

Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy

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SUMMARY

Forty-nine patients with atopic dermatitis entered a double blind placebo controlled cross-over study of mupirocin, a new topical antistaphylococcal antibiotic. Forty-five patients were evaluable. Quantitative bacteriological assessment before treatment showed that heavy colonization of the skin with *Staphylococcus aureus* was present in nearly all patients even in the absence of overt infection. However, the bacterial count was significantly reduced by 2 weeks' treatment with topical mupirocin, but not by the placebo. Moreover, a significant reduction of clinical severity was also observed after treatment with mupirocin, which was maintained over the following 4 weeks, although recolonization occurred during this period, with bacterial counts rising to pre-treatment levels. Despite recolonization, clinical deterioration was not observed during the trial period. No serious side-effects were observed.

Phage typing showed that 50% of patients carried more than one bacterial phage type. Recolonization in eight patients (17%) was with a 'new' strain that had not previously been isolated.

Chronic atopic dermatitis may be aggravated by a number of factors. Cutaneous infection, usually with *Staphylococcus aureus*, is one such factor and systemic antibiotics play a major role in the treatment of exacerbations. Heavy skin colonization with *S. aureus* has been reported in patients with atopic dermatitis even if the skin is not clinically infected,¹⁻⁴ and this may contribute to continuing disease activity.⁵ Patients with atopic dermatitis may show increased adherence of staphylococci to both skin and mucous membrane surfaces⁶⁻⁹ and *S. aureus* may have specific binding sites, possibly protein A or teichoic acid, on the cell wall, for fibronectin¹⁰ and laminin¹¹ in the tissues.

Although antibacterial activity of *Pseudomonas fluorescens* was demonstrated almost a century ago, antimicrobial metabolites have only recently been isolated and developed commercially. Mupirocin (Bactroban) has a novel mode of action in that it inhibits the enzyme isoleucyl

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transfer-RNA synthetase and thus stops bacterial protein synthesis.¹² It is rapidly metabolized systemically and is excreted mainly as the biologically inactive monic acid in the urine. It has a wide range of activity against Gram positive bacteria and is active against staphylococci, including methicillin resistant *S. aureus*.¹³

The present study was undertaken to assess the degree of skin colonization with *S. aureus* in patients with atopic dermatitis and to determine how this changed with topical therapy with 2% mupirocin ointment. Concomitant changes in the clinical severity of the dermatitis were also recorded.

METHODS

Clinical details

Patients aged 2 years or older were included in the study provided they had the typical clinical features and classical relapsing course of atopic dermatitis. They were excluded if systemic antibiotic therapy was required for overt secondary infection of the skin, if they had received topical or systemic antibiotics for any reason during the preceding 4 weeks, or if potent topical steroids (British National Formulary category 1) were needed to control disease activity. All patients or their parents gave informed consent and the study was approved by the ethical committees of both hospitals at which patients were studied.

Forty-nine patients were entered into a double-blind placebo-controlled cross-over study and were randomly allocated to receive placebo first (P/M group) or mupirocin first (M/P group). Patient details are given in Table 1. At the initial visit 2 weeks before the start of the trial all patients were given a standard topical steroid therapy (0.5% clobetasol butyrate ointment, Eumovate) which was continued throughout the trial. Patients were asked to apply the topical steroid twice daily during the 2 week pre-trial period and once daily while using mupirocin or placebo. Patients were re-assessed clinically, by the same observer, at 2-week intervals for 10 weeks. For the first two weeks of the trial (weeks 0-2) patients in the M/P group applied

TABLE 1. Details of patients who received placebo followed by mupirocin (P/M group) and mupirocin followed by placebo (M/P group)

