

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: NDA 20-769**

**PHARMACOLOGY REVIEW(S)**

**Division of Dermatologic and Dental Drug Products (HFD-540)  
Pharmacology/Toxicology Forward Planning Meeting**

**NDA Number:** 20-769 **Date:** October 15, 1996  
**Drug Name:** Hydrocortisone butyrate **Reviewer:** Javier Avalos  
**CAS Number:** 13609-67-1  
**Drug Type:** New Formulation  
(i.e. NME, new formulation, new indication)  
**Drug Class:** Glucocorticoid  
**Indication:** Relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses  
**Route of Administration:** Topical  
**Date CDER Received:** September 3, 1996  
**User Fee Date:** September 3, 1997  
**Expected Date of Draft Review:** ~~December~~ 18, 1996 *(end of January)*  
**Sponsor:** Yamanouchi Europe B.V.  
Elisabethhof 19 (P.O. Box 108)  
2350 Leiderdorp  
Netherlands  
011-71-45-57-45

**Fileability:**

- |  | <u>YES</u> | <u>NO</u> |
|--|------------|-----------|
| On initial overview of the NDA application:  | <u>YES</u> | <u>NO</u> |
| (1) On its face, is the pharmacology/toxicology section of the NDA organized in a manner to allow substantive review to begin?<br>Comments?  | <u>X</u>   | ___       |
| All pharm/tox studies are referenced to previously approved NDAs.<br>(NDA 18-514, 18-652, and 19-116)  |            |           |
| (2) Is the pharm/tox section of the NDA indexed and paginated in a manner to allow substantive review to begin?  | <u>X</u>   | ___       |
| (3) On its face, is the pharm/tox section of the NDA legible so that substantive review can begin?   | <u>X</u>   | ___       |
| (4) Are all required (*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity*, effects on fertility*, juvenile studies, acute studies*, chronic studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc)? | ___        | <u>X</u>  |

**Comments?**

PK studies with the Locoid Lipocream were not conducted. The Sponsor will need to demonstrate that the PK for the lipocream formulation does not differ from other formulations of hydrocortisone.

- (5) If the formulation to be marketed is different from the formulation used in the toxicology studies, has the Sponsor made an appropriate effort to either repeat the studies using the to be marketed product or to explain why such repetition should not be required? \_\_\_\_\_ X  
Comments? The Sponsor should demonstrate that the PK for the lipocream formulation does not differ from other formulations of hydrocortisone.
- (6) Are the proposed labeling sections relative to pharm/tox appropriate (including human dose multiples expressed in either mg/m<sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57? X \_\_\_\_\_  
Comments? Class label for corticosteroids.
- (7) Has the Sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the Sponsor? \_\_\_\_\_ X  
Comments? In the Pre-NDA meeting (10/8/1992), the proposed plan for the NDA would be acceptable as long as the PK would not be different from what was reported in previously NDAs.
- (8) On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the Sponsor submitted a rationale to justify the alternative route? X \_\_\_\_\_
- (9) Has the Sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? \_\_\_\_\_ X  
Comments? The statement would be by reference to the other NDAs.
- (10) Has the Sponsor submitted the data from the nonclinical carcinogenicity studies, in the STUDIES electronic format, for the review by Biometrics? \_\_\_\_\_ X  
Comments? The Sponsor has not performed carcinogenicity studies on the test substance.
- (11) Has the Sponsor submitted a statement(s) that the pharm/tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns? \_\_\_\_\_ X  
Comments? The statement would be by reference to the other NDAs.

(12) From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not. X \_\_\_\_\_

(13) If the NDA is fileable, are there any issues that need to be conveyed to Sponsor? If so, specify: X \_\_\_\_\_

PK studies with the Locoid Lipocream were not conducted. The Sponsor will need to demonstrate that the PK for the lipocream is not different from previously approved NDAs.

(14) Issues that should not be conveyed to the Sponsor:

\_\_\_\_\_  
Reviewing Pharmacologist/Toxicologist

\_\_\_\_\_  
Pharmacology/Toxicology Team Leader

**Review and Evaluation of Pharmacology and Toxicology Data  
Division of Dermatologic and Dental Drug Products (HFD-540)**

JUL 23 1997

**NDA 20-769 (000) - LABELING ADDENDUM**

**Drug Name:** Locoid (Hydrocortisone butyrate lipocream) Lipocream, 0.1%  
**Drug Category:** Glucocorticoid

**Sponsor:** Yamanouchi Europe B.V.  
Elisabethhof 19 (P.O. Box 108)  
2350 Leiderdorp  
Netherlands  
011-71-45-57-45

**Introduction:** The label submitted by the Sponsor is deficient in the "Mutagenicity" and "Pregnancy: Teratogenic Effects" sections as proposed. A description of the findings from the mutagenicity and teratogenic toxicity studies is not reported in the proposed label. Two mutagenicity studies have been conducted with hydrocortisone while three reproductive toxicity studies have been conducted with hydrocortisone butyrate.

**Recommendation:** In the Carcinogenesis, Mutagenesis, and Impairment of Fertility section, the following sentence should be amended:

This sentence should read:

In the \_\_\_\_\_ section, the following sentence should be amended to read:

Following this sentence, the following sentences need to be included:

Javier Avalos, Ph.D.  
Toxicologist

cc:  
HFD-540  
HFD-540/Pharm/Jacobs  
HFD-540/Pharm/Avalos  
HFD-540/CSO/Anderson  
HFD-540/MO/Huene  
HFD-540/Chem/Pappas

For Concurrence Only  
HFD-540/DD/JWilkin  
HFD-540/Team Leader/Jacobs

JAN 24 1997

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

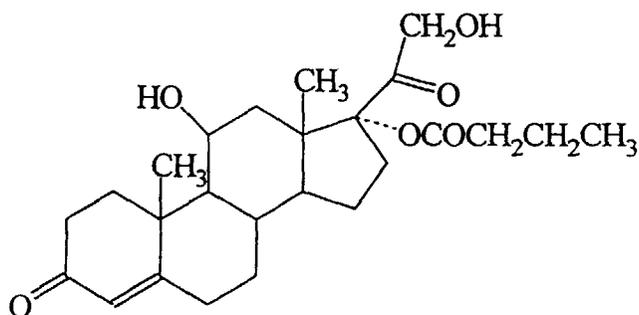
NDA 20-769 (000)

Date Submitted: August 30, 1996  
Date CDER Received: September 03, 1996  
Date Assigned: September 16, 1996  
Number of Volumes: 19

Drug Name: Locoid (Hydrocortisone butyrate lipocream) Lipocream, 0.1%

Chemical Name: 11,21-dihydroxy-17-(1-oxobutoxy)-(11 $\beta$ )-pregn-4-ene-3,20-dione

Chemical Structure:



Molecular Weight: 432.54  
Molecular Formula:  $C_{25}H_{36}O_6$   
CAS Number: 13609-67-1  
Drug Category: Glucocorticoid

Sponsor: Yamanouchi Europe B.V.  
Elisabethhof 19 (P.O. Box 108)  
2350 Leiderdorp  
Netherlands  
011-71-45-57-45

Indication: Relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses

Route of Administration: Topical

Submissions Cross-referenced: IND IND IND IND  
NDA 18-514; NDA 18-652; NDA 19-116.

**Background:** Hydrocortisone butyrate (0.1%) in a cream, ointment, and lotion formulation as Locoid Cream, Locoid Ointment, and Locoid Lotion have been approved since 1982, 1982, and 1987, respectively. The Locoid Cream formulation was discontinued on June 1, 1978. The indication of all the formulations is the relief of inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses.

**Index of Studies:**

All non-clinical studies (pharmacokinetics, pharmacology, toxicology, and reproductive toxicity) are included in the IND (IND and NDA by reference to NDAs 18-514 (Locoid cream-0.1%), 18-652 (Locoid ointment-0.1%), and 19-116 (Locoid lotion-0.1%). Pharmacology/Toxicology reviews and literature article are appended. The reviews appended evaluated the following studies:

**IND**

**NDA 18-514:**

1. Acute toxicity of hydrocortisone 17-alpha-butyrate (H 17B), studied in mice and rats by i.p., s.c., and p.o. routes.
2. Study of the acute toxicity of oral and intraperitoneal HCB in the mouse.
3. Short term local tolerance and irritation studies in rats.
4. Subchronic (28 days) oral toxicity study of HCB in rats (Dr. C.J. Van Eeken).
5. Subchronic (30 days) subcutaneous toxicity study of HCB in dogs (Dr. C.J. Van Eeken).
6. Topical toxicity of HCB in rabbits (at least 21 weeks) (Dr. C.J. Van Eeken).
7. Subacute (35 days) toxicity (subcutaneous) of HCB in rat (T. Aoyama, *et al.*).
8. Chronic (7 months) toxicity (subcutaneous) experiments of HCB (in suspension) in rats (T. Aoyama, *et al.*).
9. Skin irritation experiment in rats.
10. Local tolerance (30 days) on the shaved back of rabbits by epicutaneous application of Locoid cream, ointment, and lotion (S. Ferri, *et al.*).
11. Subacute (2 months) topical toxicity studies with HCB cream, ointment, and lotion in rats (M. Mollet).
12. Experiment (30 days) on the maximum no effect level after subcutaneous administration in rats (J. Takhaski, *et al.*).
13. Comparative study of the embryotoxic and teratogenic effects of cutaneously applied HCB and hydrocortisone in the rat (Dr. C.J. Van Eeken).
14. A comparative study of the embryotoxic and teratogenic effects of topically applied HCB and hydrocortisone in the rabbit (Dr. C.J. Van Eeken).
15. Teratological studies on HCB in mice and rats (T. Aoyama, *et al.*).

