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Protective effect of a topically applied anti-oxidant plus an anti-inflammatory agent against ultraviolet radiation-induced chronic skin damage in the hairless mouse

D. L. BISSETT, R. CHATTERJEE, and D. P. HANNON, *The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45239-8707.*

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Synopsis

Female albino hairless mice (Skh:HR-1) exposed chronically to sub-erythemal doses of ultraviolet radiation develop visible skin changes, histological alterations, and tumors. Topical treatment of mice with binary combinations of an anti-oxidant (alpha-tocopherol, ascorbic acid, or 2,4-hexadien-1-ol) and an anti-inflammatory agent (hydrocortisone, naproxen, or ibuprofen) prior to each UVB radiation exposure reduced significantly the severity of the observed photodamage events. The combinations provided protection additive of the effects of the individual components. UVA radiation-induced photodamage was inhibited effectively by the anti-inflammatory agent alone. Addition of an anti-oxidant did not increase this level of protection.

INTRODUCTION

Activated oxygen species and oxygen radicals have been implicated in ultraviolet (UV) radiation damage to skin (1–4). These species, in particular superoxide and singlet oxygen, are probably involved in chronic photodamage, since topical anti-oxidants that scavenge these species are photoprotective in the hairless mouse (2,3). Also, oxygen radicals are probably a primary factor in chronic photodamage, since chelators, which prevent iron-catalyzed production of oxygen radicals, are dramatically photoprotective in the mouse (4).

Inflammatory cells also appear to play a role in photodamage. With chronic UV radiation exposure of mouse skin, there is an increase in dermal cellularity, including inflammatory cells (5,6). We have observed that topical anti-inflammatory agents are protective against chronic photodamage (7). This suggests that the inflammatory cell infiltrate contributes to the damage, although it is not clear what specific role these cells play in the damage process.

Since either anti-oxidants or anti-inflammatory agents alone provide protection, it is likely that a combination of the two would have greater effectiveness. In this report, we describe the protective effect in hairless mice of the combination against UV radiation-induced chronic skin damage. The degree of protection is quantified using both histological and visible perception methods.

MATERIALS AND METHODS

ANIMALS

Female albino hairless Skh:HR-1 mice were obtained from the Temple University Health Sciences Center, Philadelphia, PA. They were housed five to a cage in a room with controlled temperature and humidity and with a 12-hour 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 end of experimental work, they were sacrificed by CO_2 asphyxiation.

IRRADIATION AND TOPICAL TREATMENT

The procedure for irradiation of the dorsal skin of mice with UVB or UVA radiation has been described previously (6,8). Briefly, mice were irradiated individually under a bank of four Westinghouse FS-40 sunlamps (UVB radiation, peak of emission near 315 nm) or four General Electric F-40 black lights (UVA radiation, no detectable emission below 340 nm). The output of the sources was monitored with an International Light (Newburyport, MA) model IL1350 radiometer with model SED240 (UVB) and SED015 (UVA) sensors. Mice were irradiated three times weekly (Monday, Wednesday, and Friday) with 30 mJ/cm^2 UVB radiation per exposure (approximately one half the mouse MED) or five times weekly (Monday–Friday) with 15 J/cm^2 UVA radiation per exposure.

For mice receiving topical treatment with anti-inflammatory agents and/or anti-oxidants, the dorsal skin of the mice ($n = 10$ per group; neck to tail area) was treated with 0.1 ml of test material solution two hours prior to each UV radiation exposure. Solutions were w/v% (pH 6–7) in an ethanol:propylene glycol:water vehicle [typically 2:1:1 (v:v:v)], the ratio of the components varying somewhat, depending upon the solubility of the particular test material. The test solution was delivered to the skin using a Pipetman® (Rainin Instrument Co., Woburn, MA) and spread evenly over the dorsal skin with the flat edge of the disposable pipet tip.

VISIBLE SKIN GRADING

Skin wrinkling (UVB radiation-induced event) and skin sagging (UVA radiation-induced event) in hairless mice were assessed as described previously (6–8). Briefly, wrinkling and sagging were evaluated using grading scales (0–3, with half-grade increments), where 0 is normal and 3 is the maximum visible skin change observed in our work (6). Skin lesions were diagnosed as tumors if they were circular, red, raised, and greater than 1 mm in diameter. These lesions were counted.

HISTOLOGY

The histological methods and grading scales were those used previously (6,7,9). Briefly, biopsies (2 × 10 mm) from visibly non-tumor-bearing dorsal skin were taken at sacrifice (CO_2 asphyxiation), fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6 to 10 microns. Sections were then stained with a variety of stains.