	P/M group (n = 21)	M/P group (n = 24)
Sex	12M 9F	12M 12F
Age: Mean	20.4	22.4
(years) (and range)	(2-52)	(2-56)
Race: Caucasian	19	22
Indian	2	1
Chinese	0	1
Other atopic disease:		
Asthma	8	10
Hay fever	6	4
None	7	10

mupirocin ointment and patients in the P/M group applied the polyethylene glycol ointment base (placebo). The trial ointment was applied in the morning and the topical steroid at night to all affected sites. After 2 weeks the groups were crossed over and the P/M group received mupirocin and the M/P group received placebo for a further 2 weeks (weeks 2-4). All the patients used clobetasol butyrate only twice daily for the remaining 4 weeks of the study period. The only other topical preparations allowed were the patients' usual emollients which were continued unchanged throughout the trial.

Patients were assessed clinically on a 0-3 scale for six features (extent, erythema, pustulation, excoriations, dryness and cracking) at 16 body sites. An estimate of the percentage body surface area involved was made using the rule of nines, which estimates the body surface area using nine or multiples of it, i.e. arms and head 9% each, anterior trunk, posterior trunk and legs 18% each.

Each patient was scored by the same observer at each visit to avoid inter-observer variation. Observers were blind as to the topical preparation used and the bacteriological findings. Patients were also asked to make a subjective assessment on a five point scale (much better, better, same, worse, much worse) of itch, their sleep disturbance and the appearance of their skin.

Bacteriological assessment. Bacteriological results were scored blind by the same bacteriologist at weeks 0, 2, 4, 6 and 8 on samples taken from two skin sites on each patient, the site 'worst affected' by atopic dermatitis and a 'control' site—an area of clinically normal skin unaffected by eczema.

TABLE 2. Bacterial isolates from the worse affected skin sites at week 0

Isolates*	Total	Number of patients with colony counts of <i>S. aureus</i>	
		< 10 ²	≥ 10 ²
M/P group			
<i>S. aureus</i>	20	2	18
Coagulase negative staphylococcus	1		
<i>Clostridium perfringens</i>	1		
<i>Acinetobacter</i> spp.	1		
P/M group			
<i>S. aureus</i>	18	2	16
Coagulase negative staphylococcus	1		
β-haemolytic streptococcus (Lancefield Group G)	2		
<i>Clostridium perfringens</i>	2		

* Cultures from two patients in the M/P group and four patients in the P/M group grew two organisms, while cultures from three patients in the M/P group and two in the P/M group showed no growth.

Quantitative assessment of S. aureus

Numbers of *S. aureus* were estimated by applying 55 mm diameter contact plates (Sterilin Ltd. Middlesex, U.K.) directly to the skin sites. The plates contained cysteine, lactose, electrolyte deficient (CLED) agar (Oxoid Ltd, U.K.) which gives good colony differentiation of *S. aureus* and prevents the swarming of *Proteus spp.*

After aerobic overnight incubation at 37°C, the number of colonies of *S. aureus* per contact plate, i.e. per 24 cm², was counted, which represented the number of viable colony-forming units of *S. aureus* in the area of skin sampled.

Qualitative assessment

Swabs (Albuswabs, Exogen, Clydebank, U.K.) were taken from worst affected and control sites. Each swab was used to inoculate blood agar, blood agar with gentamicin (10 mg/l) MacConkey agar and Robertson's meat broth. One blood agar plate was incubated aerobically and one anaerobically and the remaining media were incubated aerobically at 37°C overnight.

The blood agar, with and without gentamicin, was important for detecting β -haemolytic streptococci, which are less easily identified on CLED agar.

Swabs taken from the patients' anterior nares at week 0 were plated on blood agar and incubated aerobically at 37°C overnight.

Organisms were identified by routine laboratory methods. Sensitivity to commonly used antibiotics was tested by the comparative disc diffusion method.¹⁴ Minimum inhibitory concentrations (MICs) of mupirocin for all *S. aureus* isolates were determined by the broth dilution method,¹⁵ and phage typing was carried out according to standard methods.