**NDA 18-652:**

1. Anti-inflammatory effects of HCB after topical, systemic and also in pellet granuloma tests.
2. Pharmacokinetics were examined in dogs, rats, and humans with HCB.

3. Studies in NDA 18-514 were cross-referenced.
4. Four other studies are summarized by the Sponsor and quoted by the reviewer in his review. These studies are 1) a six month topical toxicity study in rabbits using the ointment preparation, 2) topical application of cream ointment or lotion for 30 days in rabbits and rats, 3) systemic (oral) toxicity study in rats (number of administrations not given), and 4) a subcutaneous toxicity study conducted in dogs (number of administrations not given).

NDA 19-116:

Studies were cross-referenced to NDA 18-514 and 18-652.

Literature Report not submitted in any submission:

Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation-induced chronic skin damage in the hairless mouse. Bissett, D.L., Chatterjee, R., and Hannon, D.P. *Photodermatol Photoimmunol Photomed* 1990;7:153-158.

### **Summary of Studies:**

#### Local Irritation-

As noted in the IND (IND                      , a moderate degree of irritation occurred that decreased with time in animals treated with the lipocream formulation. However, the cream, lotion, and ointment were "well-tolerated" by rabbits after 30 daily topical applications.

#### Pharmacokinetics-

From the reviews of previous submissions, the biopharmacokinetics of hydrocortisone butyrate was examined in dogs, rats, and humans. Hydrocortisone butyrate is converted to hydrocortisone 21-butyrate and later to hydrocortisone. The majority of metabolism occurs with the liver and serum esterases. The serum half-life is about 6-10 hours. Pharmacokinetic data of hydrocortisone is also available in Goodman's and Gilman's Pharmacology text book. Hydrocortisone is reversibly bound up to 90% by two plasma proteins, costeroid-binding globulin (CBG) and albumin. CBG is a high affinity for steroids and low binding capacity while albumin has a high affinity but relatively large binding capacity.

The metabolism of hydrocortisone, in general, involves oxidation followed by conjugation to form water-soluble derivatives. Reduction of the 4,5 double bond in the A ring of hydrocortisone also occurs. This reduction can transpire at both hepatic and extrahepatic sites to yield inactive compounds. The 3-ketone on the A ring can also be reduced to a hydroxyl group. This step only occurs in the liver. Conjugation (sulfate or glucuronide) of the reduced A ring steroids at the 3-hydroxyl group mostly occur liver and, to a lesser extent, in the kidney. The sulfate esters and glucuronide conjugates are the predominant form excreted in the urine. In humans, biliary and fecal excretion of steroids is of no quantitative importance.

#### Single Dose Toxicity Studies -

In summary, the acute toxicity of hydrocortisone butyrate was evaluated in mice and rats. LD<sub>50</sub> values were determined after a oral, intraperitoneal, or subcutaneous

administration. These values ranged from 498 mg/kg to greater than 3 g/kg in mice and greater than 3 g/kg in rats. Males animals were more sensitive than female animals. In addition, a lower LD<sub>50</sub> value was obtained after an intraperitoneal administration.

#### Repeated-Dose Toxicity Studies-

The potential subchronic and chronic (28 days to 7 months) toxicity of hydrocortisone butyrate was determined after topical, subcutaneous, and oral administrations. Five of the seven studies examining the potential toxicity of hydrocortisone butyrate after multiple administrations were conducted in rats (3 subcutaneous, 1 oral, and 1 topical). The other two studies were conducted either in dogs (subcutaneous; 30 days) or rabbits (topical; 6 months). Dose-dependent corticosteroid findings were reported in all studies performed. The findings included decreased body weights; thymus, pancreas, spleen, skin, and adrenal atrophy; increased infections; increased urea, triglyceride levels, potassium calcium, glucose, and alkaline phosphate values; and gender differences (males are more sensitive). The lowest dose administered in rats after a topical (2 months), subcutaneous (3 months), and oral (28 days) exposure was 0.02, 0.003, and 1 mg/kg/day, respectively. A no-observable-effect-level (NOEL) was only determined with the subcutaneous route. The NOEL for this route was 0.03 mg/kg/day for male rats. A NOEL was not obtained for female rats.

#### Carcinogenicity Studies

No carcinogenicity studies were conducted with this product.

#### Reproductive Toxicity Studies-

Three reproductive toxicity studies were reviewed in NDA 18-514. In summary, hydrocortisone butyrate was administered topically to rabbits and rats or subcutaneously to rats and mice. In a segment II study, topical administration of 0, 0.1%, 1%, or 10% hydrocortisone or hydrocortisone butyrate as an ointment (a gel of liquid paraffin with 10% polyethylene) to pregnant Wistar rats (days 6-15) resulted in no teratogenic findings. Animals were necropsied on day 20. Deaths of the dams were reported in the high dose group for either drug. In the 1% treated group, fetal weights were lower. No marked differences between the hydrocortisone butyrate and hydrocortisone were noted. The same formulation and concentrations were applied on New Zealand rabbits (days 6-18) and animals were sacrificed on day 25. Deaths in the 1% and 10% treatment groups were noted. A dose-dependent increase in fetal resorptions was reported. No teratogenic findings were noted in either rats or rabbits following topical administrations. Subcutaneous administrations of 0, 0.2 and 1 mg/kg/day hydrocortisone butyrate (in suspension) were given to pregnant mice from day 7 to day 13 of gestation. Rats were given subcutaneous administrations of 0, 0.2, and 9 mg/kg/day hydrocortisone butyrate from day 9 to day 15 of gestation. A portion of the mice was killed on day 18 and a portion of the rats on day 20. Those animals not killed at the early time were killed after weaning. Rat offspring of the second group were also killed at weaning while the mice offspring were killed on day 42. No deaths were reported in any group, but decreases in body weights were observed only in rats of the high dose group. Also, increased fetal deaths and resorptions were only observed in the high dose rat group. Rat fetuses also

had an increased number of ossifications in caudal vertebrae while no differences were noted in mice. However, mice fetuses had an increased number of cervical ribs (45.6%) and clubbed legs (1 case) in the high dose group. These findings also occurred in animals (rats and mice) treated with hydrocortisone 21-acetate (8.4 mg/kg/day).

Although only a few teratogenic findings were observed in these studies, corticosteroids as a group are teratogenic when administered systemically at relatively low dosage levels and when potent corticosteroids are applied topically. The pregnancy category for corticosteroids is C.

### Mutagenic Potential

The genotoxic potential of hydrocortisone butyrate is not described in any of the previous reviews (IND , NDA 18-514, NDA 18-652, and NDA 19-116). In addition, the Sponsor does not identify these type of studies in their NDA application (NDA 20-769). However, results from a mutagenicity study are briefly presented in the proposed label. The label states: "Studies to determine mutagenicity with prednisolone and hydrocortisone have revealed negative results". The Sponsor should submit these studies for review and the label should describe the findings of these studies in more detail (e.g., type of mutagenicity assay conducted, strain or cell type used).

### Published Article-

The article describes a photoprotective effect in hairless mice of topical anti-inflammatory agents against chronic skin damage induced by UVB and UVA. Female albino hairless Skh:hr-1 mice (10/group; 10 weeks old) were treated with 0.1 ml of 0, 0.5%, 1%, or 2% hydrocortisone (w/v% in 2:1:1 (v:v:v) propylene glycol:ethanol:water vehicle) 2 hours prior to each irradiation. The 0.1 ml volume of treatment provided approximately a 2 mg/cm<sup>2</sup> coverage of the skin. Two other test compounds were also evaluated (0.5%, 1%, or 2% ibuprofen and 1% or 2% naproxen). These compounds will not be discussed in this review. Irradiation was conducted on a set of animals with UVB light (Westinghouse FS-40 sunlamps) 3 times weekly (Monday, Wednesday, and Friday) with 30 mJ/cm<sup>2</sup> per exposure (approximately ½ the mouse MED). Another set of animals was irradiated with UVA light (GE F-40 black lights) 5 times weekly (Monday-Friday) with 15 J/cm<sup>2</sup> per exposure. Irradiation treatments with UVB or UVA continued for 24 weeks or 26 weeks, respectively. Skin wrinkling (UVB radiation), skin sagging (UVA radiation), skin tumors (UVB radiation), and histological evaluation (UVB or UVA radiation) of visibly non-tumor-bearing dorsal skin were analyzed. The histological parameters evaluated were epidermal thickness, glycosaminoglycan (GAG) content, dermal cellularity, elastosis, collagen damage, and dermal cyst changes. Skin penetration of test material was also examined in mice as well as in human abdominal skin.

A three-fold greater penetration of test material through mouse skin than through human skin was reported. Values for the human penetration were reported (ug/cm<sup>2</sup>), but no values were given for mice. The following statement was given for mouse penetration: "Because of its greater permeability, mouse skin is expected to permit even more of the materials to enter the skin". The difference in penetration was implied to be the result of the

differences (3-fold) in stratum corneum thickness between the 2 species.

