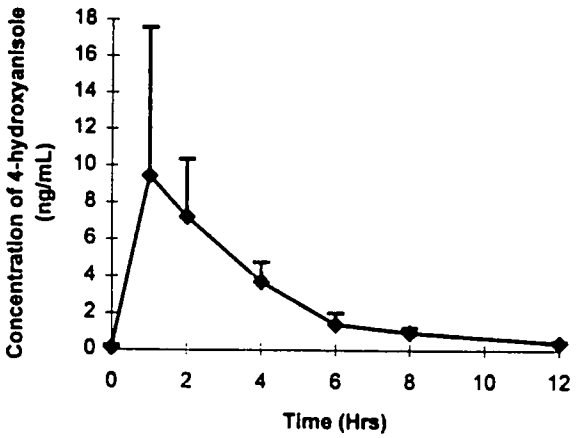

Figure: Mean (SD) plasma concentration of 4-hydroxyanisole (expanded for the first 12 hours) after topical application of 4-hydroxyanisole/[^{3}H] treinoin to the backs of healthy volunteers.

Recovery of radioactivity from urine, feces and application site

The mean recovery of radioactivity from urine was 3.25% and in feces was 1.17% of the dose over the 168 hr collection period. Mean recovery from the application site was 88%. Mean overall recovery from urine, feces and application site was 92.4%. The tables for the cumulative recovery of radioactivity for each subject is shown in the Appendix on page 18.

Percutaneous absorption of [^{3}H] tretinoin

The mean percutaneous absorption of [^{3}H] tretinoin from the formulation was found to be 4.43% of the dose (range [redacted] Previous clinical studies have

shown the percutaneous absorption to range from 0.5% to 7%[3,4,5]. The percutaneous absorption calculated from the total excretion of radioactivity in urine and feces is shown below. The individual subject data is given in the Appendix on page 19.

Statistic	Total excretion in urine, U (% of dose)	Total excretion in feces, F (% of dose)	Percutaneous absorption D=U+F (% of dose)	Total recovery (%applied)
Mean	3.25	1.17	4.43	92.4
SD	3.74	1.47	4.84	2.75

Comment

- Tretinoin metabolites concentrations in plasma were not determined in this study. However, reference 1 and 2 suggest that after single dose or long term treatment with topical retinoin formulations the endogenous levels of 13-cis-retinoic acid or 13-cis-4-oxo-retinoic acid were not effected. Endogenous levels of 13-cis-retinoic acid or 13-cis-4-oxo-retinoic acid have been reported as 1.63 ng/mL and 3.68 ng/mL, respectively[6].
- No formal gender or race analysis was done, however out of the 8 subjects used this human study, two were females, one black and one Indian. No observable differences were seen amongst them.
- The exact to be marketed formulation has not been used in this study, the difference lies only the amount of overage for tretinoin (it has been increased from)

VII. Conclusions

No significant increase beyond endogenous levels of tretinoin were seen in this study. The systemic exposure from 4-hydroxyanisole is 66.9 ng.hr/ml. The maximum exposure to 4HA in mice was 16.6 times the exposure in humans in this pharmacokinetics study and in rats was 34.6 times the exposure in humans in this study. In humans the daily dose of 4HA was 0.37mg/kg (for 21 days BID), in mouse it ranged from 0.22-3.375 µg/kg (for 3 months BID) and ~~in rats it ranged from 4-40~~ mg/kg (for 6 months BID). At a dose of 40 mg/kg/day in rats only a very slight increase in neutrophil count and serum potassium was observed. Other effects were treatment site related irritation.

The human study is acceptable from the pharmacokinetics stand point.

[3] Schafer H. et al, Penetration of Vitamin A acid into human skin. Acta Derm. Venereol (Stockh) Suppl 1975, 74, 50-5.

[4] Worobec SM et al, Percutaneous absorption of ^{3}H -tretinoin in normal volunteers. [Abstratract],Clin. Res, 1990, 38,786A.