RESULTS

Four patients were excluded from the final analysis. Three had received placebo first (P/M group) and one mupirocin (M/P group). One patient in the M/P group had an acute flare requiring potent steroids during the run-in period, a second, in the P/M group, required concomitant antibiotics for a respiratory infection and a third, in the P/M group, reacted adversely to both mupirocin and placebo. Only one patient defaulted while on mupirocin. Thus, 45 patients were available for analysis.

TABLE 3. Numbers of patients colonized with *S. aureus* (i.e. colony counts $\geq 10^2$)

	Worst affected sites					Control sites				
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 0	Week 2	Week 4	Week 6	Week 8
	End of run-in	End of mupirocin	End of placebo			End of run-in	End of mupirocin	End of placebo		
M/P group	18 of 24	1 of 24	7 of 21	13 of 20	9 of 20	4 of 24	0 of 24	2 of 21	2 of 20	9 of 20
	End of run-in	End of placebo	End of mupirocin			End of run-in	End of placebo	End of mupirocin		
P/M group	16 of 21	14 of 21	1 of 20	9 of 19	8 of 18	2 of 21	4 of 21	1 of 20	2 of 19	2 of 18

Forty-six isolates were obtained from the worst affected sites of the 45 patients at week 0. *S. aureus* was isolated from 38 of the 45 patients; five patients carried two strains and one carried three strains giving a total of 45 strains. β -haemolytic streptococci of Lancefield Group G were isolated on two occasions. No β -haemolytic streptococci of Lancefield Group A were isolated. An *Acinetobacter* sp, a heavy growth of coagulase negative staphylococci, and *Clostridium perfringens* were isolated on one, two and three occasions, respectively (Table 2). Small numbers of coagulase negative staphylococci and diphtheroids in mixed culture were regarded as normal flora.

Control non-affected sites yielded similar results. Twenty-five positive isolates were obtained at week 0 of which 23 were *S. aureus*. *Clostridium perfringens* and a heavy growth of coagulase negative staphylococci were each isolated on one occasion. Small numbers of coagulase negative staphylococci and diphtheroids in mixed culture were again regarded as normal flora.

With the exception of one isolate, all the *S. aureus* isolates were sensitive to mupirocin with MICs between 0.125 and 0.5 mg/l. The more resistant isolate (MIC 32 mg/l) was isolated from the worst affected site of a patient in the M/P group 4 weeks after stopping mupirocin. No methicillin resistant strains of *S. aureus* were isolated.

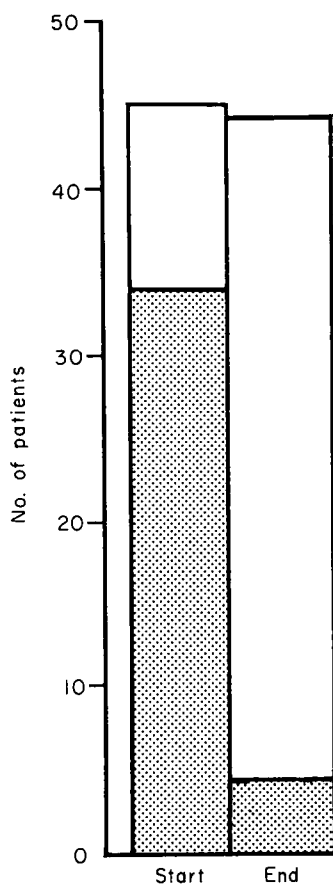


FIGURE 1. Numbers of patients colonized by staphylococci before and after mupirocin treatment (M/P and P/M groups combined) at worst affected sites. ■ ≥ 100 colonies/24 cm²; □ < 100 colonies/24 cm².

TABLE 4. Phage typing of 45 strains of *S. aureus* from the worst affected area at week 0

Phage type	No of strains (%)	
Group III	14	(31%)
Group I	4	(9%)
Group II	3	(7%)
Other	9	(20%)
Non-typable	15	(33%)

Full phage type details of all strains available on request.