Exposure of hydrocortisone followed by UVB reduced the severity of UVB irradiation effects. Dose-dependent increases in photoprotection (anti-wrinkling) with increasing concentration of test material was observed. Body weights were significantly reduced (13% or 12%) in the animals treated with 2% hydrocortisone compared to vehicle or untreated control animals, respectively. Time to tumor and number of tumors was delayed (22.2 weeks vs. 19.1 weeks) or decreased (4 tumors vs 9 tumors at week 24) in animals treated with 1% hydrocortisone compared to the vehicle treated animals. Histological parameters (i.e., collagen, elastin, GAGs, epidermal thickness, and dermal cellularity) taken at week 20 were also statistically significantly decreased in animals treated with 1% hydrocortisone followed by UVB compared to vehicle treated plus irradiation animals.

Hydrocortisone (0.5%) also reduced the severity of UVA effects in UVA irradiated animals. The onset of visible damage was delayed in animals treated with 0.5% hydrocortisone. Histological parameters (elastin, epidermal thickness, and dermal cellularity) measured at week 26 were also reduced in animals treated with 0.5% hydrocortisone.

In summary, application of 0.5% or 1% hydrocortisone prior to UVB or UVA irradiation for 26 or 24 weeks reduced UVA and UVB-induced visible wrinkling, visible sagging, tumor formation, and histological alterations.

## Comments

The pharmacologic and toxicity properties of hydrocortisone are well understood. In the nonclinical studies conducted with hydrocortisone butyrate, similar pharmacologic findings were reported in all treated species (dogs, rats, mice, and rabbits). These changes were observed following topical, oral, and subcutaneous administrations. The teratogenicity of hydrocortisone butyrate is also similar to other corticosteroids. The findings with hydrocortisone butyrate included an increased number of ossifications in caudal vertebrae in rats, and an increased number of cervical ribs and clubbed legs in mice. Although only a few teratogenic findings were observed in these studies, corticosteroids as a group are teratogenic when administered systemically at relatively low dosage levels and when potent corticosteroids are applied topically. The pregnancy category is

The genotoxicity potential of hydrocortisone butyrate has not been reviewed. Pharmacology/toxicology reviews of previous NDA submissions (NDA 18-514, NDA 18-652, and NDA 19-116) do not include any mutagenicity reports. Additionally, the Sponsor does not indicate that any mutagenicity studies were conducted in the NDA. However, a reference is made to a mutagenicity study in the proposed label. The Sponsor should submit this study for review.

Although the Sponsor did not evaluate the potential enhancement of UV irradiation, a study found in the literature examined the photoprotective effect of hydrocortisone. The application of 0.5% or 1% hydrocortisone prior to UVA or UVB irradiation for 24 or 26 weeks reduced UVA- and UVB-induced visible wrinkling (UVB) or sagging (UVA), tumor formation (UVB only), and histological alterations. The study was conducted for up to 26 weeks and animals were irradiated with either UVA or UVB. Irradiation was conducted 5

times a week with UVA and only 3 times a week with UVB. In the current protocol recommended by the Agency, animals are treated with test material and solar simulated light 5 times a week for 40 weeks, and observed for an additional 12 weeks. The great differences between both protocols exist. However, the findings in the literature article are encouraging and do not necessitate the re-evaluation of the potential enhancement of UV carcinogenesis.

**Conclusion:** From a pharmacological standpoint, this NDA is approvable.

**Recommendations:**

1. The Sponsor is recommended to submit any mutagenicity studies conducted with hydrocortisone butyrate for review and the label should describe the findings of these studies in more detail (e.g., type of mutagenicity assay conducted, strain or cell type used).

Javier Avalos, Ph.D.  
Toxicologist

cc: NDA 20769  
HFD-540 Div File  
HFD-540/Pharm/Jacobs  
HFD-540/Pharm/Avalos  
HFD-540/CSO/Anderson  
HFD-540/MO/Huene  
HFD-540/Chem/Pappas

For Concurrence Only:  
HFD-540/DD/JWilkin  
HFD-540/Team Leader/Jacobs

REVIEW & EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA

NDA 19-116 (Original Submission, dated 9/2/83)

Date Review Completed: 2/13/84

Applicant: Gist-Brocades n.v.  
Delft, The Netherlands

Drug: LOCOID<sup>R</sup> Lotion (0.1% Hydrocortisone 17-butyrate)

Composition:

	<u>mg/ml</u>
Hydrocortisone	1.00
Isopropylalcohol	
Citric acid (anhydrous)	
Sodium citrate (anhydrous)	
Glycerin (85%)	
Povidone K 90 (100%)	
Purified water, q.s. ad	

Related Submissions: INDs:  
NDAs: 18-514; 18-652;

Preclinical Studies: The applicant states: "The preclinical studies underlying this submission have all been included in the NDA submissions for the hydrocortisone butyrate cream and ointment, NDA 18-514 & 19-652." The applicant gives, in this submission, a very brief overview of the salient features of the studies reviewed already in the framework of the evaluation of the above-cited INDs and NDAs.

Recommendation: For the reasons elaborated in my review of NDA 18-652, I do not object to the approval of this drug. The Summary of Basis for Approval should be the same as for NDA 18-652.

✓ Lorant Buko, D.V.M., M.Sc.

cc: Orig. IND

HFN-140

HFN-140/MO

CSO

HFN-220

HFN-102/Glocklin

HFN-140/LBuko/smc/2/22/84

R/d init.by:JMDavitt

1655a

REVIEW & EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA

NDA 18-652 (Original Submission dated June 12, 1981)

140

Date Review Completed: 12/21/81

Sponsor: Gist-Srocades NV  
Wateringseweg 1  
P.O. Box 1  
2600 MA Delft-Holland

Sponsor of Corresponding IND

Drug: Locoid<sup>R</sup> Ointment 0.1%

Generic Name of Active Ingredient: hydrocortisone 17-butyrate

Composition of the Drug: Hydrocortisone 17-butyrate mg  
Plastibase 50W\* up to gm

\*A gel of mineral oil with 5% polyethylene

Related Submissions: IND's

NDA 18-514

Preclinical Studies

Pharmacology: The subject compound exerted anti-inflammatory effects by various routes, i.e. topical, systemic and also in pellet granuloma tests. Its potency is essentially equivalent to the middle range topical corticosteroids available in the USA, but it has less systemic effects than the fluorinated analogues.

Pharmacokinetics: The studies in dogs, rats and humans indicated that the drug is converted in the body to hydrocortisone 21-butyrate, and later broken down to hydrocortisone. However, a portion of the drug remains intact and exerts its anti-inflammatory effect as such. Its serum half-life is about 6-10 hrs. The enzymes responsible for the hydrolysis of the drug are liver and serum esterases.

Toxicity Studies

Sponsor's Statement in Vol. 1.1; 23 (Item 10):

"The reports of the preclinical investigations with hydrocortisone 17-butyrate have all been included in full in the cream submission, NDA 18-514, to which reference is made."

Reviewer's Remarks: For the detailed discussion of the above investigations, the reader is referred to my review of the above NDA. However, since the sponsor summarized the highlights of the animal toxicity studies in the pertaining IND with special attention to this formula and to the comparative efficacy and safety evaluation of the related drugs (Locoid Ointment, Locoid Cream, and Locoid Lotion) this summary will be quoted below.

"A six months topical toxicity study was conducted in rabbits utilizing the ointment preparation. There was no evidence of systemic toxicity other than some signs of adrenal suppression and thymus atrophy. While the skin in the treated rabbits was somewhat thinner than the controls, because of the frequent shaving and irritation of the application procedure, the thinner skin on the treated rabbits may be a reflection of its anti-inflammatory effect rather than evidence of skin atrophy. However it is not possible from this study to make a determination between these choices. The trauma of application appeared to be the most serious effect, which occurred within the control group receiving the vehicle alone and active preparation group.

Additional toxicity studies were conducted utilizing topical application of cream, ointment or lotion for 30 days in rabbits and rats. Under these conditions, all three preparations showed essentially equivalent effects.

Systemic toxicity studies were conducted by the oral route in rats. Evidence of adrenal suppression at the high dose was seen, as reflected by adrenal size and thymus involution. At doses of 1 and 5 mg/kg little adverse effects were observed.

A subcutaneous toxicity study was conducted in dogs. Under these conditions because administration did not depend on absorption, the most profound corticosteroid effects were observed and there was evidence of toxicity reflecting the biological activity of systemic corticosteroids. The toxicity data would appear to indicate that hydrocortisone 17-butyrate is a biologically active corticosteroid. When applied topically the systemic effects seems small. The same is true to a large extent by oral administration. In these animal studies a direct comparison was not made with fluorinated steroids. However the systemic effects appeared to be less. The pharmacology studies indicate a lower level of systemic effects than might be expected from some of the fluorinated steroids now used in dermatology."

Comments & Recommendations: The safety of this drug is identical to that of the cream (NDA 18-514). Therefore, I do not object to the approval of this drug. My elaboration on the Summary for Basis of Approval is also the same as for NDA 18-514.

cc: Orig. NDA

HFD-140

HFD-140/CSO

MO

Uorant Buko, D.V.M.

HFD-130

HFD-102/VGlocklin

HFD-140/U.Buko/smc/2/5/32

R/d Init.by: JMDavitt

4403A

REVIEW & EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA

140

NDA 18-514

1-6-51

Sponsor: Gist-Brocades nv, Delft, The Netherlands

Organization Performing Preclinical Studies: An international team from Europe (Netherlands, Italy, France) and from Japan. For the detailed information in this regard, see my corresponding IND review (IND

Drug: Locoid Cream

Name of the Active Ingredient: Hydrocortisone 17-Butyrate (also referred to as "HCB" and as "H 17B").

