TOPICAL ANTIBIOTICS AND TOPICAL ANTIMICROBIAL AGENTS IN ACNE THERAPY

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In recent years, there has been a steady increased use of topical antibiotics and topical antimicrobial agents in the treatment of acne (1—8). Benzoyl peroxide was the first to achieve prominence and currently is available in numerous gels, lotions and cream formulations at 5% and 10% concentrations. More recently, topical antibiotics such as tetracycline, erythromycin and clindamycin have been reported to be useful in acne therapy (1—5). Topical antibiotics are now widely prescribed in the U.S.A. despite the fact that as of this moment only one has been officially cleared by the Food and Drug Administration. The widespread use of topical antibiotics consists primarily of individually formulated compounds of various concentrations and vehicles. Despite the lack of uniform preparations, topical antibiotics are currently the "in" therapy for acne.

Numerous clinical studies have been conducted with benzoyl peroxide and topical antibiotics and there is little doubt that these agents are of value. However, it is virtually impossible to compare studies because of differences in clinical grading and therapeutic assessment schemes. For example, the combination photographic and 0 to 8 scale used in the studies on topical tetracycline (1, 2) are vastly different from the percent reduction in inflammatory papules system of Stoughton (5), or the weeks to 50% improvement of Fulton, and the excellent, good, fair and poor ranking system of Mills and Kligman (4). While clinical efficacy is, of course, the ultimate question for any drug, clinical studies are the most difficult assay to perform. Acne is a multifactorial disease in which bacteria (*P. acnes*) play a central role but by no means are bacteria the only factor. Differences in clinical efficacy from one study to another at times reflect differences in patient population and the relative importance of the various factors involved. At other times, differences in results reflect differences in the grading technique of the investigators. A prime example of the disparity that can develop during a multicenter clinical trial can be seen in Frank's summary of 300 patients treated with topical 0.5% tetracycline (1). One investigator found a 60% reduction in clinical severity after 13 weeks of therapy compared to only an 18% reduction in the eyes of another whereas 3 others were in more general agreement (37 to 46% improvement).

In the past year, we have evaluated multiple topical antibiotic and topical antimicrobial agents for their ability to suppress *Propionibacterium acnes* in-vivo. Our aim has been to gain basic information on the relative ability of various agents to penetrate sebaceous follicles and suppress *P. acnes*. As a further measurement of activity on *P. acnes* we measured the effect of these agents on the composition of skin surface lipids. Approximately 95% of the free fatty acids found in skin surface lipids result from hydrolysis of sebaceous gland triglycerides by *P. acnes* lipase (9). While there is a current argument regarding the relative importance of free fatty acids in the pathogenesis of acne lesions, there is uniform agreement that surface free fatty acid levels are a function of *P. acnes* lipase activity and as such measurement of free fatty acid levels is an indirect measurement of *P. acnes* activity. We sought objective measurements of the relative in-vivo efficacy of various agents in suppressing *P. acnes* and surface fatty acid levels. Our premise for these studies was that any antimicrobial agent which is effective in acne therapy must penetrate skin sufficiently to lower *P. acnes* levels and surface free fatty acid levels. With this premise, we measured in-vivo effects on volunteers free of acne who had high levels of *P. acnes* and surface free fatty acids in order to avoid the technical problems of culturing *P. acnes* in inflamed skin.

MATERIALS

We evaluated the following agents applied twice daily — 2% erythromycin free base, 2% clindamycin phosphate, 2% clindamycin free base, 2% neomycin free base, 2% declomycin free base, 2% novobiocin free acid in equal parts ethanol and water, 2.5%, 5% and 10% benzoyl peroxide lotion, and 5% and 10% benzoyl peroxide gel formulations.