S. aureus colonization

The colony counts of *S. aureus* per 24 cm² (contact plate surface area) were arbitrarily divided into two mutually exclusive and exhaustive categories: counts $\geq 10^2$, designated 'colonized', and counts $< 10^2$ including 0 (Tables 2 and 3). Differences in the number of colonized sites between the patient groups, and within patient groups at different times, were analysed using Fisher's exact test.

At week 0, 6 of the 45 patients (13%) were colonized at the 'control' clinically normal site while 39 patients (87%) carried less than 10^2 colonies and 20 patients (44%) did not carry any *S. aureus* at these sites.

On the 'worst affected' sites, at week 0, 34 of the 45 patients (76%) were colonized while 11

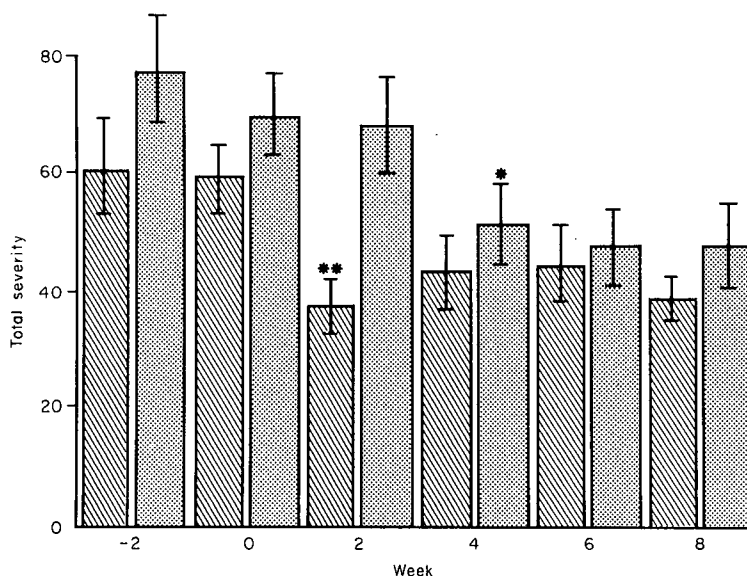


FIGURE 2. Clinical severity scores in the M/P group (▨) and P/M group (■). Bars are means $\pm$ SEM. After treatment with mupirocin, clinical activity fell and was significantly different from week 0 * $P < 0.05$, ** $P < 0.001$.

patients (24%) carried less than 10^2 colonies. In only 7 of the 45 patients (16%) was no *S. aureus* isolated from the worst affected site at week 0.

There was no significant difference between the number of sites colonized in the two groups, either 'worst affected' or control, at week 0, but in both groups the number of worst affected sites colonized was significantly greater than the number of control sites ($P < 0.0001$). In the P/M group there was no significant difference between the number of sites colonized at week 0 and week 2, i.e. before and after application of the placebo ($P > 0.05$). Thus there was no evidence that the polyethylene glycol ointment base had anti-staphylococcal activity.

In contrast, there was a significant fall in the number of worst affected sites colonised in both groups after 2 weeks of mupirocin therapy ($P < 0.0001$). *S. aureus* could be recovered from only 4 of 44 patients (9%) at this time (Fig. 1).

Recolonization to pre-treatment levels was found at week 6, 2 weeks after the end of mupirocin treatment in the P/M group and 4 weeks after the end of mupirocin treatment in the M/P group. At this time the number of colonized sites in each group did not differ significantly from that before treatment ($P > 0.05$).

Phage typing was carried out on all strains of *S. aureus*. The results for the 45 strains isolated from the worst affected site at week 0 are shown in Table 4. The majority belonged to group III; there were three from group II and four from group I. At week 0, 12 of the 45 patients (27%) carried the same phage type in the nose as that in the worst affected area and 14 of the 45 (31%) had the same phage type in the worst affected and the control sites. In only eight patients (18%) was the same phage type present in all three sites.

