

The *In vivo* Effect of UVB Radiation on Skin Bacteria in Patients with Atopic Dermatitis

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Fourteen patients suffering from atopic dermatitis under treatment with UVB radiation were subjected to aerobic bacterial cultures in order to investigate whether this ultraviolet waveband has any *in vivo* germicidal effects, and, if so, whether there is a correlation with clinical improvement. Treatments were given 3 times a week for 8 weeks. Bacterial samples were collected before, midway and after the termination of therapy. On the latter two occasions, cultures were performed 30 min and 24 h post-UVB irradiation. The main bacteria found were *Staphylococcus epidermidis* and *S. aureus*. *S. aureus* carriage was found in 12 patients in lesional, dermatitic skin, and in 11 patients in clinically non-lesional skin. UVB radiation was found to have an antimicrobial effect primarily concerning *S. aureus*. Bacterial counts of this organism in lesional skin were decreased from a mean of 1.3×10^3 to 1.2×10^1 bacteria per cm^2 skin at the 8-week 30-min count ($p < 0.01$) and 7.5×10^1 at the 8-week 24-h count ($p < 0.05$). The treatment yielded a statistically significant clinical improvement. **Key words:** *Staphylococcus aureus*; *Staphylococcus epidermidis*; Phototherapy; Antimicrobial effect.

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The role of microorganisms, in particular *Staphylococcus aureus*, as pathogenetic factors in atopic dermatitis (AD) has

been a matter of discussion (1–3). The prime reason for this is the finding that the skin of AD patients is widely colonised by *S. aureus*. While the skin in healthy individuals has an *S. aureus* colonisation rate of 3–8% (4–6), lesional, clinically non-infected skin of patients with AD is colonised with this microorganism in 80–93% of cases (3,6–8) and the non-affected skin in 51–78% (3,5,6–8). Different antimicrobial methods have therefore been employed for the treatment of atopic dermatitis. Among these are topical antibiotic agents, which have been reported to be efficacious for the treatment of AD (3,9). Ultraviolet radiation (UVR) is another antimicrobial method (10,11). This mode of treatment has also been shown to be effective for the control of AD (12,13). UVR, however, is also thought to have other modes of action besides its antimicrobial properties.

Previous studies concerning the effect of UVR on microorganisms have been performed *in vitro* (e.g. 10,11), included diseases other than AD, such as psoriasis (14), or employed UVR waveband ranges other than UVB (10,14). We report here the results of a study where patients treated for AD with ultraviolet B radiation (UVB; 280–315 nm) were subjected to quantitative aerobic cultures before, during and after treatment. Parallel to this, a clinical assessment was made. The objective of the study was to investigate whether UVB has *in vivo* germicidal effects in AD patients, and, if so, whether there is a correlation between this property and clinical improvement.

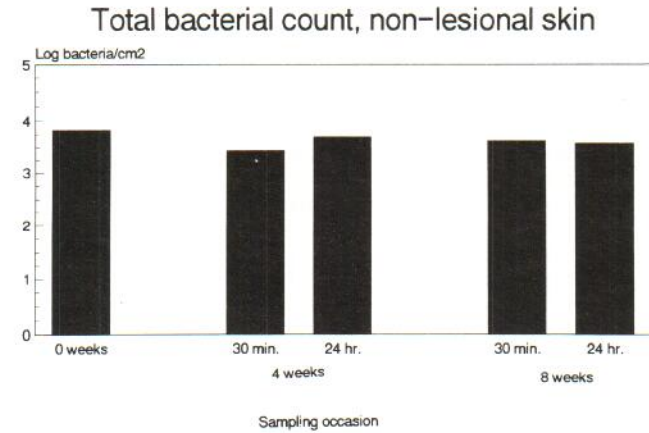


Fig. 1. Logarithms of mean total bacterial counts in non-lesional skin in patients with atopic dermatitis treated with UVB light for 8 weeks. 30 min. and 24 hr. denotes sampling 30 min and 24 h after UVB irradiation, respectively. No statistically significant differences were found.

MATERIAL AND METHODS

Patients

The following exclusion criteria were applied: UVR treatment or sun-bathing during one month prior to the study; use of systemic corticosteroids during the study; use of oral or topical antibiotics or antimycotics during and one month before the study; use of topical preparations containing salicylic acid one week before and during the study; and age below 15 years. A requirement for inclusion was the presence of dermatitis lesions on the patient's chest. All patients were instructed not to use any other topical preparations than Uniderm® ointment (containing only hydrocortisone 1%, liquid paraffin and white petrolatum), Essex ointment (the base of Uniderm® ointment) or white petrolatum on the culture sampling site (chest) during the study. All patients fulfilled the diagnostic atopic dermatitis criteria of Hanifin & Rajka (15). Informed consent was obtained from all subjects.