Category: Topical Anti-inflammatory Agent

Composition (per gram):

Hydrocortisone 17-butyrate.....mg  
Gel of liquid paraffin with 1% of polyethylene (Plastibase<sup>R</sup>50W)....mg

Toxicology:

I Acute Toxicity Studies:

A. Acute Toxicity of Hydrocortisone 17-alpha-Butyrate (H 17B), Studied in Mice & Rats by i.p., s.c. and p.o. Routes:

The LD<sub>50</sub> values (mg/kg) obtained were, as follows (male, female) in mice; i.p. - 1550, 1750; s.c. - 3,000, 2,500; p.o. - 3,000 and in rats above 3,000 by the three routes of administration.

B. Study of the Acute Toxicity of Oral & Intraperitoneal HCB in the Mouse:

It was not possible to determine the LD<sub>50</sub> by the oral route in the male mouse. A dose of 2 g/kg caused death in only 1/10 animals.

In the female mouse, the dose of 2 g/kg by the oral route did not cause death of any animals. By the i.p. route, the lethal dose 0<sup>+</sup> is close to 0.25 g/kg in the male mouse, and above 0.50 g/kg in the female mouse.

C. "Survey of Short-Term Local Tolerance & Irritation Studies":

Groups of 10 male Wistar Imamichi rats with body weights of about 200 g were used. The hair on the back of the rats was shaved off over an area about 10 x 15 cm<sup>2</sup>, and this area was divided into 6 parts.

An area of 1.5 x 1.5 cm<sup>2</sup> in the center of the right side was abraded with a sterilized injection needle, with caution to avoid bleeding. The left side was not abraded. About 0.5 g of the cream containing 0.1% H 17B or of the cream vehicle were spread on 2 cm diameter cotton patch discs, and these were applied to the respective skin areas. They were covered with adhesive plaster. The evaluation of the skin irritation was carried out 24 hrs. and 72 hrs. after the application; the observations were made by two persons to avoid subjective judgement.

After evaluation of the skin symptoms, Evans blue was injected into the saphenous vein to examine the blood vessel permeability at the sites. Histopathological examination was carried out after H-E staining of skin specimens from a proportion of the animals.

Results:

1. Skin Irritation: Observations made after application of a cream containing 1% H 17B during the 24 & 72 hrs. showed no swellings and no remarkable erythema. To explain more precisely, the observation made after the application of H 17B cream for 24 hrs. was a very slight erythema of a non-abraded site in two cases, but the observations after application for 72 hrs. showed absolutely no abnormalities. Therefore, the above-mentioned findings are thought to be due to the physical irritation of hairshaving and abrasion. Note: the sponsor indicates 0.1% on p.75, and 1.0% here (on p.83).
2. The Vascular Permeability of the Dye: In the area of the skin to which H 17B cream had been applied, no increase of the vascular permeability of the dye was observed.

Histopathological Findings: In the area of the skin to which H 17B cream had been applied, no adverse phenomena like congestion, cell infiltration and degeneration or necrosis of cells and tissue were observed.

## Acute Toxicity Studies

<u>Administration Period</u>	<u>Route</u>	<u>Test Animal</u>	<u>Animals per Dose Group</u>	<u>Dosage Form</u>	<u>Drug Dosage</u>	<u>Parameters Measured</u>	<u>Comments &amp; Results</u>
single appl. 7 days observation	i.p.	rat	7M + 7F	HCB susp. in 0.5% CMC	not stated	mortality LD50 (L&W) general symptoms autopsy	M&F LD50 *3.0 g/kg
single appl. 7 days observation	s.c.	rat	7M + 7F	as above	as above	mortality LD50 (L&W) general symptoms autopsy	M&F LD50 *3.0 g/kg
Single appl. 7 days observation	oral	rat	7M + 7F	as above	as above	mortality LD50 (L&W) general symptoms autopsy	M&F LD50 *3.0 g/kg
single appl. 7 days observation	i.p.	mouse	7M + 7F	as above	as above	mortality LD50 (L&W) general symptoms autopsy	LD50 M-1.55 g/kg F-1.75 g/kg
single appl. 7 days observation	s.c.	mouse	7M + 7F	as above	as above	mortality LD50 (L&W) general symptoms autopsy	LD50 M-3.00 g/kg F-2.50 g/kg

## Acute Toxicity Studies (Cont.)

<u>Administration Period</u>	<u>Route</u>	<u>Test Animal</u>	<u>Animals per Dose Group</u>	<u>Dosage Form</u>	<u>Drug Dosage</u>	<u>Parameters Measured</u>	<u>Comments &amp; Results</u>
single appl. 7 days observation	oral	mouse	7M + 7F	as above	as above	mortality LD <sub>50</sub> (L&W) general symptoms autopsy	M&F LD <sub>50</sub> *3.0 g/kg
single appl. 7 days observation	oral	mouse	5M +5F	HCB susp. in 1% tra. gum	500 1000 2000 mg/kg	mortality LD <sub>50</sub>	Not possible to determine LD <sub>50</sub> because of low oral toxicity.
single appl. 7 days observation	i.p.	mouse	5M + 5F	as above	100 250 500 mg/kg	mortality LD <sub>50</sub>	M LD <sub>0</sub> approximately 250 mg/kg F LD <sub>0</sub> *500 mg/kg
single appl. 1 month observation	i.p.	mouse	10M + 10F	as above	100 250 500 1000 mg/kg	mortality LD <sub>50</sub> (L&W)	Retarded toxicity is observed. M LD <sub>50</sub> 498 mg/kg. F LD <sub>50</sub> approx. 5300 mg/kg

\* = more than

## II Subacute & Chronic Toxicity Studies:

### A. Sub-Chronic Oral Toxicity Study of HCB in Rats: (Dr. C.J. Van Eeken)

HCB was given orally for 28 days in doses of 0, 1, 5 and 25 mg/kg/day as a suspension in aqueous polyvinyl-pyrrolidone. Each test group consisted of 10 male and 10 female Wistar-TNC random-bred SPF rats.

All animals survived with evidence of dose-related growth inhibition. There was a rise in the erythrocyte count, with dose-related decrease in the thymus, spleen and adrenal weights. These findings reflect the expected pharmacological activity of exogenous corticosteroids. The differences from control values are small in the 1 mg/kg group, and moderate in the highest dose group of 25 mg/kg. The increase in urea observed in the highest male dose group is probably the result of glucogenesis from proteins. Since the plasma BUN did not rise, there appears to be no indication of impaired renal functions. No significant rise in blood sugar was observed at the doses of hydrocortisone 17-butyrate used. There were no indications of infections arising as a consequence of corticosteroid administration. No abnormalities were observed on macroscopic and microscopic examination of tissues.

### B. Sub-chronic Subcutaneous Toxicity Study of HCB in Dogs: (Dr. C.J. Van Eeken)

HCB was administered s.q., as a suspension, to male and female beagle dogs, at 0, 1, 5 and 25 mg/kg for 30 days. There were 10 animals/group. Parameters studied included behaviour, mortality, autopsy results, food intake, body weight, clinical chemistry, hematology, gross and histopathological examination of organ weights.

Early mortality was observed and was progressive in all dosage groups. Infections were the cause of death. The body weights of the males observed showed a dose-related decrease, as did food intake. The blood studies reflected a dose-related effect.

The general condition of the dogs at autopsy was poor. Inflamed areas and abscesses were observed in many dogs, especially from the highest dosage group, accompanied by swelling of the leg(s) in some cases. A high incidence of brain edema and of pneumonia was observed. No abnormalities were found in the liver; adrenals were normal. There was reduction of lymphoid tissue and effects on the hemopoietic system. A distinct sex difference in various parameters was observed.

C. Topical Toxicity of HCB in Rabbits: (Dr. C.J. Van Eeken)

HCB as a 0.1% ointment was applied daily, under aluminum foil, for 6 months, to the shaved backs of male and female NZ white rabbits. Two dosage groups, each consisting of ten animals, were used - one group receiving the ointment base. The site of application measured 8 x 12 cm. Mortality, cutaneous reaction (photographs) and changes in weight were recorded. Animals were autopsied, organ weights determined and histology of the skin performed.

One rabbit from each group died (week 20 & 21). Hair growth was normal in the placebo group; minimal regrowth occurring in the HCB group. Body weight increase in the HCB H 17B group was numerical and significantly greater. Of the weights of heart, liver, kidneys, spleen and adrenals, only the last-mentioned were significantly reduced.

At autopsy the animals were in poor conditions. Numerous abscesses and bacterial infections were found in various organs. Occurring in both groups, these phenomena were ascribed to the repeated shaving and daily rubbing and a tapeworm infestation rather than to the treatment with hydrocortisone 17-butyrate.

Acanthosis was seen in both groups. The frequency of mitosis in the epinuclear diameter of the basal layer cells. The rabbits treated with HCB showed a lower frequency of mitosis and a lower inflammatory reaction score; both changes toward the normal. The thickness of the epidermis was reduced. Areas of skin atrophy were seen.

D. Subacute Toxicity of HCB in the Rat: (T. Aoyama, et al.)

Groups of 12 male & female rats were injected s.c. daily with 0.0, 0.1, 0.5, 2.2 & 10 mg/kg of HCB (in suspension) for 35 days.