SUBJECTS

We used panels of volunteers who were free of acne and who had high counts of *P. acnes* (10^5 per cm^2) and corresponding high values of surface free fatty acids. Panels consisted of at least 10 subjects. We have avoided using acne patients for these studies because of the multiple technical difficulties in measuring *P. acnes* and free fatty acids in patients with inflammatory acne lesions. Free fatty acid levels and *P. acnes* levels are often lowest in the most severe cases particularly in acne conglobata (10, 11). The currently widely used methods for assessing *P. acnes* and free fatty acid levels utilize surface collection methods. In the presence of severe inflammation, *P. acnes* is routinely reduced — possibly secondary to increased oxygen tension and other aspects of inflammation which appear to be inhibitory to *P. acnes*. For example, on the scalp, seborrheic dermatitis and even in the clinically non-inflamed (though histologically inflammation is prominent) condition called dandruff, *P. acnes* is sharply reduced (12). In highly inflamed acne, the surface *P. acnes* and free fatty acid levels are often significantly lower than surface and sub-surface measurements. Table 1 demonstrates the differences in surface and sub-surface measurements. The sub-surface measurements were obtained by placing a drop of cyanoacrylate glue on a glass slide which is then placed on the skin surface and held in place for 1 minute after which it is peeled off the skin surface revealing imprints of each follicle. Follicles which are involved in the acne process and which are becoming impacted with coherent masses of follicular horny cells are seen as waxy plugs which are interspersed among thin vellus hairs from vellus follicles. These "follicular plugs" are dissected away under a dissecting microscope and are then processed for quantitative microbiology after homogenization in 0.1% Triton-X-100 and for lipid analysis by standard thin layer chromatography after extraction with hexane. As can be seen in Table 1 the sub-surface *P. acnes* count in a follicular plug often exceeds the total count obtained by the surface detergent scrub for a cm^2. Likewise the sub-surface free fatty acid level (expressed as the free fatty acid to triglyceride ratio) was usually greater than the surface measurement would indicate. Unfortunately, this technique is too tedious and difficult to obtain uniform "plugs" on multiple occasions from the same patient to make it feasible for routine use in evaluating antibiotics in acne patients. While surface collection methods have decided limitations, they are reproducible, technically feasible and sufficient to demonstrate significant changes during effective therapy.

QUANTITATIVE MICROBIOLOGY

Quantitative cultures were obtained by the detergent scrub technique of Williamson and Kligman (13). This method is reproducible and will demonstrate significant differences with effective anti-microbial therapy but is limited by the fact that it does not sample the entire follicle as has been recently stressed

Table 1. *Comparison surface scrub and cyanoacrylate.*

Acne	Surface scrub				Cyanoacrylate	
	P.acnes/cm^2		FFA/TG Ratio		*P.acnes* per follicular plug	FFA/TG Ratio per follicular plug
	Forehead	Cheek	Forehead	Cheek	Cheek	
Mild inflammatory	3.5274	5.8004	0.08	0.26	6.0000	0.43
	5.3647	6.3233	0.59	0.58	5.8325	1.33
	4.6021	5.6243	0.03	0.02	5.5563	—
	3.3233	4.4372	0.01	0.40	5.6435	0.40
	5.8915	4.1013	0.55	0.36	5.9823	3.71
Severe inflammatory	3.2264	5.1015	0.01	0.10	5.6021	1.47
	7.1015	6.4994	0.35	0.30	6.8062	1.82
	5.1681	5.2264	0.11	0.36	5.5051	0.44
	5.4694	5.5786	0.03	0.17	5.6435	0.81
	1.8004	4.8004	0.04	0.66	5.8062	0.20
	3.6243	4.0223	0.43	0.09	5.7782	1.24
	3.5274	5.8004	0.19	0.09	6.0000	—

by Puhvel et al (14). As detailed above, we have found the same limitation applies to sampling acne patients when surface scrub cultures are compared to sub-surface cultures of follicular costs obtained by cyanoacrylate glue. Cultures were obtained one hour after cleansing the surface of the forehead and cheek by wiping for 30 seconds with sterile gauze soaked with 0.1% Triton-X-100 to remove surface debris and bacteria. These areas were protected by a plastic weighing boat with perforations to prevent undue collection of moisture. The collection fluid was diluted in ten fold steps in 0.05% buffered Triton-X-100 plated on Brain Heart Infusion Agar and cultured anaerobically for 7 days. *P. acnes* was identified by colonial morphology, susceptibility to *P. acnes* bacteriophage and when indicated by biochemical testing (15).

