The Effectiveness of Topical AC-11® in Protecting Against Carcinogenesis in Hairless Mice Exposed to Repeated Doses of Solar-Simulating Radiation

Summary

Introduction

Nonmelanoma skin cancers (basal cell and squamous cell carcinomas) are the most common skin cancers as well as the most common human cancers (Almahroos and Kurban, 2004). The incidence of basal cell and squamous cell carcinoma increases with increasing exposure to sunlight, in individuals with fair skin, and in those with a genetic predisposition to sunlight sensitivity (Armstrong and Kricher, 2001; Vink and Rosa, 2001).

Exposure to sunlight results in both oxidative and photochemical (cyclobutyl pyrimidine dimers, 6-4 photoproducts) DNA damage (Black et al, 1997; Black, 2004). Specific mutations have been noted to occur in the *p53* tumor suppressor gene at dipyrimidine sites in basal cell and squamous cell carcinomas (You et al, 2000). More than 90% of squamous cell carcinomas and about 50% of basal cell carcinomas have mutated *p53* genes (Kraemer, 1997). An unrepaired mutation at the *p53* gene may be responsible, at least in part, for the progression of DNA damage to mutation to cancer.

AC-11 is an aqueous extract of the botanical, *Uncaria tomentosa*. It has been shown to both enhance the repair of cyclobutyl pyrimidine dimers in human living skin equivalents and to decrease oxidative DNA damage in humans and animals (Mammone et al, 2004; Pero et al, 2002; Pero et al, 2005; Sheng et al, 2001; Sheng et al, 2000a; Sheng et al, 2000b). This study was designed to assess the effectiveness of AC-11 in inhibiting the formation of UV-induced malignancies and pre-malignant lesions in the hairless mouse model.

Methods

Fifty Skh-1 hairless mice were repeatedly exposed to solar-simulating radiation (290 nm - 400 nm) using a 1,600 watt xenon arc lamp fitted with filters as a radiation source.

Thirty mice were treated with AC-11 in 3 concentrations (0.5%, 1.5%, and 3.0%). AC-11 was formulated in a non-irritating, dye-free, perfume-free, and fragrance-free vanishing cream vehicle. Ten mice were treated with vehicle only and 10 were untreated. AC-11 or the vehicle only were applied daily to the backs of each mouse for the duration of the study.

After establishing the minimal erythemal dose of solar-simulating radiation, study mice were initially exposed to 0.9 x the minimal erythemal dose 5 times a week for 2 weeks. The dose was increased by 20% and repeated 5 times a week during the next 2-week period. The 20% increase in radiation dose and the exposure schedule was continued for additional 2-week intervals. A total dose of 738 J/cm^2 of solar-simulating radiation was administered.

Tumor progression was assessed according to the following scale with the assigned score for each response noted in parentheses: mild erythema (0); intense macular erythema (0); light scaling accompanying erythema (1); firm scaling, palpable keratosis (2); raised palpable plaque, corresponding to early malignant development (3); and extensive tumor development (4). Mild erythema and intense macular erythema are not considered precancerous and were thus assigned a tumor progression score of zero.

Tumor progression scores were reported as means ± standard errors and compared using Kruskal-Wallis one-way analysis of variance. A p value of less than 0.05 was considered significant.

Results

Of the 50 mice exposed to solar simulating radiation, 5 were biopsied (one from each of the different AC-11 concentrations used, one from the vehicle-only group, and one from the untreated controls), for histological examination and not included in the assessment of clinical response. Other animals were not evaluable for reasons not related to the study or the test material. In total, 21 AC-11-treated and 14 vehicle-only controls or untreated controls were evaluable.

Controls suffered more damage than did AC-11-treated mice (Table 1). The percent of animals that had a more severe clinical response was skewed to the animals who did not

receive topical AC-11. For example, most of the AC-11-treated animals (52.4%) were found to have the least severe response observed (light scaling accompanying erythema) following a total cumulative dose of 738 J/cm^2. By comparison, only 14.3% of controls were found to have the same dermal manifestations following the same dose. More importantly, the percent of animals with the most severe clinical response (raised palpable plaques corresponding to early malignant development) occurred substantially more frequently in controls than in AC-11-treated animals (21.4% vs. 4.8%).

