The Mast Cell. Its Role in Health and Disease. International Symposium. Davos, April 1979.
8. Soter, N. A.: The efficiency of oral disodium cromoglycate in human mastocytosis. *In* The Mast Cell, Its Role in Health and Disease. International Symposium. Davos, April 1979.
9. Warin, R. P. & Hughes, R. C. W.: Diffuse cutaneous mastocytosis. Br Dermatol *75:* 296, 1963.
10. Waters, W. J. & Lacson, P. S.: Mast cell leukaemia presenting as urticaria pigmentosa. Report of a case. Pediatrics *19:* 1033, 1957.
11. Willemze, R., Ruiter, D. J., Scheffer, E. & Van Vloten, W. A.: Diffuse cutaneous mastocytosis. Report of a case with clinical histopathological and ultrastructional aspects. Br Dermatol *102:* 5, 601, 1980.
12. Wilson, C. W. M., Matsp, Prendergast, C. & Stuart, M.: Buccal absorption and action of sodium cormoglycate. *In* The Mast Cell, Its Role in Health and Disease. International Symposium Davos, April 1979.

A Double-Blind Study of the Effects of Benzoyl Peroxide 20% and Eusol and Liquid Paraffin on the Microbial Flora of Leg Ulcers

Diana C. Daltrey and W. J. Cunliffe

School of Health and Applied Sciences, Leeds Polytechnic, and Department of Dermatology, Leeds General Infirmary, Leeds, England

Received February 23, 1981

Abstract. Leg ulcers in 30 patients were treated for 6 weeks with either benzoyl peroxide 20% (Benoxyl 20) or Eusol and liquid paraffin. The types and numbers of micro-organisms/cm² leg ulcer surface were assessed initially and after 2 and 6 weeks of treatment. There was no significant difference between the preparations as regards their ability to reduce the surface count of aerobic micro-organisms. The only significant result was a reduction in the number of lesions colonized by *Staphylococcus aureus* when treated with benzoyl peroxide.

Key words: Leg ulcer; Microbiology; Therapy; Eusol; Benzoyl peroxide

Benzoyl peroxide is known to have an antibacterial effect *in vitro* (1), and has been used to treat cutaneous ulcers (3, 7). Our aim was to examine and compare any changes in the bacterial flora of leg ulcers during treatment with either benzoyl peroxide, or Eusol and liquid paraffin. The latter was employed as a comparative treatment since it is widely used in dermatological practice.

METHOD

Subjects

Thirty patients with ulcers on the lower legs entered the study; 17 received treatment with 20% benzoyl peroxide and 13 with Eusol and liquid paraffin (equal parts); the latter preparation is extensively used in the United Kingdom for the treatment of leg ulcers. This study was part of a larger clinical investigation. Full clinical and therapeutic details will be reported elsewhere. The 30 patients in this study were the only group to have detailed microbiological investigations carried out. In all but 3 subjects the ulcers were predominantly venous in origin but the clinical status of the patients did not influence either the pretreatment microbiological data or the response to therapy.

Bacteriology

Samples were taken from the centre of each lesion immediately after removal of the dressing at time 0, 2 and 6 weeks. Samples were always taken by the same person (D. D.). One cm² of the ulcer surface was sampled through a sterile metal template using an alginate swab moistened in saline, followed by a dry alginate swab. (If the ulcer was smaller than the template, the whole ulcer was swabbed and the area measured by planimetry.) Both swabs were aseptically broken off into 20 cm³ nutrient broth + 1% sodium hexametaphosphate and the swabs were dissolved by vigorous vortexing. Serial tenfold dilutions of the swab solution in nutrient broth were made immediately. Counts were performed by the method of Miles and Misra (6) on blood agar and MacConkey agar aerobically, and on vitamin K_1 blood agar (K_1BA) and neomycin vitamin K_1 blood agar (NeoK_1 BA) anaerobically (9). Since the count procedure involved much aeration, and it was not possible to perform a second strictly anaerobic count procedure, a plain cotton swab was used to sample the area around the template for strictly anaerobic bacteria. This swab was inoculated immediately onto prereduced K_1BA and NeoK_1BA plates. All anaerobic plates were incubated in GasPak jars (90% H_2 + 10% CO_2) at 37°C and inspected after 48 hours and 1 week. Aerobic plates were incubated at 37°C and inspected after 24 hours and 48 hours.