Twenty-six patients were included in the study. Out of these, 12 were excluded: 3 due to lack of time for treatment, 2 due to a journey, 1 due to a car accident, 2 due to alleged irritation elicited by UVB, 2 due to oral antibiotic treatment given to them by other physicians during the trial and 2 patients were lost to follow-up.

The final study population comprised 14 patients, 7 men and 7

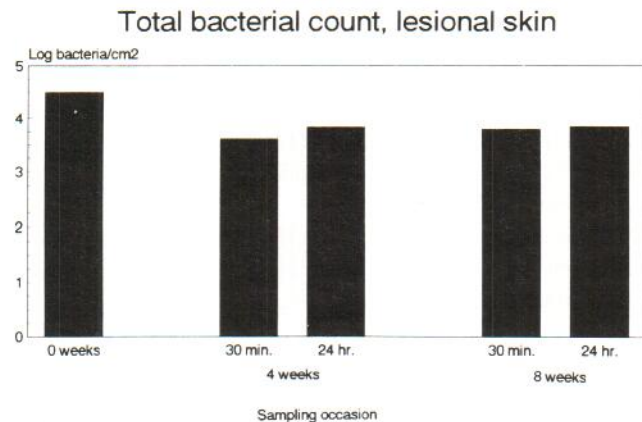


Fig. 2. Logarithms of mean total bacterial counts in eczematous skin in patients with atopic dermatitis treated with UVB light for 8 weeks. 30 min. and 24 hr. denotes sampling 30 min and 24 h after UVB irradiation, respectively. Statistically significant differences: 0 weeks vs. 4 weeks 30 min; 0 weeks vs. 8 weeks 30 min; and 0 weeks vs. 8 weeks 24 h (all $p < 0.05$). Remaining differences not significant.

S. aureus count, non-lesional skin

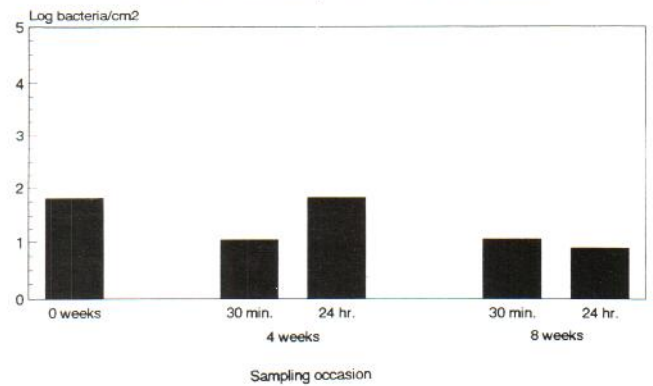


Fig. 3. Logarithmic populations of *S. aureus* in non-lesional skin in patients with atopic dermatitis treated with UVB light for 8 weeks. 30 min. and 24 hr. denotes sampling 30 min and 24 h after UVB irradiation, respectively. Statistically significant differences: 0 weeks vs. 8 weeks 30 min; 0 weeks vs. 8 weeks 24 h; and 4 weeks 24 h vs. 8 weeks 24 h (all $p < 0.05$). Remaining differences not significant.

women with a mean age of 22.9 (SD 4.8) years. The mean total disease duration was 19.1 (SD 6.8) years and the duration of the current exacerbation was 4.6 (SD 5.8) months. Four patients had skin type II (always burns, sometimes tans), 9 skin type III (sometimes burns, always tans) and 1 skin type IV (seldom burns, always tans). Nine patients suffered from bronchial asthma and/or allergic rhinoconjunctivitis. According to the severity grading system proposed by Lange-land (16), 1 patient had a mild, 8 had a moderate and 5 had a severe disease.

Microbiology

Culture samples were obtained from all patients before the start of phototherapy, after 4 weeks and after the termination of therapy (8 weeks). On the latter two occasions, cultures were performed 30 min and 24 h after UVB irradiation. Twenty-four hours before this irradiation and 24 h before the pretreatment sampling, no topical preparations, including the use of soap, were allowed. The sampling site was in all patients the chest. Samples were taken from lesional and non-lesional skin at approximately the same location each time.