[5] Kemper C. et al. Percutaneous absorption of ^{3}H -tretinoin following long term administration of topical tretinoin. [Abstract], Dermatologica, 1990, 181, 351.

[6] Eckhoff C. et al. Identification and quantification of all-trans- and 13-cis-retinoic acid or 13-cis-4-oxo-retinoic acid in human plasma. J. lipid Res. 1990,31,175-82.

/S/ 4/1/98

Veneeta Tandon, Ph.D.
Pharmacokineticist
Division of Pharmaceutical Evaluation III

Team Leader: E. Dennis Bashaw, Pharm. D. /S/ 6/2/98

CC: NDA 20-922 (ORIG)
HFD-540/Div File
HFD-540/CSO/Cross
HFD-880(Bashaw/Tandon)
HFD-880(Lazor)
HFD-344(Viswanathan)
CDR ATTN: B.Murphy

AP

APPEARS THIS WAY
ON ORIGINAL

APPENDIX

Table 1

SUBJECT DEMOGRAPHY

Treatment=All Subjects

	Subject Number	Age (yr)	Height (cm)	Weight (kg)	Gender	Race	Build
	1	46	175.3	102.6	Male	Black	Large
	2	43	177.8	81.0	Male	White	Medium
	3	42	167.6	70.7	Female	White	Medium
	4	51	170.2	74.3	Female	White	Medium
	5	31	175.3	76.5	Male	INDIAN	Medium
	6	57	186.7	69.8	Male	White	Medium
	7	53	188.0	94.5	Male	White	Large
	8	40	175.3	94.5	Male	White	Large
N		8	8	8			
MEAN		45	177.0	83.0			
STD		8.3	7.16	12.51			
MIN		31	167.6	69.8			
MAX		57	188.0	102.6			

Table 2: Dose of 2% 4-Hydroxyanisole/0.01% [^{3}H]Tretinoin Topically Applied to the Backs of Healthy Volunteers

Subject	Wt. of Solution Applied (g)	4-Hydroxyanisole Applied (mg)	4-Hydroxyanisole Surface Density ($\mu g/cm^2$)	[^{3}H]Tretinoin Applied (μg)	[^{3}H]Tretinoin Surface Density ($\mu g/cm^2$)	Radioactivity Applied (μCi)
1						
2						
3						
4						
5						
6						
7						
8						
Mean	0.678	14.92	37.3	91.5	0.23	212
SD	0.010	0.23	0.6	1.4	0.00	3

Table 3: Individual and Mean (SD) Plasma Concentration Data for Tretinoin

INDIVIDUAL SUBJECT DATA
BMS-181159 STUDY DE133-008 (SFI0198)
TABULATION CODE (3698) 3E
BMS-181159: HUMAN DERMATOLOGY STUDY

PLASMA(K3EDTA) CONCENTRATION OF BMS-181159 (NG/ML) FOR SUBJECTS:

TIME (HR)	0001	0002	0003	0004	0005	0006	0007	0008	N	MEAN	SD	%RSD
CENTER:	001	001	001	001	001	001	001	001				
0.00									8	0.00	0.00	.
1.00									8	0.00	0.00	.
2.00									8	0.00	0.00	.
4.00									8	0.00	0.00	.
6.00									8	0.00	0.00	.
8.00									8	0.00	0.00	.
12.00									8	0.00	0.00	.
24.00									8	0.00	0.00	.
36.00									8	0.00	0.00	.
48.00									8	0.00	0.00	.
72.00									8	0.00	0.00	.
96.00									8	0.00	0.00	.
120.00									8	0.16	0.45	282.84
144.00									8	0.00	0.00	.
168.00									8	0.00	0.00	.
									8	0.16	0.45	282.84

Table 6: Individual and Mean (SD) Plasma Concentration Data for 4-Hydroxyanisole

INDIVIDUAL SUBJECT DATA
BMS-181159 STUDY DE133-008 (SFI0198)
TABULATION CODE (3698) 3E
BMS-181159: HUMAN DERMATOLOGY STUDY

PLASMA(K3EDTA) CONCENTRATION OF 4-HYDROXY ANISOLE (NG/ML) FOR SUBJECTS:

TIME (HR)	0001	0002	0003	0004	0005	0006	0007	0008	N	MEAN	SD	%RSD
CENTER:	001	001	001	001	001	001	001	001				
0.00									8	0.12	0.15	132.35
1.00									8	9.01	8.11	89.98
2.00									8	7.20	3.15	43.77
4.00									8	3.69	1.05	28.52
6.00									8	1.39	0.63	45.08
8.00									8	0.95	0.26	27.44
12.00									8	0.40	0.08	19.30
24.00									8	0.10	0.08	79.47
36.00									8	0.29	0.47	161.99
48.00									8	0.10	0.06	56.44
72.00									8	0.09	0.06	73.59
96.00									8	0.15	0.12	79.98
120.00									8	1.17	2.71	232.38
144.00									8	0.17	0.13	77.40
168.00									8	0.19	0.32	164.19

Table 4: Individual and Mean (SD) Plasma Concentration Data of Total Radioactivity (pg-eg of Tretinoin/mL)

INDIVIDUAL SUBJECT DATA
BMS-181159 STUDY DR133-008 (SPI0268)
TABULATION CODE (1) DERMAL/90 DO
HUMAN BMS-181158/181159 DERMAL STUDY

PLASMA(KJEDTA) CONCENTRATION OF BMS-181159 EQUIVALENT (PG/ML) FOR SUBJECTS:

TIME (HR)	1	2	3	4	5	6	7	8	N	MEAN	SD	%RSD
0.00									8	0	0	.
1.00									8	2	3	192
2.00									8	10	18	177
4.00									8	45	75	168
6.00									8	65	97	150
8.00									8	72	102	141
12.00									8	90	118	132
24.00									8	55	64	115
36.00									8	48	52	108
48.00									8	44	47	107
72.00									8	38	39	104
96.00									8	33	35	104
120.00									8	29	32	111
144.00									8	27	28	106
168.00									8	23	26	112

* The 6 hr plasma samples for subjects 5 and 6 appeared to have been mislabeled at the time of collection. These samples were included in the calculation of the mean plasma concentration at 6 hr, but were excluded from pharmacokinetic analysis.

Table 8: Mean (SD) Cumulative Percent Recovery of Total Radioactivity in Urine, Feces and at the Application Site Following Topical Administration of 4HA/[^{3}H]Tretinoin to Healthy Subjects (N=8)

Specimen	Time (hr)	Cumulative Recovery (% Dose)								
		Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Mean (SD)
Urine	0 - 4									0.11 (0.18)
	0 - 8									0.61 (0.93)
	0 - 12									1.05 (1.58)
	0 - 16									1.09 (1.92)
	0 - 24									2.03 (2.67)
	0 - 48									2.61 (3.18)
	0 - 72									2.86 (3.38)
	0 - 96									3.01 (3.49)
	0 - 120									3.12 (3.59)
	0 - 144									3.19 (3.67)
	0 - 168									3.25 (3.74)
Feces	0 - 24									0.00 (0.01)
	0 - 48									0.33 (0.69)
	0 - 72									0.55 (1.04)
	0 - 96									0.76 (1.26)
	0 - 120									0.87 (1.41)
	0 - 144									1.07 (1.46)
	0 - 168									1.17 (1.47)
Application Site	12									88.01 (5.40)
Total	0 - 168									92.44 (2.75)

Table 9: Percutaneous Absorption of [^{3}H]Tretinoin From a 12-hr Application of 2% 4-Hydroxyanisole/0.01% [^{3}H]Tretinoin

Subject No.	Total Excretion in Urine (% of Dose)	Total Excretion in Feces (% of Dose)	Percutaneous Absorption (% of Dose)
1			
2			
3			
4			
5			
6			
7			
8			
Mean	3.25	1.17	4.43
SD	3.74	1.47	4.84
N	8	8	8
Median	1.5	0.68	2.18
Geometric Mean	1.74	0.7	2.49
95% C.I for Median	0.62-4.89	0.29-1.69	0.92-6.72