SKIN SURFACE LIPID MEASUREMENTS

The surface of the forehead and cheek was wiped for 30 seconds with gauze soaked with 0.1% Triton-X-100 to remove bacteria and debris. After the area was dry, a 30 second wiping with gauze impregnated with hexane was used to remove surface lipid. Two areas on the forehead and one on each cheek were then protected with a perforated plastic weighing boat taped sealed to the skin. The perforations prevented accumulation of moisture under the weighing boat. The subjects were maintained at rest in the same temperature, humidity environment for each sampling. This one hour "sebum excretion" was used since there was less variability in the FFA/TG ratio at the one hour sample when compared to the measurement of the casual level (coefficient of variation of the casual FFA/TG level is 22% compared to 15% for the one hour measurement). There was no significant difference in *P. acnes* counts obtained 1 hour after cleansing and delipidization of the surface. However, the 1 hour measurement reflects *P. acnes* percolating upward to the surface along with sebum and thus is a measure of deeper reservoirs of *P. acnes*.

RESULTS

The results of the various topical antibiotics are summarized in Table 2. Values are expressed as the mean logarithmic count of *P. acnes per* cm^2 and the lipid results are expressed as the free fatty acid/triglyceride ratio. 2% erythromycin, 2% clindamycin free base and phosphate produced a significant reduction in *P. acnes* as measured by student's paired t-test.

With respect to free fatty acid levels, however, we did find that 2% erythromycin and both 2% clindamycin free base and phosphate produced a greater reduction in the FFA/TG ratio. We interpret these findings to mean that sufficient quantities of erythromy-

Table 2. *Topical antibiotics.*

Agent	Day:	Treatment 0	14	28
PROPIONIBACTERIUM ACNES				
2% Erythromycin Free Base		5.9768	5.7974	5.3193
2% Clindamycin Phospate		5.8241	5.5911	5.0669
2% Clindamycin Free Base		5.7923	5.5026	4.6338
2% Neomycin Free Base		5.4712	5.2523	5.3617
2% Novobiocin Free Acid		5.7460	5.6310	5.8034
2% Declomycin Base		5.7575	5.8150	5.6423
Control (ETOH—H_2O)		5.9786	5.7992	6.0383
FREE FATTY ACID/TRIGLYCERIDE RATIO				
2% Erythromycin Free Base		0.83	0.41*	0.37*
2% Clindamycin Phosphate		1.52	0.92*	0.72*
2% Clindamycin Free Base		0.90	0.52*	0.47*
2% Neomycin Free Base		0.69	0.57	0.48
2% Novobiocin Free Acid		0.65	0.65	0.58
2% Declomycin Base		0.75	0.57	0.62
Control (ETOH—H_2O)		1.18	1.20	1.19

*significant by Student's t-test

cin and clindamycin penetrated the sebaceous follicles to partially inhibit *P. acnes*, i.e., enough antibiotic penetrated to produce more of an effect on the metabolism of *P. acnes* than its viability. More significant, however, is the rather feeble results these agents produced in *P. acnes* levels. None of those judged to be clinically effective proved to be effective in suppressing *P. acnes* more than one log.

Table 3 summarizes the results obtained with various formulations and concentrations of benzoyl peroxide. In contrast to the topical antibiotics, we found a significant reduction of *P. acnes* after 7, 14, and 28 days of therapy. Free fatty acid levels were also significantly lowered but not until 14 and 28 days of therapy (the 7 day values just failed to achieve statistical significance). The follow up data is of interest in that both the *P. acnes* counts and free fatty acid levels rapidly return to pre-treatment levels. In contrast, our laboratory has previously shown that with systemic antibiotics, return to pre-treatment levels of *P. acnes* required 2 weeks or more (9). This suggests that systemically administered antibiotics which require a longer period (approximately 4 weeks) to reduce *P. acnes* can accumulate and concentrate in follicles and thus exert a long lasting effect and that topical benzoyl peroxide fails to accumulate and its activity is short lived. A surprising finding was the lack of a difference in efficacy for the various concentrations.