The general condition of the rats in the highest dosage group deteriorated. Two animals died in the lowest dosage groups, 4/24 died in the 2.2 mg/kg group. The surviving rats from 10.0 mg/kg dosage group were sacrificed earlier, so no statistical analyses were performed on hematological and serum biochemical parameters and organ weights after autopsy for this dosage group. Inhibition of weight gain and reduction of food and water intake were observed in the two highest dose groups.

Serum analysis of the females showed slight differences from the control group. Serum triglyceride levels were significantly higher in the males and related to the dosage. Occasional, not dose-related increases of total protein and albumin, deviations from globulin control values, increase of potassium, calcium, glucose and alkaline phosphate values were observed in the three dosage groups analyzed.

Leucocytes were significantly decreased in the 2.2 mg/kg group. Lymphocyte values showed a tendency to decrease; neutrophils showed a tendency to increase.

On autopsy, a reduction of the thymus was observed. The weight of thymus, spleen, adrenals and pancreas were significantly reduced, especially in the higher dosage groups. In the 0.5 and 2.2 mg/kg groups, the weight of the liver/100 gm of body weight in the males were: hypertrophy and fatty infiltration of the liver, atrophy of white spleen (sic) and decrease in lymphocytes, atrophy of adrenals and some cases of vacuolization of cells as well as decrease of thymus cells.

The above phenomena of toxicity are due to well-known pharmacological effects of corticosteroids.

E. Chronic Toxicity Experiments of HCB in Rats:  
(T. Aoyama, et al.)

Groups of 10 male and 10 female rats were treated daily with 0.0, 0.1, 0.5, 2.5 & 6.25 mg/kg HCB (in suspension) s.q. for 7 months.

Weight gain suppression and weakness were observed in the highest dosage group. Mortality in the highest dosage group only was larger than in the control group (9/20 and 5/20, respectively). Casualties in the males were higher than in the females.

Males and females showed suppression of increase of body weight related to the dose. Tendency of reduction of food and water intake was observed in the 6.35 mg/kg group.

SGOT & SGPT values showed a tendency to increase, significant cases were found only in the females in the higher dosage groups. Changes of biochemical serum parameters were less than in the subacute experiment. Leucocytes were highly significantly reduced in the 6.25 mg/kg group. Neutrophils tended to increase; lymphocytes were decreased in the two highest dose groups.

Autopsy findings pointed to significant weight reduction of the adrenals in all dosage groups. Decrease in the weights of the spleen and the lungs were observed.

Histopathological examination of organs revealed hypertrophy of liver cells which were vacuolized. Fat droplets infiltration was seen in 4 cases. In the two highest dosage groups, atrophy of the white spleen (sic) pulp was observed whereas lymphocytes had decreased remarkably. The red spleen pulp showed a decrease in the red blood cells.

In the two highest dosage groups, substantial atrophy of the adrenal cortex was unnoticed. Only slight deviations occurred in the adrenal medulla. Lymph nodes in the vicinity of the bronchioles were atrophied and were hardly visible. Thymus cells showed a tendency to decrease; an increase of the reticular tissue was observed. Atrophy of the thymus was more pronounced than in the subacute experiment.

Heart, pancreas, thyroid gland, kidney, brain, reproductive organs, bone marrow and stomach in the treated groups did not differ significantly from those in the control group.

The observed phenomena of toxicity are considered due to well-known pharmacological effects of corticosteroids.

F. Skin Irritation Experiment:

The hair on the backs of 10 rats was shaved, and this area divided into 6 parts. In 3 parts on one side skin was abraded. About 0.5 g of HCB cream and the vehicle on cotton patch discs were applied to the respective areas and fixed and covered with adhesive tape. Observations of skin irritation was performed by two persons 24 hrs. & 72 hrs. after application. After the observations, Evan's blue was injected i.v. and the penetration into the blood vessels was examined.

Histopathological examination was performed after H-E staining of skin specimens from a proportion of the animals.

Three cases of light erythema were seen after 24 hrs. in the 0.1% cream treated group. No abnormalities were seen 72 hrs. after application. Permeability was increased in the HCB cream treated areas. Histopathological examination of the skin did not reveal phenomena like congestion, cell infiltration and degeneration or necrosis of cells and tissues.

Conclusions: HCB cream is not a skin irritant in the above experiment.

G. Local Tolerance on the Shaved Back of Rabbits by Epicutaneous Application of Locoid Cream, Ointment & Lotion: (S. Ferri, et al.)

To fulfill registration requirements in Italy, a limited study of local tolerance was done in that country. The full report and an English translation are included in Item 10, Vol. 1.5, pp. 1-54. Both documents were part of IND The sponsor has no access to detailed individual animal data.

Four groups of rabbits ("Fulvo di Bologna" breed) were used. The dorsal skin was shaved; applications were made daily for 30 days. The shaving was repeated weekly. 24 hrs. after the last application, the animals were killed and pieces of the skin from the treated area were taken for histological examination. No macro- or microscopic lesions were observed; cream, ointment and lotion were well-tolerated.

H. Subacute Topical Toxicity Studies with HCB Cream, Ointment & Lotion:  
(M. Mollet)

For 2 months, groups of 5 male and female rats were treated with either 0.1% HCB cream, ointment or lotion or with the vehicle of the comparison compound hydrocortisone 21-acetate ointment. Two dosages of HCB were applied: 0.02 mg and 0.14 mg/kg/day, respectively. Behaviour and body weight were observed daily. Hematology and blood biochemical parameters were determined after treatment. After autopsy, microscopic and histological examinations of skin and principal organs were made, as well as weight determinations of adrenals and thymuses.

No animals died. The weight curves were parallel to those of the controls, the weight being higher than in the hydrocortisone acetate (HA) group. Hematology showed significant changes of hemoglobin levels, and in a few cases, decrease of erythrocytes. Significant changes were seen in potassium, sodium, calcium and chlorine levels. In a few cases, proteins and glucose were reduced. Weight reduction of the adrenals and thymuses was observed, which was more pronounced and constant in the HA group. Histology did not reveal significant changes in the organs apart from some reduction of collagen in skin samples.

The observations are considered normal pharmacologic effects of corticosteroids.

I. Experiment on the Maximum No-Effect Level in Rats:  
(J. Takhaski, et al.)

From the subacute and chronic toxicity studies performed in Japan and reviewed above, it was estimated that the maximum no-effect level in rats amounted to 0.1 mg/kg or slightly less. The present study was performed to confirm this. Rats divided into 5 groups of 10 males + 10 females each, received daily for 3 months, s.c. injections of 0.0, 0.1, 0.03, 0.01 & 0.003 mg/kg HCB (in aqueous suspension), respectively.

Body weight gain and food intake in the treated groups were not different from those in the control group. Mortality (0, 5 & 10/group) was not drug-related and was mostly due to pneumonia.

Apart from the significant increase in glucose in the males of the highest dose group and an increase of chlorine levels in the highest dose group, no differences were seen in the serum biochemical parameters (total protein, albumin, globulin, A/G ratio, SGOT, SGPT, alkaline phosphatase, neutral fat, BUN, sodium potassium, calcium), between treated and untreated groups. At autopsy, microscopic observations did not reveal abnormalities. Weights of organs (thymus gland, spleen, adrenals, liver, pancreas, lung and testes in males) were not significantly reduced. Adrenals and thymus weights tended to decrease in the male highest dose group.

Histopathological observations showed a tendency for the cell number of the thymus gland to decrease in the highest dosage group. In the adrenals, changes were not observed. Slight focal cellular infiltration or fatty degeneration in liver lobules were observed in some animals divided over all groups. These changes were considered to be accidental. Changes in lungs were small, although pneumonia was observed in some animals, including controls.

On the basis of the above findings, the "maximum no-effect level" was established at 0.03 mg/kg.

### Reproduction & Teratology:

#### I Comparative Study of the Embryotoxic & Teratogenic Effects of Cutaneously-Applied HCB & Hydrocortisone in the Rat: (Dr. C.J. Van Eeken)

Pregnant SPF Wistar TNO random-bred rats were treated daily (days 6 thru 15 post-coitum) with ointments containing 0.1%, 1% & 10% hydrocortisone or its butyrate (HCB) on the shaved skin of the back. Each treatment group consisted of 10 rats. An additional control group, consisting of 26 rats, received the ointment base, plastibase, a gel of liquid paraffin with 50% polyethylene. No occlusion was used.

Observations for general behaviour and mortality were made. The rats were sacrificed on day 20 and effects on maintenance of pregnancy, contents of uterine horns examined. Fetuses were ~~examined~~ examined for weight, sex, macroscopic abnormalities and microscopic evidence for cleft palate.

One death occurred in the dams of the 10% hydrocortisone group. Two fetuses in the 10% HCB group were dead. Female fetal weight in this group was significantly lower than that of the other groups. In the 1% hydrocortisone group, the male fetal weight was numerically lower. In this group, a higher incidence of resorptions was noted.

No teratogenic abnormalities were observed, nor marked differences between hydrocortisone 17-butyrate and hydrocortisone.

#### II A Comparative Study of the Embryotoxic & Teratogenic Effects of Topically-Applied HCB & Hydrocortisone in the Rabbit: (Dr. C.J. Van Eeken)

Ointments containing HCB or hydrocortisone, in concentrations of 0.1, 1 & 10%, were applied daily to the shaved backs of NZ white rabbits. The application was with occlusion, and was made on days 6-18 post-insemination.

The number of deaths and abortions were noted. After 25 days, the rabbits were autopsied and the maintenance of pregnancy and the contents of the uterine horns noted. The fetuses were weighed, their sex determined and examined for cleft palate and other abnormalities.