Table 1. Response to solar-simulating radiation at day 77 in hairless mice treated with or without topical AC-11. The total cumulative dose was 738 J/cm^2.

	Controls* (n = 14)		AC-11† (n = 21)	
Clinical Response Category	Count	Percent	Count	Percent
Mild Erythema	0	0.0	0	0.0
Intense Macular Erythema	0	0.0	0	0.0
Light Scaling Accompanying Erythema	2	14.3	11	52.4
Firm Scaling, Palpable Keratosis	9	64.3	9	42.9
Raised Palpable Plaque Corresponding to Early Malignant Development	3	21.4	1	4.8
Extensive Tumor Development	0	0.0	0	0.0

* Controls included 7 mice that were treated with vehicle only and 7 that were untreated.
† AC-11 was applied topically in concentrations of 0.5%, 1.5%, and 3.0% to 7, 6,and 8 evaluable animals, respectively.

A significant ($p < 0.02$) difference was noted in the mean tumor progression scores when controls (2.07 ± 0.62) were

compared to AC-11-treated animals (1.52 ± 0.60; Figure 1). There was no difference in mean tumor progression scores when responses to the different concentrations AC-11 were compared. There was no difference in mean tumor progression scores when controls treated with vehicle-only were compared to untreated controls, suggesting that the vehicle had no significant effect on tumor progression.

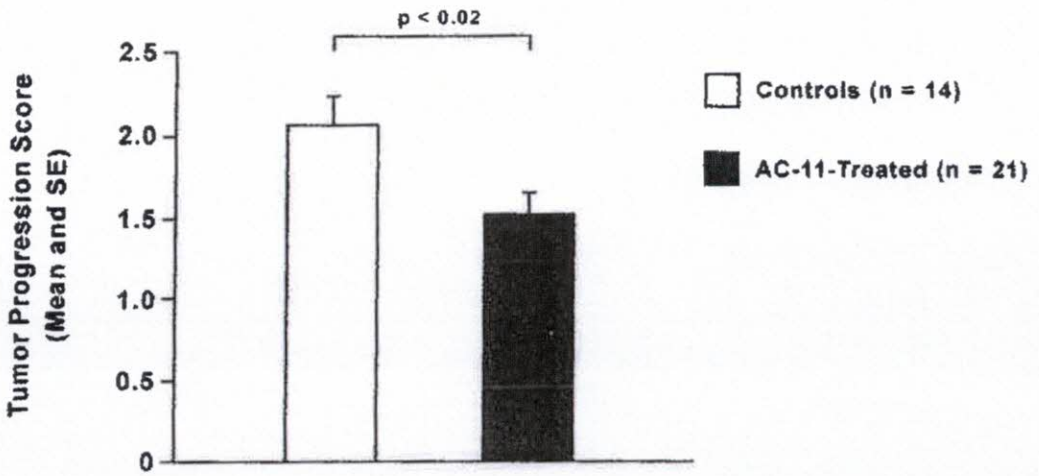

Figure 1. Mean (and standard error) tumor progression scores in hairless mice exposed to a cumulative dose of 738 J/cm^2 of solar-simulating radiation administered over 77 days. Control animals (n = 14) included untreated and vehicle-only-treated animals. Topical application of AC-11 (total n = 21) included animals treated with 0.5%, 1.5%, and 3.0% concentrations of AC-11. Tumor progression scores were assigned as follows: 4 = extensive tumor development; 3 = early malignancies (raised palpable plaques); 2 = firm scaling, palpable keratosis; 1 = light scaling with erythema.

Histological findings were consistent with the development of squamous cell carcinoma. Although it tended to be obscured by extensive hyperplasia, signs of collagen damage in irradiated areas and changes in dermal architecture were evident.

Conclusion

Results obtained in the hairless mouse model of UV-induced carcinogenesis suggest that topical AC-11 may be an effective adjunct to the standard precautions of limiting sun exposure and the application of sunscreens to minimize the risk of nonmelanoma skin cancer.

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