DISCUSSION

These studies were undertaken to obtain data on the relative in-vivo effect of topical antibiotics and topical benzoyl peroxide in reducing *P. acnes* and surface free fatty acid levels. Our aim was to establish the relative efficacy of currently available formulations of topical antimicrobial agents. A great deal of confusion surrounding the relative merits of topical and systemic antimicrobial agents stems from variables in patient population and different methods of clinical and antimicrobial analysis employed by various investigators. Assessment of clinical efficacy is complicated by the multitude of factors other than *P. acnes* which are involved and by the variety of criteria used to judge clinical results. Measurement of antimicrobial effects in acne patients is further complicated by the technical problem of the difficulty in recovering the anaerobic diphtheroid *P. acnes* from inflamed skin. In this study, we demonstrated that surface col-

Table 3. *Topical Benzoyl Peroxide.*

Agents	No. of Subjects	Day:	Treatment 0	7	14	28	Follow-up 3	7
Propionibacterium Acnes								
Lotion								
Control	10		5.8388	5.6636	5.8971	5.7220	—	—
2.5%	10		6.2546	4.5658*	4.6284*	4.2531*	—	—
5.0%	30		6.0169	4.5728*	4.3321*	4.3923*	5.2347*	5.8363
10.0%	20		5.9726	4.4794*	4.5986*	4.3002*	5.0169*	5.6739
Gel								
Control	10		6.0408	5.9199*	5.7991*	5.9803*	—	—
5.0%	20		5.8244	4.7177*	4.4847*	3.5528*	4.7760*	5.7079
10.0%	10		6.1082	4.3979*	4.2146*	4.0314*	5.2530*	6.1692
Free Fatty Acid/Triglyceride Ratio								
Lotion								
Control	10		0.74	0.70	0.68	0.69	—	—
5.0%	30		0.88	0.63	0.51*	0.36*	0.74	0.87
10.0%	20		0.96	0.66	0.56*	0.45*	0.82	0.91
Gel								
Control	10		0.83	0.81	0.83	0.83	—	—
5.0%	20		0.77	0.58	0.46*	0.36*	0.63	0.71
10.0%	10		0.82	0.57	0.44*	0.38*	0.72	0.79

*significant by Student's t-test

lections often showed less *P. acnes* in a 3.8 cm^2 area than that found in a single follicular cast obtained with cyanoacrylate glue. In order to eliminate these multiple variables we used subjects free of acne who had high levels of *P. acnes* and corresponding high levels of surface free fatty acids.

In the case of benzoyl peroxide, we demonstrated a significant reduction of *P. acnes* and surface free fatty acid levels. Interestingly, we found no significant difference between 2.5, 5 and 10% concentrations and no differences between different vehicles. Gels were not significantly more effective than lotions. The magnitude of the reduction of *P. acnes* with all benzoyl peroxide preparations was in the range of one and a half logarithms. In the past, we and others have expressed these results in terms of geometric mean per cent reduction or a 90 to 99% reduction. This type of data presentation tends to magnify and exaggerate the significance of the results. Another way of expressing the same result is in terms of logarithmic per cent reduction which results in a 25 to 30% reduction, a far less striking figure. On the face of the published data on reductions of *P. acnes* with benzoyl peroxide and systemic antibiotics one would conclude that benzoyl peroxide should be superior. The effect is swifter (a matter of days versus 3 to 6 weeks) and quantitatively impressive. Our clinical impression, however, is that systemic antibiotics are more effective, particularly in severe cases. In a recent evaluation of a 5% benzoyl peroxide in patients with moderately severe inflammatory acne (the type of patient normally placed on systemic antibiotics) good to excellent clinical improvement was seen in only 30% (Table 4). By comparison 7 of 8 treated with 600 mg demeclocycline daily for 9 weeks and 5 of 6 treated with 1200 mg daily achieved similar results (Table 5).