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 10- to 12-week-old mice, and the upper end of the scales being the maximum observed change in the parameters in previous work (6). The histological parameters and the corresponding stains and grading scales (half-grade increments) follow: epidermal thickness (H&E stain), 0–3 scale; glycosaminoglycan content (Mowry stain), 0–4 scale; dermal cellularity (H&E stain), 0–3 scale; elastosis (Luna stain), 0–5 scale; and collagen damage (Van Gieson stain), 0–5 scale. The scores for all parameters were summed to arrive at a histological damage index, a convenient way to express histological data in a single number.

STATISTICS

The Student t-test was used to determine the statistical significance of differences among treatment groups.

RESULTS AND CONCLUSIONS

UVB PHOTOPROTECTION

Binary combinations of an anti-inflammatory agent (naproxen, ibuprofen, or hydrocortisone) with an anti-oxidant (alpha-tocopherol, ascorbic acid, or 2,4-hexadien-1-ol) were applied topically to mice prior to each UVB radiation exposure to determine photoprotective effect. The combinations were more effective against mouse skin wrinkling than were the individual test materials (Table I). The level of protection by the combination appears to be additive of the individual component effects.

A plot of the data for a typical example, 0.5% hydrocortisone + 5% alpha-tocopherol, is presented in Figure 1. Noteworthy is the delay in onset of wrinkling by the combination. There was no delay for the individual components.

Skin biopsies were taken for histological evaluation at week 20, the end of the study shown in Figure 1. The histological results parallel the visible skin-wrinkling data; i.e., the combination was overall more protective against alterations than were the individual components (Table II). As expected, dermal cellularity scores were reduced for the treatments involving an anti-inflammatory agent. Of particular interest are the collagen histological scores, which correspond well with the observed skin-wrinkling grades. Other data from our lab (2,9,10) also suggest a similar association.

The combination treatment groups were stopped before a good assessment of tumor development could be made. However, most vehicle control mice had at least one visible

Table I
Anti-Wrinkling Effect of Anti-Oxidant Plus Anti-Inflammatory Agent vs Individual Components

Treatment	% Wrinkling reduction[a] when vehicle control wrinkle grade is:		
	0.5	1.0	1.5
5% Ascorbic acid	70	54	23
5% Alpha-tocopherol	82	53	29
5% Hexadienol	64	48	25
0.5% Hydrocortisone	38	35	26
1% Ibuprofen	68	40	29
1% Naproxen	64	48	26
0.5% Hydrocortisone + 5% tocopherol	100[b]	73[b]	60[b]
1% Ibuprofen + 5% tocopherol	100[b]	87[b]	67[b]
1% Naproxen + 5% tocopherol	88	73[b]	52[b]
0.5% Hydrocortisone + 5% ascorbate	100[b]	90[b]	73[b]
1% Naproxen + 5% ascorbate	91	80[b]	42
0.5% Hydrocortisone + 5% hexadienol	82	75[b]	47[b]

[a] All wrinkle grade reductions shown here are significantly different from the vehicle control.
[b] These wrinkle grade reductions are significantly different from those for the individual components.

tumor at study termination (week 20), while there was a greatly reduced incidence of tumors in the combination treatment groups. For example, at week 20, 70% of vehicle control mice had at least one tumor, while only 10% of the 0.5% hydrocortisone + 5% alpha-tocopherol group had tumors (one mouse had a single tumor).