Over the period of the study 22 of the 45 patients (49%) were found to carry more than one strain of *S. aureus*. After treatment with mupirocin 12 of the 45 (27%) were recolonized with the same strain as that originally found in the nares, 14 of 45 (31%) with that from the worst affected area and 9 of 45 (20%) with that from the control site. Eight patients were recolonized with a 'new' strain that had not previously been isolated.

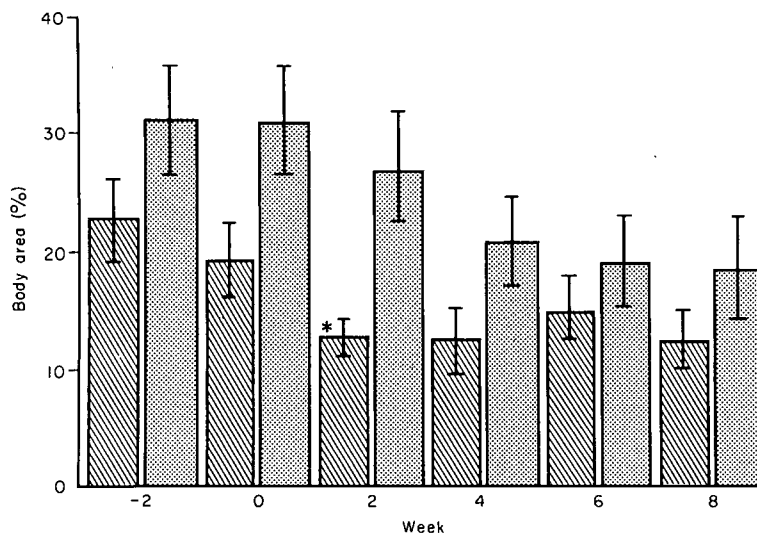


FIGURE 3. Percentage body area affected in M/P group (▨) and P/M group (▩). Bars are means $\pm$ SEM. *Significantly different from week 0, $P < 0.05$.

TABLE 5. Patients' assessments of skin appearance, itch and sleep disturbance

	Weeks 0-2 M/P group No. of patients					Weeks 0-2 P/M group No. of patients					Weeks 2-4 P/M group No. of patients				
	Better	Same	Worse	χ^2 [†]	P	Better	Same	Worse	χ^2 [†]	P	Better	Same	Worse	χ^2 [†]	P
Skin appearance	19**	5	0	19.0	<0.001	10	8	3	3.8	NS	8	10	3	2.3	NS
Itch	13**	10	1	10.3	<0.001	4	13	4	0	NS	9	10	2	4.5	NS
Sleep disturbance*	12**	10	0	12.0	<0.001	3	13	3	0	NS	2	14	3	0.2	NS

*Two patients in each group had no sleep disturbance.

**A significant proportion of patients showed an improvement ($P < 0.05$; Fishers exact test).

† χ^2 : within group test of differential change. All have 1 degree of freedom.

Clinical pre-treatment severity scores showed that the P/M group was slightly more severely affected, mean score $\pm$ SEM 69.9 ± 6.9 than the M/P group, mean score 59.5 ± 6.4 and also had a greater mean surface area involved, $31.0 \pm 4.7\%$ versus $19.1 \pm 3.0\%$ in the M/P group. After the 2-week run-in period, clinical severity fell in the P/M group, but remained virtually unchanged in the M/P group. After the first trial period, clinical severity was virtually unchanged in the P/M group (using placebo) mean score 68.0, but fell significantly in the M/P group (using mupirocin) to a mean of 37.6. This was significantly different both from the M/P group pre-treatment baseline ($P < 0.001$) and from the P/M group ($P < 0.002$). After the cross-over period at 4 weeks, the P/M group improved significantly ($P < 0.05$) whereas the M/P group, (now on placebo) showed a slight, but not statistically significant deterioration. Clinical activity then remained stable in both groups for the remainder of the trial (Fig. 2). Calculation of the change in clinical severity scores over the whole trial period taking visit 0 as the base-line value, showed that the change in clinical severity was virtually identical for the two groups.