Table 4. *Multiple Dose Oral Declomycin Clinical Response and Free Fatty Acid Changes*

— Good —			— Fair —			— Poor —		
Dosage	FFA/TG Ratio		Dosage	FFA/TG Ratio		Dosage	FFA/TG Ratio	
	0	6 wks		0	6 wks		0	6 wks
1200	0.24	0.13	1200	0.34	0.13	1200	0.33	0.31
1200	0.39	0.13	600	0.34	0.33	600	0.21	0.13
1200	0.86	0.24	600	0.17	0.10	150	0.52	0.17
1200	0.15	0.06	600	0.22	0.11	150	0.60	0.75
600	0.45	0.23	150	0.26	0.18	150	0.27	0.32
600	0.15	0.10	150	0.28	0.33	150	0.85	0.54
600	1.22	0.53	150	0.28	0.24			
600	0.21	0.05						
% Reduction: 60%			% Reduction: 25%			% Reduction: 9%		

Table 5. *Clinical Results with Topical Antimicrobial Agents and Systemic Declomycin in Papulopustular Acne.*

Agent	N	Excellent	Good	Fair	Poor
5% benzoyl peroxide	25	8%	20%	56%	16%
2% erythromycin (ETOH + H_2O)	30	10%	40%	40%	10%
benzoyl peroxide + retinoic acid*	390	67%	25%	7%	1%
1% clindamycin**	29		55% to 80% improvement		
0.5% tetracycline***	300		18% to 60% improvement (13 weeks)		
systemic declomycin					
150 mg.	7	0	43%	57%	0
600 mg.	8	50%	37%	13%	0
1200 mg.	6	68%	16%	16%	0

* Hurwitz, 1976
** Stoughton, 1976
*** Frank et al, 1976

This discrepancy between clinical and microbiological evaluation points out the weakness of current methodology for measuring *P. acnes*. Surface collection methods are reproducible and useful for showing differences with therapy but must be interpreted with caution. Surface collection methods do not reach the deeper recesses of sebaceous follicles and a significant reduction in *P. acnes* demonstrated by surface collection methods does not necessarily mean that a similar effect has occurred in the deeper portions of the follicle. Likewise, a significant reduction as measured by surface collection methods does not guarantee that an agent will be clinically useful. Clinical benefit must be separately established in clinical trials. However, it is safe to say that an agent which does not suppress *P. acnes* at all is unlikely to be successful in a clinical trial.

In the case of topical antibiotics, only erythromycin and clindamycin produced a statistically significant reduction in *P. acnes*. Novobiocin, neomycin, demeclocycline and ethanol showed no reductions. The effect of erythromycin and clindamycin on free fatty acid levels was more impressive than the effect on *P. acnes*. All three formulations significantly reduced surface free fatty acid levels after two weeks of therapy. We interpret these results to indicate that enough antibiotic penetrated sebaceous follicles to affect the metabolism of *P. acnes* more than its viability. These agents are bacteriostatic, not bacteriocidal as is benzoyl peroxide, and organisms dying in-vivo can be resuscitated upon transfer to an enriched medium. The results with topical antibiotics are clearly inferior to that obtained with systemic antibiotics which achieve a 1 logarithm or greater reduction in 4 to 6 weeks. Despite the relative feeble effect on *P. acnes* levels, topical erythromycin and clindamycin appear to be useful in the clinical management of acne. In fact, we found 2% erythromycin to be slightly more effective than 5% benzoyl peroxide even though the latter was more effective in suppressing *P. acnes*. This may indicate that the recent evidence supporting the concept that antibiotics may have anti-inflammatory capabilities in skin may be relevant and an important aspect of the clinical benefit achieved with antibiotics in the management of acne (17). The patients used in this study had papulo-pustular acne of the type normally requiring systemic antibiotics showing good to excellent results but the majority not faring so well. The available benzoyl peroxide preparations on the other hand appear to be more efficient in suppressing *P. acnes*, but again clinical results are quite variable when patients with extensive papulo-pustular acne are studied. Despite these limitations, we view the future of topical antibiotics and topical antimicrobial agents in a sanguine fashion. With better formulation and more understanding of skin permeability in acne patients, therapeutic efficacy will undoubtedly be enhanced.