The technique for quantitative bacterial cultures used is that described by Williamson & Kligman (17). Samples were collected using a

S. aureus, lesional skin

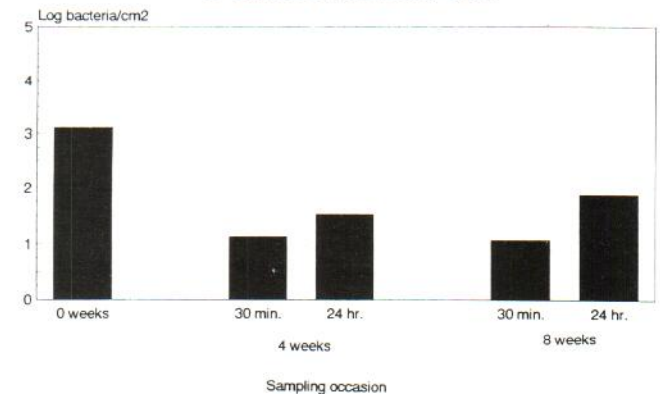


Fig. 4. Logarithmic populations of *S. aureus* in eczematous skin in patients with atopic dermatitis treated with UVB light for 8 weeks. 30 min. and 24 hr. denotes sampling 30 min and 24 h after UVB irradiation, respectively. Statistically significant differences: 0 weeks vs. 4 weeks 30 min ($p < 0.01$); 0 weeks vs. 8 weeks 30 min ($p < 0.01$); 0 weeks vs. 8 weeks 24 h ($p < 0.05$); and 8 weeks 30 min vs. 8 weeks 24 h ($p < 0.05$). Remaining differences are not significant.

stainless steel ring with an internal diameter of 2.6 cm, covering a 5.5 cm² area of skin. The ring was held in place on the skin with moderate pressure from two fingers. One milliliter of sterile 0.075 M phosphate buffer, pH 7.9, containing 0.1% Triton X-100 was poured into the ring, and the skin was gently rubbed with a blunt sterile glass rod for 1 min. The fluid was removed using a Pasteur pipette. Ten-fold dilutions were inoculated onto a blood agar medium. The dishes were incubated at 37°C and examined after 2 days. After incubation, colonies of different morphological types were counted and selected for identification as to the bacterial type. Gram staining was done and appropriate biochemical tests performed for identification, as described by Stockes (18).

Phototherapy

A cubicle containing 14 Philips TL 12/40 W and 14 Philips TL 12/20 W fluorescent tubes (Philips, Roosendaal, The Netherlands), emitting mainly in the UVB region, was used for phototherapy (emission spectrum given in ref. 12). The irradiance at body distance was 0.85 mW/cm². Measurements were performed with an International Light radiometer/photometer IL 1350 using an IL SED 240 as the probe (International Light Inc., Newburyport, Mass., U.S.A.). As a rule, treatments were given 3 times a week for 8 weeks. A mean of 20.8 (SD 4.3) treatments were given. The mean initial, final and total doses were 19.8 (SD 6.0) mJ/cm², 149 (SD 118) mJ/cm², and 2405 (SD 1584) mJ/cm², respectively.

Clinical assessment

Patients were assessed by a doctor on entry to the study and after its completion. Eight effect variables were recorded: pruritus, lichenification, xerosis, scaling, vesiculation, excoriations, erythema and an overall evaluation. Each of these was assigned a score from 0 to 3: 0, none; 1, slight; 2, moderate; and 3, severe. The sum of the scores was designated the total score. The percentage of skin involvement was calculated by the rule of nine.

Statistical analysis

For comparison of bacterial counts, Student's *t*-test (two-tailed) was employed on logarithmic count values. For analysis of clinical variables, Wilcoxon's matched-pair signed rank test (two-tailed) was used. The correlation analysis was performed with Spearman's rank correlation test.