Five rabbits died; 2 from the 1% HCB group, 1 from the 1% and 2 from the 10% hydrocortisone group. In 3 of these, lung edema was observed.

One abortion was observed in each of the following groups: 1% & 10% HCB and 10% hydrocortisone. The incidence of pregnancy was not different for the dosage groups. The increase in the number of resorptions was found to be dose-related. In these dose groups, no fetuses, but only resorptions or microscopic evidence of pregnancy were found.

Between the remaining groups, no significant differences were found regarding the distribution of live or dead fetuses. Excluding the highest dose groups for obvious reasons, the sex ratio of the fetuses in all dose groups was similar. Mean fetal weight did not differ among the groups. No evidence of teratogenic activity was observed, but for both compounds a similar dose-related embryotoxic effect was present.

### III Teratological Studies on HCB in Mice & Rats: (T. Aoyama et al.)

A 20-day s.c. dose ranging study was done in non-pregnant mice (6/group) and rats (10/group), to establish the maximum dosage in this teratology study. A dose selected from these experiments was given thereafter to some pregnant animals to establish survival rate of offspring.

Maximum dosages chosen were 1 mg/kg in mice and 9 mg/kg in rats. In the teratology and embryotoxicity experiment, groups of mice were subcutaneously receiving, from day 7 to day 13 of pregnancy, 0.0, 0.2 & 1 mg/kg/day hydrocortisone 17-butyrate (in suspension) and for comparison, one group was treated with 0.995 mg/kg of hydrocortisone 21-acetate. Groups of rats were given, from day 9 to day 15 of pregnancy, 0.0, 0.2 & 9 mg/kg/day, s.c., of the butyrate, and for comparison, 8.4 mg/kg hydrocortisone 21-acetate. A portion of the mice were killed on day 18 and a portion of the rats on day 20, and observations were made. The animals not killed were allowed to have normal parturition and lactation. At the moment of weaning, the mother animals were sacrificed and examined. The living offspring of the mice was sacrificed on day 42 after birth, while offspring of the rats were killed at the moment of weaning.

Body weight and food intake of drug-treated pregnant mice did not differ from those in the control group. In rats, the highest HCB dosage group showed a decrease in body weight and food intake during the period of administration.

Visual inspection of the sacrificed pregnant mice and rats did not reveal abnormalities. Implantation sites were equal in treated and untreated animals. No significant differences were observed between treated and untreated groups with respect to the number of live fetuses and sex ratio. The percentage of dead fetuses and resorptions was only significantly increased in the highest dose group of rats. Mean body weights of rat fetuses was significantly lower in the treated groups when compared with controls.

External malformations (clubbed legs) occurred in the 1 mg/kg HCB-treated mouse group (1 case), and in the hydrocortisone 21-acetate reference mouse group.

In the mice, there was no difference in the number of ossifications in caudal vertebrae in treated and untreated groups; in the rats these numbers were significantly reduced in the HCB-treated groups.

Only in mice were fetuses with cervical ribs observed, the number being significantly increased in the 1 mg/kg 17-ester group (45.6%) and the 21-acetate treated group (57.4%). Type of malformation and its incidence in HCB-treated animals was not shown to be different from those in hydrocortisone 21-acetate treated animals. According to the author, however, HCB has a tendency to be more toxic than hydrocortisone 21-acetate. Examination of the mother animals who were allowed to litter normally and were sacrificed at the time of weaning, revealed no abnormalities. Neither deviations in function of sense organs, nor changes in survival rate at weaning, nor external abnormalities were seen in newborn rats or mice.

The number of live young at birth, the birth rate and number of live young at weaning were significantly reduced in the highest dosage group of the rats only. These findings are thought to be related to the significant increase of fetal death and resorption before birth in rats.

Further examination of newborns did not reveal abnormalities, apart from an increase in body weight of the newborn rats in the drug-treated group, but this is thought to be related to the small litter size per mother.

This study supports the findings of the sponsors' own experiments showing that the teratogenic and/or embryotoxic potential of HCB is not significantly different from that of hydrocortisone (acetate).

Comments & Recommendations:

1. In an unusually great number of studies, the applicant adequately demonstrated that the drug is relatively safe, and that it could be marketed for human therapy in indicated cases.
2. For an elaboration on the results of the comparative toxicity studies, the reader is referred to my Pharmacologist's Portion of the Summary for Basis of Approval.
3. To my surprise, the NDA submission lacks discussion of the most sophisticated explanation of the effects of anti-inflammatory compounds, and did not perform any study for the elaboration of this question. My hypothesis involving the above sophisticated explanations will be presented in an addendum to this review. n 1

cc: Orig. NDA

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# Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation-induced chronic skin damage in the hairless mouse

Bissett DL, Chatterjee R, Hannon DP. Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol Photoimmunol Photomed* 1990; 7: 153-158.

Albino hairless mice (Skh:HR-1) exposed chronically to suberythemal doses of ultraviolet (UV) radiation display visible and histological alterations in the skin. One alteration is an increase in dermal cellularity, including inflammatory cells. This suggested a role for inflammation in chronic photodamage. We evaluated the photoprotective effect of topical hydrocortisone, ibuprofen, and naproxen against photodamage. All 3 agents protected against UVB radiation-induced visible wrinkling, tumor formation, and histological alterations. Hydrocortisone and naproxen were also evaluated for protection against UVA radiation-induced visible skin sagging and histological alterations. Both were very effective. These data indicate that chronic topical application of anti-inflammatory agents provides broad solar UV spectrum photoprotection.

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Key words: skin aging; ultraviolet radiation; anti-inflammatory agent; skin inflammation; photoprotection

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Accepted for publication October 8, 1990

The Skh hairless mouse has been used as a model to study the photoaging effects of chronic low-dose ultraviolet (UV) radiation on the skin (1, 2). There are dramatic changes in the appearance of the skin surface, which have been described as wrinkling and sagging (1), in addition to the appearance of skin tumors (3). Histologically, there are a number of alterations in the epidermis and dermis (1, 2), which are similar to those observed in sun-exposed human skin (4-8). These visible and histological changes in the mouse have been used as photodamage markers for evaluation of chronic photoprotection by sunscreens (1, 9-13) and antioxidants (14, 15).

One of the histological alterations induced by chronic UV exposure is increase in dermal cellularity, including inflammatory cells (1, 2, 16). Although the degree of cellular infiltrate is greater with UVB than with UVA radiation, both spectral regions induce an increase. The presence of inflammatory cells in the dermis of chronically exposed skin suggests a possible involvement in the chronic damaging processes leading to skin photoaging. Anti-inflammatory agents then have the potential to protect against chronic photodamage.

Presented in part at the 17th Annual Meeting of the American Society for Photobiology, Boston, Massachusetts, July 1989.

In this report we describe the photoprotective effect in hairless mice of topical anti-inflammatory agents against chronic skin damage induced by UVB and UVA radiation.

## Material and methods

### Animals

Female albino hairless Skh:HR-1 mice were obtained from the Temple University Health Sciences Center, Philadelphia, PA, USA. They were housed 5 per cage in a room with controlled temperature and humidity and with a 12-h light-darkness cycle. They were given a standard diet and water ad libitum. Mice were approximately 10 weeks old at the start of experimental work. At the completion of work, they were killed by CO<sub>2</sub> asphyxiation.

Mice were chosen at random for placement in treatment groups. Each treatment group was assigned a unique number code.

### Irradiation and topical treatment

The procedure for irradiation of the dorsal skin of mice with UVB or UVA radiation has been described previously (1). Briefly, mice were irradiated individually under a bank of 4 Westinghouse FS-

Figure

Table 1. Description of grading scales for visible skin changes

Grade	Description of skin
<i>Skin wrinkling</i>	
0	Numerous fine striations covering back and sides of body. Fine striations run length of body (head-to-tail direction).
0.5	Loss of fine striations on back along spine. Numerous fine striations along sides run length of body (head-to-tail direction).
1.0	No fine striations on back and along sides. Skin is smooth.
1.5*	No fine striations on back and along sides. Some shallow permanent wrinkles across back (perpendicular to head-to-tail direction).
2.0*	No fine striations on back and along sides. Several shallow to moderately deep permanent wrinkles across back (perpendicular to head-to-tail direction).
2.5*	No fine striations on back and along sides. Numerous moderately deep to deep permanent wrinkles across back (perpendicular to head-to-tail direction).
3.0*	No fine striations on back and along sides. Numerous deep permanent wrinkles across back (perpendicular to head-to-tail direction).
<i>Skin sagging</i>	
0	Numerous fine striations covering back and sides of body. Fine features run length of body (head-to-tail direction). Skin has pale purple-pink coloration.
0.5	Slight reduction in fine striations on back. Numerous fine features along sides run length of body (head-to-tail direction). Slight spotty blanching of skin on back.
1.0	Moderate reduction in fine striations on back and along sides. Slight blanching of skin on entire back.
1.5	Most fine striations gone. Slight nodular wrinkling on back, with no orientation to nodules. Moderate blanching of skin on entire back.
2.0	All fine striations gone. Moderate nodular wrinkling on back, with no orientation to nodules. Complete blanching of skin on entire back. Slight loose folds of skin (head-to-tail direction) on sides.
2.5	All fine striations gone. Moderate to severe nodular wrinkling on back, with no orientation to nodules. Complete blanching of skin on entire back. Moderate loose folds of skin (head-to-tail direction) on sides.
3.0	All fine striations gone. Severe nodular wrinkling on back, with no orientation to nodules. Complete blanching of skin on entire back. Large loose folds of skin (head-to-tail direction) on sides.