The count of aerobic and facultatively anaerobic bacteria/cm² was determined and the presence or absence of strict anaerobes was noted. Aerobic isolates were fully identified, anaerobic Gram-negative rods were presumptively identified using antibiograms (9): other anaerobic isolates were loosely grouped as anaerobic and microaerophilic cocci, or Gram-positive rods.

RESULTS

The range of organisms isolated and the changes in flora during treatment are shown in Table I. Each type of organism was found with similar frequency

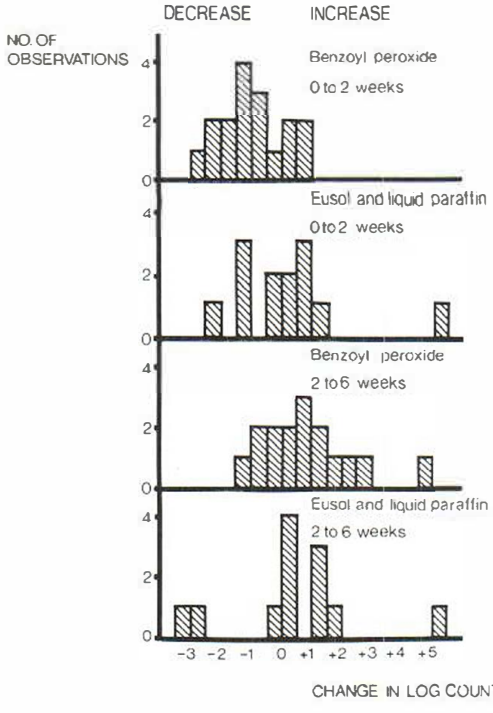

Fig. 1. Change in log. no. of aerobic bacteria/cm² ulcer surface during treatment with benzoyl peroxide or Eusol and liquid paraffin.

in the two treatment groups. The commonest isolates were *Pseudomonas aeruginosa*, Streptococcus group D and *Staphylococcus aureus*. The anaerobic isolates were mostly Bacteroides and anaerobic cocci. Pseudomonas, once acquired, never disappeared from any lesion. There was a statistically significant drop in the number of lesions colonized by staphylococci after benzoyl peroxide treatment ($p<0.001$). Staphylococci disappeared from all nine benzoyl peroxide treated lesions where they were present as part of a mixed flora: however, in one lesion *S. aureus* was the sole colonizing organism and it persisted through 6 weeks of benzoyl peroxide treatment.

Most of the leg ulcers were colonized by several different micro-organisms. The median number of isolates before treatment was 2 (range 0–8): after treatment with benzoyl peroxide or Eusol for 6 weeks the median numbers were 2 (range 1–8) and 3 (range 2–8) respectively.

Fig. 1 indicates that benzoyl peroxide tended to reduce the log count of aerobic bacteria/cm² more than Eusol over the first 2 weeks of treatment, but this trend was not statistically significant at the 0.05 level (Mann-Whitney U). The decrease in counts in benzoyl peroxide treated lesions could not be entirely accounted for by disappearance of Staphylococcus. There was no difference between the treatments thereafter.

DISCUSSION

Eusol and liquid paraffin is widely used in the treatment of leg ulcers (8) and is therefore a useful preparation with which to compare new treatments. Chlorinated lime formulations are bactericidal when used in sufficient concentration: however, most chlorine compounds are unstable and their activity is readily reduced by the presence of organic matter (4). Interest in peroxides as antibacterial agents for skin lesions stemmed mainly from the work of Meleney, who used zinc peroxide to treat chronic ulcerative burrowing lesions of the abdominal wall. Zinc peroxide was effective against micro-aerophilic streptococci and some anaerobes *in vitro* and *in vivo*, but did not kill the aerobes tested (*E. coli*, staphylococci and α-haemolytic streptococci) (5).

Measurement of bacterial population size on an ulcer surface is difficult, since there is no guarantee that the organisms will be homogeneously distributed over the surface and there is no ideal non-destructive sampling method available. However, by standardizing all parts of our procedure we believe that we achieved a system that could monitor change effectively, although it could not measure absolute values accurately. The results were therefore analysed by non-parametric statistical methods. In this type of system small fluctuations in count must not be considered significant; we were looking for a uniform and sustained decrease in count.