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DISCUSSION

Cunliffe, Leeds: Dr. Leyden, our data is remarkably similar. However, we have not shown a correlation between the number of bacteria and the degree of acne. I agree with you that — if you look at controls, whatever they might be in acne — yes, there is a difference. But nevertheless I certainly believe that bacteria are important. We believe it is what they are doing at the duct. We believe that the microenvironment they live in is important. The pH, the oxygen tension and the nutrients control what they produce in the follicle and this in turn influences their effect on the development of hyperkeratinization and possibly the inflammatory lesion.

Leyden, Philadelphia: I would like to discuss the question as to why the *P. acnes* population does not increase as acne becomes more severe. I feel that the main substrate for *P. acnes* is sebum and just as in any culture system you can enrich the medium ad infinitum, but you do not see ad infinitum growth. With any bacterial system, you get an increase and then you get a plateau. With more severe acne there is sebum. However, that extra sebum just does not result in more bacterial growth. A steady-state is reached beyond which there is no further growth.

Strauss, Iowa: You did not mention in reference to the topical tetracycline that you have an artificial system of grading these patients from 1—8, instead of 1—4. The average improvement was, I think, about one and a half grades on a scale of 8. So you probably did not have that much improvement, if you consider our usual grading system.

Shuster, Newcastle-upon-Tyne: What happens to patients given systemic tetracyline if you add benzoyl peroxide? Do you get an additional effect? Is the effect explained by some additional effect on the organisms?

Leyden, Philadelphia: It is a difficult question to answer. Bacteriologically, with the methods we have, you do not add anything. But you do add something clinically, because bacteria are only one part of the disease and the antibiotic tetracycline can only affect that aspect of it as you can get a beautiful reduction of *P. acnes* and still get pimples. Once you have got a pimple, if you put something on it, like benzoyl peroxide in a gel, it will go away faster than if you do not put anything on it.

Cunliffe, Leeds: Does the addition of topical therapy really help? Firstly, I realize you do not like clinical impressions, Dr. Shuster — but my impression is, like Jim Leyden's, that it does something. We have done one clinical study, in which we compared tetracycline alone with tetracycline plus benzoyl peroxide. The total count of inflamed and the non-inflamed lesions was significantly decreased at the end of three months, both with tetracycline and with the combined therapy, so we could not answer the question.

I wonder if Jim Leyden has looked at the effect of combined benzoyl peroxide and Retin®-A gel on the bacterial flora?

Leyden, Philadelphia: Take a culture and make sure it does not dry out and then have it plated on media that are specific for gram-negatives. That will not kill any competitive organisms and the recovery rate is fine. We measured vitamin-A-acid plus benzoyl peroxide to see if we would get a better effect on *P. acnes*. We are doing a cross-over study with benzoyl peroxide right now.

Plewig, Munich: The most stounding message I got this afternoon was from you Jim Leyden, because in the American literature there are several reports of successful topical treatment with antibiotics. So far we were unable to confirm clinically the effectiveness of these compounds when tested in large series of patients.

Holm, Umeå: I would also like to comment upon the problem with the combined therapy. First, we would like to know what the real mechanism is for the tetracycline effect on the *P. acnes*. We know e.g. that there

is an accumulation of tetracycline on the cell wall of the microorganisms and that tetracyclines are chelating agents, binding divalent cations. That is probably the reason why we can use low doses and still have an effect in long-term therapy. Furthermore, there is an accumulation in the lipids. We could therefore speculate that the effect is due to a combination of these mechanisms. I suggest that you first try tetracyclines and then the topical treatments, to elucidate the mechanisms separately.