\* The first appearance of tumors is usually at a grade of 1.5. Higher grades are almost always accompanied by tumors.

40 sunlamps (UVB radiation, peak of emission near 315 nm) or 4 General Electric F-40 black lights (UVA radiation, no emission below 340 nm). The output of the sources was monitored with an International Light (Newburyport, MA) model 700A research radiometer. Mice were irradiated 3 times weekly (Monday, Wednesday, and Friday) with 30 mJ/cm<sup>2</sup> UVB radiation per exposure (approximately 1/2 the mouse MED) or 5 times weekly (Monday-Friday) with 15 J/cm<sup>2</sup> UVA radiation per exposure.

For mice receiving topical treatment with vehicle or anti-inflammatory agents (Sigma Chemical, St. Louis, MO, USA), the dorsal skin of the mice ( $n=10$  per group; neck to tail area) was treated with 0.1 ml of test solution [w/v% in 2:1:1 (v:v:v) propylene glycol:ethanol:water vehicle or vehicle alone; prepared weekly] 2 h prior to each irradiation. Test solution was delivered to the skin

using a Pipetman (Rainin Instrument, Woburn, MA, USA) and spread evenly over the dorsal skin with the flat edge of the disposable pipet tip. The 0.1 ml volume of treatment provides approximately a 2 mg/cm<sup>2</sup> coverage of the skin, which is a standard set for sunscreen usage in the United States

Table 2. Description of grading scales for histological alterations

Grade	Description of tissue section
<i>Epidermal thickening</i>	
0	Normal epidermal thickness
1.0	Slight increase in thickness
2.0	Moderate increase in thickness
3.0	Large increase in thickness
<i>Glycosaminoglycan (GAG) content</i>	
0	Normal red staining of dermis at dermal-epidermal junction
1.0	Diffuse light blue staining of dermis at dermal-epidermal junction
2.0	Thin continuous zone of light blue staining of dermis at dermal-epidermal junction
3.0	Focal areas of dark blue staining of dermis at dermal-epidermal junction
4.0	Continuous zone of dark blue staining of dermis at dermal-epidermal junction
<i>Dermal cellularity</i>	
0	Normal cell number
1.0	Slight increase in cell number
2.0	Moderate increase in cell number
3.0	Large increase in cell number
<i>Dermal cyst changes</i>	
0	Normal cystic area
1.0	Increase in size of cysts
2.0	Slight to moderate increase in number of cysts
3.0	Moderate to large increase in number of cysts
4.0	Slight to moderate loss of cysts
5.0	Moderate to total loss of cysts
<i>Collagen damage</i>	
0	Normal appearing collagen
1.0	Tinctorial change in collagen at dermal-epidermal junction
2.0	Granular appearance - thin open collagen network at dermal-epidermal junction
3.0	Diffuse focal areas of loss of collagen staining at dermal-epidermal junction
4.0	Large focal areas of loss of collagen staining at dermal-epidermal junction
5.0	Loss of all collagen staining at dermal-epidermal junction
<i>Elastin - upper dermis</i>	
0	Normal appearing fine structured elastin
1.0	Slight loss of fine terminal elastin
2.0	Slightly thickened fibers
3.0	Moderately thick fibers with slightly altered orientation
4.0	Heavy fiber proliferation with moderately altered orientation and tinctorial change (to purple) of collagen
5.0	Heavy fiber proliferation with greatly altered fiber orientation and tinctorial change (to purple) of collagen
<i>Elastin - lower dermis</i>	
0	Normal appearing fine branching elastin
1.0	Slight to moderately thickened fibers
2.0	Increased elastin staining, primarily around adnexal structures
3.0	General overall increase in elastin staining
4.0	Heavy proliferation of elastin
5.0	Heavy proliferation of elastin with greatly altered fiber orientation

Table 3. Human skin penetration of topical anti-inflammatory agents

Treatment	Cumulative penetration ( $\mu\text{g}/\text{cm}^2$ ) <sup>a</sup> at:	
	2 hours	24 hours
1% ibuprofen	38	418
1% naproxen	8	104
1% hydrocortisone	13	121

<sup>a</sup> Values represent means of 3 replicates; a value of 1280 represents penetration of 100% of the applied test material; typical standard deviation in these assays is 25% of the cumulative penetration values.

(17). Each treatment solution was labeled with the group number of the mice to receive it, and treatments were done based on that coding.

The anti-inflammatory agents used absorb in the UVC region but not in the UVB or UVA regions. Their approximate absorption maxima are: hydrocortisone, 242 nm; ibuprofen, 218 nm; and naproxen, 230 nm. These compounds thus do not function as solar UV sunscreens.

#### Visual skin evaluation

Skin wrinkling (UVB radiation-induced event) and skin sagging (UVA radiation-induced event) in hairless mice were assessed as described previously (1). For convenience in grading, mice are held perpendicular by the tail with their feet resting against a solid surface to diminish movement. The grading scales are shown in detail in Table 1. In the scales, 0 is normal and 3 is the maximum visible skin change observed in our work (1).

Skin lesions were diagnosed as tumors if they were circular, red, raised, and greater than 1 mm in diameter. These lesions were counted. In other work we have evaluated these types of lesions histologically to identify them. They are papillomas and squamous cell carcinomas. We occasionally observe flesh-colored lesions. In our experience, these

Table 4. Anti-wrinkling effect of anti-inflammatory agents

Treatment (n=10)	% wrinkling reduction when vehicle control wrinkle grade is:			
	0.5	1.0	1.5	2.0
0.5% ibuprofen	38	16	— <sup>**</sup>	—
1% ibuprofen	62*	40*	27*	—
2% ibuprofen	50*	30*	12	—
1% naproxen	64*	39*	30*	—
2% naproxen	80*	40*	20	—
0.5% hydrocortisone	43*	32*	27*	20*
1% hydrocortisone	78*	50*	47*	35*
2% hydrocortisone	76*	65*	64*	—

\* Significantly different ( $P \leq 0.05$ ) from vehicle control. \*\* These experiments were terminated before reaching this point.

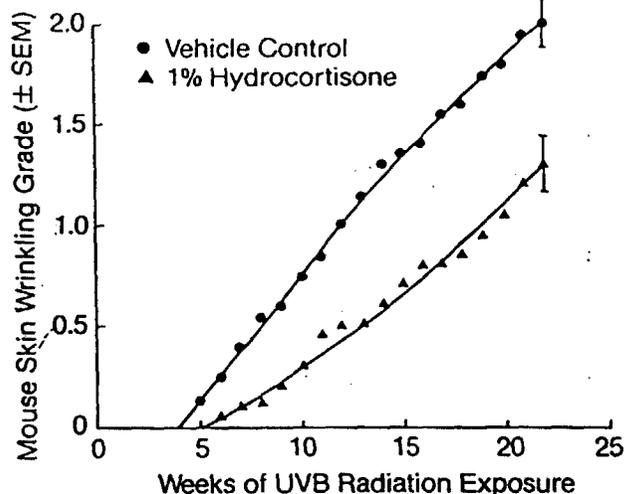


Fig. 1. Effect of topical hydrocortisone against UVB radiation-induced skin wrinkling in the hairless mouse ( $n=10$ ).

tend to regress and disappear. These were not counted as tumors in our work.

Visual evaluation was done blind based on group number by an individual not involved in the treatment and irradiation work.

#### Skin penetration

Skin penetration using human abdominal skin (obtained at autopsy) was done as described previously (14). This work was done to ensure that the test materials would enter skin from the vehicle in use. With our experimental set-up we observe approximately a three-fold greater penetration through mouse skin than through human skin (14), which is likely due to the three-fold difference in stratum corneum thickness between the 2 species (18).

#### Histology

The histological methods were those used previously (1, 16). Biopsies ( $2 \times 10$  mm) from visibly non-tumor-bearing dorsal skin were taken at death ( $\text{CO}_2$  asphyxiation) or from mice anesthetized by intraperitoneal injection (0.25 ml of 0.8% ketamine HCl, 0.06% xylazine, and 0.02% acepromazine maleate in sterile physiological saline). The biopsy sites in anesthetized mice were sutured with silk thread. The biopsies were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6–10  $\mu\text{m}$ . Sections were stained with a variety of stains.

The histological parameters and the corresponding stains and grading scales (half-grade increments) follow: epidermal thickness (H & E

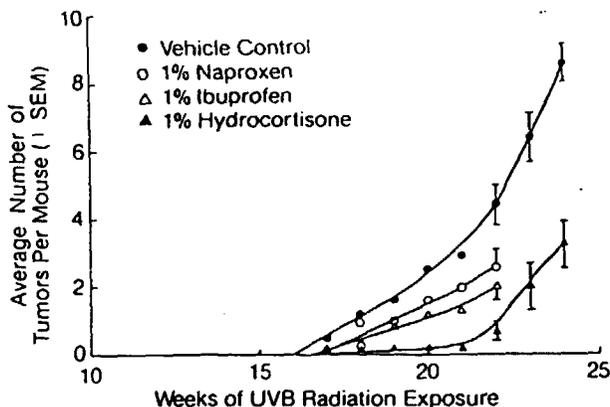


Fig. 2. Tumor yield in hairless mice ( $n=10$ ) treated topically with anti-inflammatory agents and exposed to UVB radiation.

stain), 0–3 scale; glycosaminoglycan (GAG) content (Mowry stain), 0–4 scale; dermal cellularity (H&E stain), 0–3 scale (upper and lower dermis graded separately); elastosis (Luna stain), 0–5 scale (upper and lower dermis graded separately); collagen damage (Van Gieson stain), 0–5 scale; and dermal cyst changes (H & E stain), 0–5 scale. The grading scales were based on our observations of the sequence of histological events that occur in the mouse, 0 representing the histological observations on normal 12-week-old mice and the upper end of the scales being the maximum observed change in the parameters in previous work (1). The specifics of the grading scales are detailed in Table 2. On each grading scale, half-grade increments are allowed to take into account variability across sections. The grading was done blind on coded samples by individuals not involved in the treatment, irradiation, and visible evaluations. Once all grading was completed, scores were compiled based on the code for comparison across parameters.