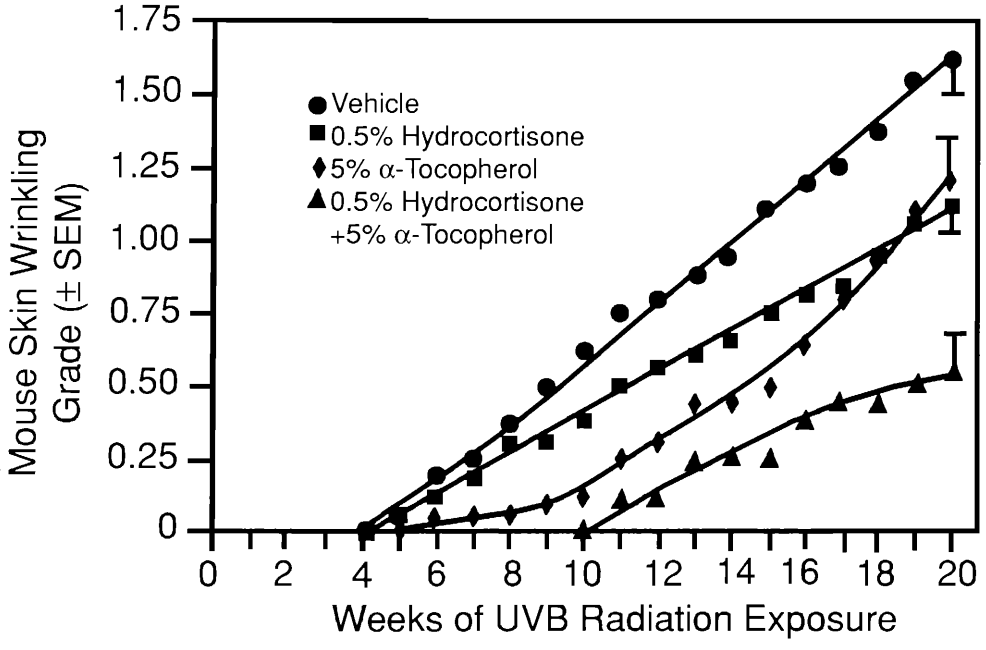

Figure 1. Protection against UVB-induced skin wrinkling by anti-inflammatory agent plus anti-oxidant.

Table II
Effect of Anti-Oxidant Plus Anti-Inflammatory Agent in Prevention of UVB-Induced Histological Changes

Treatment	Histological scores (n = 5)[a]					
	Epidermal thickening	Dermal cellularity[b]	Collagen damage	GAG increase	Elastosis[b]	Damage index[c]
Vehicle	2.0] [d]	2.7]	2.0]	1.0]	4.9]	12.6]
5% Alpha-tocopherol	1.8] [d]	2.6]	1.4]	0.6]	4.2]	10.6]
0.5% Hydrocortisone	1.5]	2.0]	0.9]	0.6]	4.8]	9.8]
0.5% Hydrocortisone + 5% alpha-tocopherol	0.9]	1.9]	0.1]	0.2]	4.4]	7.5]
No UV	0.1]	1.0]	0]	0.1]	0.6]	1.8]

[a] Baseline scores (start of experiment, 10–12-week-old mice) for all parameters are 0.
[b] Sum of scores from upper and lower dermal evaluations.
[c] Sum of all histological scores.
[d] Brackets enclose values that are equal statistically (95% confidence interval).

UVA PHOTOPROTECTION

Binary combinations of an anti-inflammatory agent (naproxen or hydrocortisone) with the anti-oxidant ascorbic acid were applied topically to mice prior to UVA radiation exposure to determine photoprotective effect. Previous data (2,3,6) indicated anti-oxidants (alpha-tocopherol, ascorbic acid, and conjugated hexadienes) were not protective against UVA, while anti-inflammatory agents were. Consistent with this is our present observation that 0.5% hydrocortisone + 5% ascorbic acid was no more effective against mouse skin sagging than 0.5% hydrocortisone alone (Figure 2).

Skin biopsies were taken for histological evaluation at week 26, the end of the study shown in Figure 2. Hydrocortisone alone and the combination were equally protective

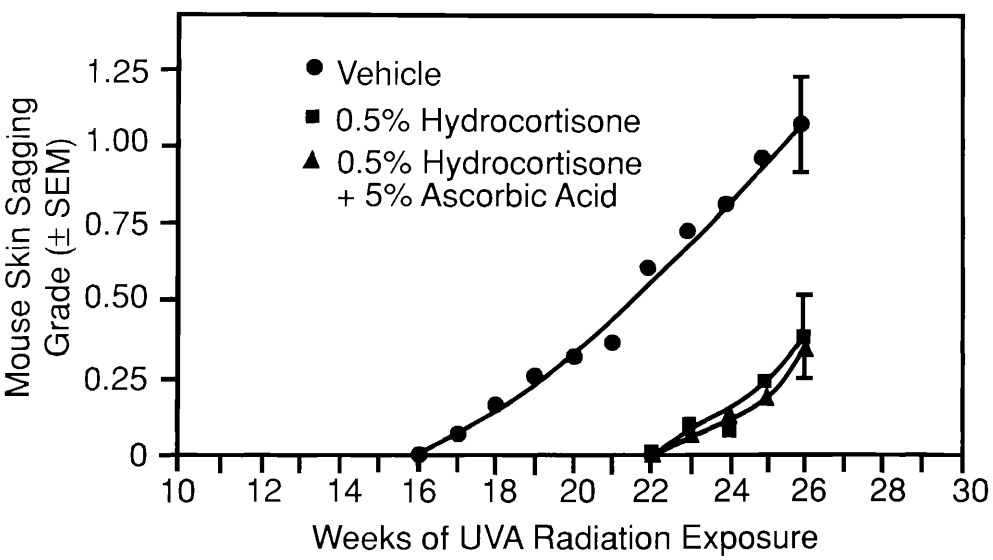

Figure 2. Protection against UVA-induced skin sagging by anti-inflammatory agent plus anti-oxidant.