RESULTS

Microbiology

The results of the bacterial counts are summarised in Figures 1–4. The total bacterial counts of non-lesional skin were not affected in any significant way by the UVB treatment (Fig. 1). The effect on lesional skin was also limited, with moderate decrease of the bacterial counts (Fig. 2). The effect of UVB on *S. aureus* counts, however, was more pronounced, a feature especially obvious for lesional skin (Fig. 4). The initial logarithmic mean of 3.11 was reduced to 1.14 and 1.08 bacteria per cm² skin when cultures were performed 30 min after irradiation at weeks 4 and 8, respectively (both *p*<0.01). As can be seen, the 24-h values are somewhat higher, but only the difference between the 30-min and 24-h week 8 values is significant. *S. aureus* was recovered from lesional skin of 12 patients and from non-lesional skin of 11 patients. The mean bacterial densities are given in Figs. 3 and 4. The majority of the total bacterial count comprised the population of *S. epidermidis*, which was found in both lesional and non-lesional skin of all patients. Sporadically encountered bacteria included *Micro-*

coccus sp., *Bacillus* sp., *Klebsiella oxytoca* and non-lipophilic diphtheroids. None of these microorganisms occurred in all samplings throughout the study.

A comparison of the antibacterial effect of UVB irradiation on non-lesional versus lesional skin revealed a significantly greater decrease in *S. aureus* counts on lesional skin when the density decrease from week 0 was compared with that of week 8, 30 min (*p*=0.004).

Clinical results

Subjected to statistical analysis were the total score, which was reduced from a pretreatment mean of 9.2 to 4.7, the overall evaluation score, reduced from 1.7 to 0.9, and the dermatitis extent, reduced from 14.6% to 6.1% (all *p*<0.001).

Correlation analysis

Designating the difference between *S. aureus* counts of lesional skin at 30 min and 24 h as Δ_1 and the difference between the overall evaluation scores at week 0 and week 8 as Δ_2 , we found a statistically significant positive correlation (correlation coefficient 0.69; *p*=0.009) between Δ_1 and Δ_2 . This means, that patients with stable *S. aureus* counts at week 8 (without exhibiting a bacterial increase from 30 min to 24 h) were the ones to show the best overall evaluation results. No statistically significant correlation *between* the total doses, final doses or the number of treatments given *and* the effect on bacterial counts could be detected.

DISCUSSION

Our study has shown UVB to have antimicrobial *in vitro* effects against *S. aureus* in patients with clinically non-infected atopic dermatitis. These effects have been paralleled with clinical efficacy. Our investigations have also confirmed previous reports (3,5–8) on the high carriage rate of *S. aureus* seen in these patients.

UVR has previously been reported to be germicidal (10–11). However, photochemotherapy (PUVA) used for treating psoriasis (14) did not significantly reduce the growth of bacteria. Also, Gerber et al (19) reported frequent sunbathing not to affect the total number of skin microorganisms. The significance of these latter results is, however, unclear due to wide inclusion criteria and a non-uniform sampling method: subjects with sunbathing habits of several times a week to twice a month were included, and the time between sampling and the most recent sunbath ranged from 1 day to 2 weeks.

In the present investigation, UVB irradiation was seen to have significant effects on *S. aureus* counts. This finding is interesting, since *S. aureus* is the microorganism most often encountered in discussions on the pathogenesis/etiology of AD (1–3). Furthermore, we have seen that the antistaphylococcal effect has a tendency to be highest immediately after irradiation as compared with 24 h later. Interestingly, we also found a correlation between good clinical efficacy and the ability to retain low *S. aureus* counts. This finding must, however, be confirmed before any conclusions are drawn from it, especially as there is a risk of the mass significance problem interfering (correlation testing being done on several parameters).

The effect of UVB on total bacterial densities was in this study marginal. Since the total counts were mainly dominated by *S. epidermidis*, it seems that UVB *in vivo* has less effect on this bacterium than on *S. aureus*. These results are in contrast to those of *in vitro* experiments (11). One possible explanation could be the fact that *S. epidermidis* is mainly found in skin hair follicles, while *S. aureus* is found on the skin surface (20), making the latter more readily accessible to UV radiation.

Statistical analysis has also shown a better antistaphylococcal effect of the UVR on lesional than on non-lesional skin. This could, however, be due to the higher initial densities of this bacterium on dermatitic skin. This finding must therefore be interpreted with caution.

Killing of bacteria has previously been attempted for the treatment of AD, with encouraging results. Mupirocin, an antistaphylococcal antibiotic, proved to be more efficacious than placebo (3). In another study (9), Wachs & Maibach compared the effect of a topical corticosteroid, a topical antibiotic and the combination of these in patients with impetiginised AD. The combination proved to be the best of these. Thus, the killing of germs, *S. aureus* in particular, seems to be of benefit to AD patients. Whether this is the mode of action for UVR or whether it is a parallel, possibly unrelated, phenomenon remains an unsolved question.

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