Similar, though less marked changes were seen in the area involved; the P/M group showed a diminution in surface area involved during both the run-in period and the placebo phase although a more marked fall was seen when mupirocin was applied. However, this was not clinically significant. In contrast, the area involved in the M/P group was unchanged during the run-in period, but fell significantly when the active treatment was applied ($P < 0.003$). In both groups the area involved remained unchanged for the rest of the trial (Fig. 3).

On subjective assessment the patients felt they had improved symptomatically in terms of skin appearance, itch and sleep disturbance while they were using topical mupirocin. However, this was only statistically significant in the group which received mupirocin first ($P < 0.001$; Fisher's Exact test) (Table 5).

No serious side-effects were observed. Two patients noticed that both the placebo and the mupirocin ointments caused stinging and an exacerbation of their symptoms. This caused one patient to withdraw from the trial.

DISCUSSION

The present study has confirmed that both eczematous and unaffected areas of skin are colonized with *S. aureus* in patients with atopic dermatitis.^{1,3,4} However, the normal areas of

skin may well be colonized by the simple transfer of organisms from the affected eczematous areas. It is difficult to compare these present quantitative studies directly with previously reported scrub techniques for sample collecting.^{3,4} The plating method has the advantage that it does not irritate eczematous skin and is quick and painless, which is an important consideration in investigating children.

The clinical improvement following mupirocin was associated with a reduction in staphylococcal colonization which raises the question of the role of *S. aureus* in this disease.¹⁶ Recolonization did not appear to be associated with clinical deterioration within the period of the study. This does not exclude a pathogenetic role for *S. aureus*, but their effect, if present, must be delayed.

Phage typing in general confirmed the finding by Aly² that most of the staphylococci belong to, or cross react with, group III. However, group I (hospital) strains were isolated from three of our patients and three strains were from group II which are associated with the 'staphylococcal scalded skin syndrome'. We also found that many of our patients with atopic dermatitis carried more than one strain of staphylococcus in different sites, which contrasts with the findings of Anderson¹⁷ who noted that different regions of the same individual were generally colonized with the same phage type. He also showed that swabs taken from seven patients at different times (range 1 to 6 weeks) were generally colonized with the same organism. This was not the pattern we found. However, our results represented recolonization after topical antibiotic treatment, which may differ from that found in the 'normal' flora of a patient with atopic dermatitis which has not been altered by treatment.

Mupirocin, but not the polyethylene glycol (PEG) base significantly reduced this staphylococcal colonization, and in 89% of patients, staphylococci were not isolated from the skin after 2 weeks application of mupirocin. Other studies have shown similar reduction of staphylococcal colonization using topical neomycin and systemic erythromycin.³ During mupirocin application, clinical activity also fell significantly. After mupirocin was discontinued, recolonization occurred fairly rapidly, but a concomitant clinical deterioration was not observed. Superinfection with other organisms was not seen during the trial, nor was this observed by Lin *et al.*¹⁸ who used mupirocin in the treatment of epidermolysis bullosa over a period of 2 years. Recently, however, there have been reports of the development of resistant strains^{19,20} and resistance may be transferable by plasmids.

A further observation in our study was that the replacement of the normal commensal lipophilic diphtheroids with *S. aureus* produced little sign of infection in atopic dermatitis. The effect of staphylococcal colonization, therefore, may simply be a direct inflammatory one, possibly induced by protein A in the cell wall.²¹

During the present study a reduction in the bacterial load was noted to be associated with clinical improvement, suggesting that control of skin colonization with staphylococci may prove clinically beneficial in the treatment of atopic dermatitis.

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