Table III
Effect of Anti-Oxidant Plus Anti-Inflammatory Agent in Prevention of UVA-Induced Histological Changes

Treatment	Histological scores (n = 5)[a]					
	Epidermal thickening	Dermal cellularity[b]	Collagen damage	GAG increase	Elastosis[b]	Damage index[c]
Vehicle	0.8][d]	2.2]	0.4 ⌉	0 ⌉	3.4 ⌉	6.8]
0.5% Hydrocortisone	0 ⌉	1.5 ⌉	0.2 ⌋	0.1 ⌋	3.1 ⌋	4.9 ⌉
0.5% Hydrocortisone + 5% ascorbic acid	0.2 ⌋	1.5 ⌋	0.4 ⌉	0 ⌉	2.4]	4.5 ⌋
No UV	0 ⌋	0.6]	0.1 ⌋	0 ⌋	0.4]	1.1]

[a] Baseline scores (start of experiment, 10–12-week-old mice) for all parameters are 0.
[b] Sum of scores from upper and lower dermal evaluations.
[c] Sum of all histological scores.
[d] Brackets enclose values that are equal statistically (95% confidence interval).

against histological alterations, as indicated by the damage index (Table III). This is consistent with their equal efficacy against skin sagging. Of all the individual histological parameters, only dermal cellularity appears to reflect the observed skin-sagging scores. This is consistent with our previous work (7). While ascorbic acid did not increase the observed level of protection in the combination treatment, there is a significantly lower elastosis score for the combination. The importance of this observation to visible skin sagging is not clear, since hydrocortisone alone was protective yet did not affect the elastosis score.

DISCUSSION

A combination of anti-oxidant and anti-inflammatory agent applied topically to mice provides UVB photoprotection that overall is approximately additive of the effects provided by the individual components. In our previous testing of either anti-oxidants (2,3) or anti-inflammatory agents (7) alone, we did not observe any delay in onset of UVB radiation-induced damage by these compounds. In contrast, the present results indicate that a combination of these two types of actives does provide a delay in onset, based on visible wrinkle evaluations. The importance of the delay in onset is not clear, but it suggests an advantage in protection for the combination. Since anti-inflammatory agents are also effective against UVA-induced damage (skin sagging), the combination provides good broad UV spectrum protection.

While it is likely that anti-oxidants are photoprotective by scavenging oxygen radicals (1–3), the protective mechanism of the anti-inflammatory agents is not clear. Anti-inflammatory agents in chronic photodamaged skin may have indirect anti-oxidant action. Oxygen radicals can arise as by-products of inflammatory cells (11). The observed reductions in dermal cellularity by topical anti-inflammatory agents would lessen the potential generation of oxygen radicals by these cells. Alternatively, proteolytic enzymes from these cells (12) may be involved in photodamage. A reduction in the dermal inflammatory cell population would also reduce the potential release of such enzymes. More work is needed to define the mechanisms of damage by UV and protection by topical treatment.

Our histological data indicate a high level of elastosis with both UVB and UVA radiation exposures. However, our results here and elsewhere (2) do not reveal an obvious association between the effectiveness against elastosis and the efficacy of a topical treatment against visible skin changes and other histological parameters. While elastosis is a useful marker for identifying photodamaged skin, it is not clear how it relates to other photodamage events. Further effort is necessary to define the association among these events.

We believe that the results observed here are relevant to human photoprotection. In human testing, both anti-oxidants and anti-inflammatory agents have been shown to be effective against acute skin photodamage (13–18). Tocopherol is protective against lipid peroxidation (13), while ascorbic acid reduces the severity of UV-induced erythema (14). Several anti-inflammatory agents also inhibit UV-induced erythema (15–18). Additionally, we have observed that both tocopherol and hydrocortisone inhibit the UV induction of human epidermal ornithine decarboxylase and that acute photoprotection predicts chronic photoprotection (manuscripts in preparation). We therefore anticipate that the acute observations on humans will translate into chronic benefits for anti-oxidants and anti-inflammatory agents, used either alone or in combination.

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