#### Statistics

Student's *t*-test was used to determine statistically significant differences between treatments.

Table 5. Anti-tumor effect of topical anti-inflammatory agents

Treatment ( $n=10$ )	Average week of tumor onset ( $\pm$ SEM)
Vehicle	19.1 $\pm$ 0.9
1% naproxen	18.8 $\pm$ 0.5
1% ibuprofen	19.7 $\pm$ 0.7
1% hydrocortisone	22.2 $\pm$ 0.7*

\* Significantly different ( $P < 0.05$ ) from vehicle control.

## Results

### Skin penetration

The anti-inflammatory agents evaluated penetrated human skin (Table 3). Because of its greater permeability, mouse skin is expected to permit even more of the materials to enter the skin.

### Mouse model: UVB photoprotection

Topical application of solutions of hydrocortisone, ibuprofen, and naproxen prior to each UVB radiation exposure reduced the severity of UVB radiation-induced skin wrinkling (Table 4, Fig. 1). At 2%, ibuprofen and naproxen were visibly irritating to mouse skin, which probably accounts for their reduced effectiveness relative to 1%. For hydrocortisone, the most effective of the 3 compounds, the level of protection increased with concentration, although by 16 weeks mice receiving 2% hydrocortisone had significantly lower body weights (28.5  $\pm$  1.4 g) vs those receiving vehicle treatment (32.8  $\pm$  2.0 g) or no UV control animals (32.3  $\pm$  3.0 g).

These anti-inflammatory agents were also effective in reducing the occurrence of UVB radiation-induced skin tumors. All 3 agents reduced the number of tumors per mouse (Fig. 2), hydrocortisone being the most effective. However, only hydrocortisone (Table 5) delayed tumor onset significantly ( $P \leq 0.05$ ), which is also apparent in a plot of tumor prevalence (Fig. 3).

Skin biopsies were taken for histological evaluation at week 20 in the experiments. Histologically, the anti-inflammatory agents reduced damage ( $P \leq 0.05$ ) as assessed in a number of parameters (Table 6). There is a good correlation between the

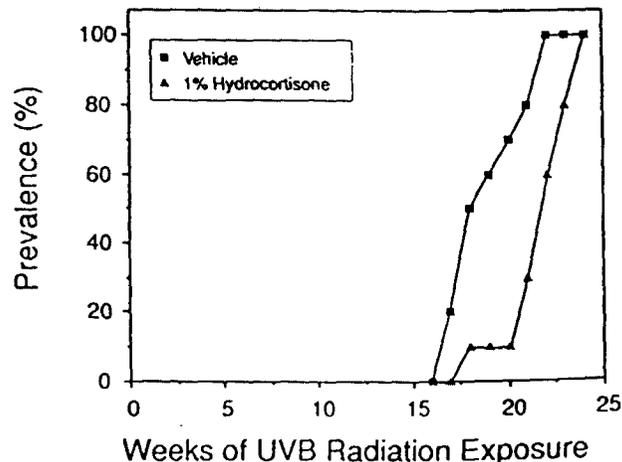


Fig. 3. Tumor prevalence in hairless mice ( $n=10$ ) treated topically with anti-inflammatory agents and exposed to UVB radiation.

Table 6. Effect of anti-inflammatory agents on UVB-induced histological changes

Treatment	Histological scores (n=5)*				
	Collagen	Elastin**	GAGs	Epidermal thickness	Dermal cellularity**
Vehicle	2.25]	7.25]	2.00]	2.00]	2.92]
1% ibuprofen	1.94]	6.30]	2.00]	2.00]	2.30]
1% naproxen	1.87]	6.16]	1.58]	1.62]	2.36]
1% hydrocortisone	0.75]	6.09]	0.00]	1.25]	0.75]
no UV control	0.51]	3.09]	0.25]	0.08]	0.41]

\* Values joined by brackets are statistically different from those for the other treatments ( $P \leq 0.05$ ). \*\* Sum of upper and lower dermal scores.

Table 7. Effect of anti-inflammatory agents on UVA-induced histological changes

Treatment	Histological scores (n=5)*				
	Collagen	Elastin**	GAGs	Epidermal thickness	Dermal cellularity**
Vehicle	0.5]	3.4]	0.2]	0.8]	2.9]
1% naproxen	0.1]	3.0]	0.1]	1.1]	1.8]
0.5% hydrocortisone	0.4]	2.4]	0.0]	0.3]	1.6]
no UV control	0.1]	1.4]	0.0]	0.2]	0.6]

\* Values joined by brackets are statistically different from those for the other treatments ( $P \leq 0.05$ ). \*\* Sum of upper and lower dermal scores.

dermal cellularity scores and the visible skin wrinkling: lower cellularity scores correspond with lower wrinkle grades. The collagen histological scores also reveal a good correspondence with visible skin wrinkling. Other data from our lab (16, 19) also suggested a similar association. The other individual histological parameters do not correspond as well with the wrinkle grades.

#### Mouse model: UVA photoprotection

Topical hydrocortisone and naproxen were evaluated for their protective effect against UVA radiation-induced skin sagging.

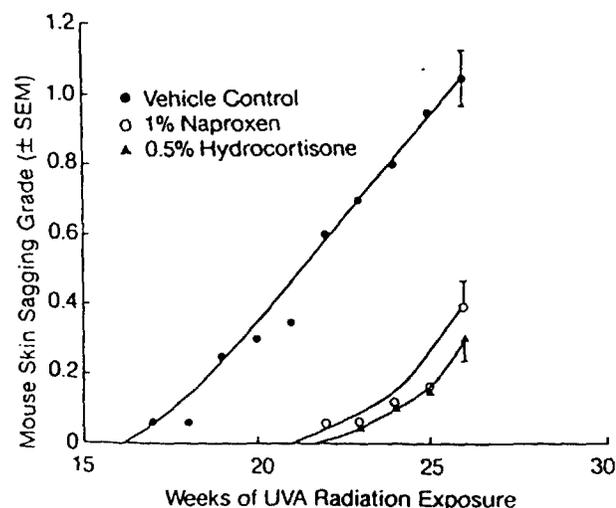


Fig. 4. Effect of topical anti-inflammatory agents against UVA radiation-induced skin sagging in the hairless mouse (n=10).

Both were very effective (Fig. 4), dramatically delaying the onset of visible damage.

Skin biopsies were taken at week 26, the end of the experiment. Histologically, hydrocortisone and naproxen reduced damage ( $P \leq 0.05$ ) as assessed in a number of parameters (Table 7). There is a good correspondence between the dermal cellularity scores and the visible skin sagging: lower cellularity scores correspond with lower sagging grades. Also graded but not included in Table 7 was dermal cyst changes. The score for no UV control mice was 1.38, and the scores for vehicle (2.25), hydrocortisone (2.08), and naproxen (2.00) were statistically equal, indicating no correspondence between cysts and visible scores. Thus, of all the histological parameters measured, only cellularity corresponded to the visible sagging grades.

#### Discussion

Our results indicate that topical anti-inflammatory agents are protective against chronic damage induced by both UVB and UVA radiation in the hairless mouse. We observe protection against visible changes (wrinkling, tumor formation, and sagging) and histological alterations. These agents thus provide broad solar UV protection of the skin.

Our results suggest that inflammatory cells are involved in the damaging processes leading to skin photoaging. The histological evaluations indicate an increased dermal content of cells, including inflammatory cells, with chronic UV exposure. How

these cells contribute to damage is not clear. For UVB radiation-induced damage, superoxide produced by the cells (20) may participate in damage. In support of this is our observation (14) that topical superoxide-scavenging antioxidants provide protection against chronic photodamage. Anti-inflammatory agents lower the dermal content of cells, including inflammatory cells, relative to vehicle treatment. A more detailed analysis though will be needed to specifically identify cell types present and their ability to produce oxygen radicals as a result of damage and treatment.

For UVA radiation-induced damage, we do not know the role of inflammatory cells. Anti-inflammatory agents do reduce dermal cellularity induced by UVA and are very potent in protection against UVA damage. We considered the possibility that dermal cyst changes (enlargement and proliferation) might contribute to damage, since UVA is particularly effective in inducing this tissue change (2). However, all UVA-exposed skin, regardless of topical treatment, had similar histological scores for cysts, indicating no correspondence between cysts and skin-sagging scores. More work will need to be done to determine the basis for UVA-induced skin changes and the role of anti-inflammatory agents in protection.

#### Acknowledgements

We gratefully acknowledge the supporting histological tumor evaluations of Drs. Greg G. Hillebrand and Gary R. Johnson of Procter & Gamble. We also acknowledge the technical assistance of James F. McBride, Mark J. Benzinger, Jayne L. Ritter, and Larry F. Patrick.

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