By these criteria neither Eusol nor benzoyl peroxide showed strong antibacterial effects. Both formulations are known to be active *in vitro* (1, 4) but previous studies have indicated that benzoyl peroxide 20% and 10% are less active *in vivo* (3, 7). Lookingbill et al., in a study of the effect of benzoyl peroxide 10% on the flora of leg ulcers, isolated a range of aerobic bacteria similar to those we found, but did not make any special attempt to isolate strict anaerobes and did not recover any (3). Anaerobes were found in 13/30 patients in our series. In view of earlier work on zinc peroxide, benzoyl peroxide might be expected to show

Table 1. *Changes in flora during treatment*

Micro-organism	No. of patients with micro-organism					
	Present throughout		Lost during treatment		Gained during treatment	
	Benoxyl	Eusol	Benoxyl	Eusol	Benoxyl	Eusol
Pseudomonas	9	5	0	0	3	5
Streptococcus	7	4	3	1	3	6
Staphylococcus	1	7	9	2	1[a]	2
Enterobacteria	4	4	1	2	5	4
Acinetobacter	0	1	1	0	2	0
Anaerobes	1	4	1	2	4	1

[a] Lost again at week 6.

greater activity towards anaerobic than aerobic micro-organisms. This did not appear to be the case in our patients (see Table I). In all cases anaerobes were found in close association with aerobic flora which may have exerted a protective effect, e.g. by possession of active peroxidase enzymes.

The disappearance of staphylococci from 9/10 benzoyl peroxide treated lesions is of interest. The persistent Staphylococcus may have been an unusually resistant strain; alternatively, loss of staphylococci may be a two-stage process involving damage by benzoyl peroxide followed by elimination by competition from hardier organisms which are present, e.g. Pseudomonas.

During this study, lesions were dressed once a day. The preparations may have exerted some antibacterial action on application to the ulcer, but subsequently may have been inactivated by exudate, slough, or even large numbers of bacteria present on the ulcer surface. Any residual bacterial population would then be able to build up in numbers between dressings.

Although this study showed no dramatic effects on bacterial counts, the preparations were of help clinically in most patients and so their mechanism of action deserves further thought. Bacteria, in the course of their pathogenic role, produce many enzymes, for example Pseudomonas produces proteases. It could well be that the drugs reduce the production of, and function of, biologically active bacterial enzymes. This possible mechanism would be similar to the treatment of acne where standard dose regimes of antibiotics are clinically effective but do not kill the bacteria (2). Other non microbiological mechanisms should also be considered for example benzoyl peroxide may increase oxygen tension in the lesion and stimulate growth of granulation tissue. This work represents only the detailed microbiological analysis in a large multicentre clinical study of benzoyl peroxide and Eusol, the clinical results will be published elsewhere.

ACKNOWLEDGEMENTS

The authors wish to thank Stiefel Laboratories (U.K) Ltd., Nurse K. Wade, J. Webster (School of Mathematics and Computing, Leeds Polytechnic) and the technicians of the School of Health and Applied Sciences.

REFERENCES

1. Cunliffe, W. J. & Holland, K. T.: An *in vivo* study of the effect of Benzoyl Peroxide 20%, Gentamicin and Phenoxyethanol against *S. aureus*, *P. aeruginosa* and *Proteus species*. Acta Dermatovener (Stockholm) *59*: 555, 1979.
2. Cunliffe, W. J. & Cotterill, J. A.: The Acnes. W. B. Saunders & Co., London, Philadelphia and Toronto, 1975.
3. Lookingbill, D. P., Miller, S. H. & Knowles, R. C.: Bacteriology of chronic leg ulcers. Arch Dermatol *114*: 1765, 1978.
4. Martindale Extra Pharmacopoeia, 27th ed. Ainley Wade, 1977.
5. Meleney, F. L.: Zinc peroxide in the treatment of microaerophilic and anaerobic infections. Ann Surg *101*: 997, 1935.
6. Miles, A. A. & Misra, S. S.: The bactericidal power of the blood. J Hyg (Lond) *38*: 732, 1938.
7. Pace, W. E.: Treatment of cutaneous ulcers with Benzoyl peroxide. Can Med Assoc J *115*: 1101, 1976.
8. Rook, A., Wilkinson, D. S. & Ebling, F. J. G.: Textbook of Dermatology, 3rd ed. Blackwell, 1980.
9. Sutter, V. L., Vargo, V. L. & Finegold, S. M.: Wadsworth Anaerobic Bacteriology Manual. University of California, 1975.