Inhibition of *Pseudomonas* Burn Wound Infection by Mafenide Dry Foam

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Abstract □ The results of an *in vivo* evaluation of 8.5% mafenide dry foam are described. Using burned guinea pigs infected with *Pseudomonas aeruginosa*, mafenide was applied every 12 hr as the dry foam or as the commercially available ointment. After 156 hr of therapy with the medicated dosage forms, the previously infected areas did not demonstrate the presence of *Pseudomonas*. However, all nonmedicated, infected controls produced positive cultures. Both medicated dosage forms demonstrated equivalent efficacy in the inhibition of *Pseudomonas* on burn wounds.

Keyphrases □ Mafenide—dry foam formulation, inhibition of *Pseudomonas* burn wound infection □ Burns—inhibition of *Pseudomonas* burn wound infection by mafenide dry foam □ *Pseudomonas* infections in burns—inhibition by mafenide dry foam

Earlier (1), the formulation and *in vitro* evaluation of medicated dry foams were reported. The dry foam was described as a light, flexible, aerated film, with hydrophilic properties that permit ease of application and removal from denuded surfaces. Furthermore, it was postulated that the local use of medicated dry foams may obviate some disadvantages associated with currently available dosage forms. This report describes the results of the *in vivo* evaluation of mafenide dry foam.

EXPERIMENTAL

***In Vivo* Evaluation**—The burned, infected guinea pig was selected as the animal model. Fifteen male guinea pigs, approximately 300 g, were randomly selected. Approximately 24 hr after shaving the dorsum of the animals, four burns were inflicted upon the back of each animal under ether anesthesia.

A burning procedure utilized steam projected through an orifice 8 mm in diameter. In this manner, steam, cooled to 80–90°, was directed onto the backs of the animals for 10 sec to obtain four full thickness thermal injuries. The animals were then returned to individual cages with ample food and water and observed for 24 hr.

After this period, three burns on each animal were inoculated by swabbing with an overnight broth culture of *Pseudomonas aeruginosa* (ATCC 9721); the fourth burn on the animal served as the burned, uninfected control. Of the three infected areas, one was medicated with 0.1 g of 8.5% mafenide ointment[1], applied by means of a sterile blade. The second infected area was medicated with a 2.5-cm square of 8.5% mafenide dry foam. The third area served as the burned, infected control.

Swab cultures were obtained every 12 hr, immediately prior to the application of the dosage form. Repeat applications were continued at these same time intervals for 156 hr.

To determine the effect of the unmedicated vehicles upon infected burns, 15 infected burns were treated with nonmedicated dry foam and 15 infected burns were treated with a nonmedicated ointment[2]. These burns were treated and cultured as described every 12 hr for 72 hr (Table I).

Identification of *Pseudomonas*—Since mixed colonies were cultured from burn areas, appropriate identification tests were performed to distinguish the test bacteria from other microorganisms. Initial differentiation was made with the Gram stain (2). The ability of *Pseudomonas* species to produce a diffusible, blue-green pigment, pyocyanin (3), also aided in the identification. In addition, most strains elaborate another pigment known as pyoverdin (fluorescein) that fluoresces upon exposure to UV light (4, 5). For this reason, a short wavelength UV lamp[3] was used as an aid in identifying and quantifying the *Pseudomonas* isolated from the guinea pig burn areas.

Visual examination of the Gram-negative bacteria permitted the differentiation of three types of bacteria: fluorescent, diffusible pigment-producing colonies; ivory-colored colonies; and yellow-orange colonies. Test for oxidase and indole were performed on representative samples of these three Gram-negative bacteria to substantiate the preliminary identification (Table II).

Table I—Results of Mafenide Therapy on Infected Burns

Hours[a]	Number of Animals[b] with Positive *Pseudomonas* Cultures[c] after Treatment[d] with: Mafenide Dry Foam	Mafenide Ointment	Uninfected Control	Infected Control[e]
12	14	4	2	15
24	1	1	5	15
36	0	0	6	15
48	0	0	5	15
60	0	0	8	15
72	0	0	8	15
84	0	0	8	15
96	0	0	10	15
108	0	0	5	15
120	0	0	8	15
132	0	0	8	15
144	0	0	8	15
156	0	0	6	15

[a] Times listed are the hours after inoculation with an overnight broth culture of *Ps. aeruginosa*. [b] Each of 15 animals received four full thickness thermal injuries. [c] A positive culture was considered to be one in which five or more *Ps. aeruginosa* colonies were isolated and identified from a burn. [d] Treatment consisted of 8.5% mafenide ointment or dry foam, applied to the involved site every 12 hr for 156 hr. In addition, one burn per animal served as the uninfected untreated control, and one burn per animal served as the infected untreated control. Student *t* computations indicate $p < 0.005$ after 24 hr of therapy. [e] Other controls: All cultures from 15 burns treated with nonmedicated ointment every 12 hr for 72 hr were positive for *Pseudomonas*, and 89 of 90 cultures obtained from 15 burns similarly treated with nonmedicated dry foam were positive.

RESULTS AND DISCUSSION

With initial surface growth of *Pseudomonas* being of primary concern in *Pseudomonas* burn wound infection (6, 7), the burned, infected guinea pig was selected as the *in vivo* model for comparing the effectiveness of mafenide dry foam with that of the corresponding ointment. Since supra-eschar bacterial colonization is usually considered to be the first stage of local sepsis, it was assumed that eradication of surface colonies would indicate the effectiveness of the mafenide-containing dosage forms. Therefore, a negative swab culture was selected as the parameter for evaluating the medicated preparations.

By developing suitable burning techniques, four thermal injuries of uniform size were inflicted onto the dorsum of each animal. In this manner, the two medicated dosage forms could be evaluated on each of 15 animals. Furthermore, the effect of diffusion from

[1] Sulfamylon Cream, Winthrop Laboratories, Inc., New York, N.Y.
[2] Neobase Ointment, Burroughs Wellcome & Co., Research Triangle Park, N.C.
[3] Mineralight, Ultra-Violet Products, Inc., San Gabriel, Calif.

Table II—Results of Microbiological Identification Tests

Organism[a]	Identification Test				
	Gram Stain	Indole	Oxidase	Fluorescence[b]	Pigment[c]
Ps. aeruginosa (ATCC 9721)	−	−	+	+	+
Unknown (fluorescent)	−	−	+	+	+
Unknown (ivory colored)	−	+	−	−	−
Unknown (yellow colored)	−	−	−	−	−

[a] Unknown organisms were isolated from burn wounds during *in vivo* studies. Representative samples of these were subjected to the tests indicated to identify *Pseudomonas*. [b] Fluorescence was observed with the naked eye using UV light. [c] Production of a diffusible pigment indicates a positive result.

one area to another was determined by utilizing one burn per animal as the infected, untreated control. It was assumed that if diffusion of medication from a treated area to an untreated area were to occur, the untreated, infected burns would not demonstrate the presence of *Pseudomonas*. However, the untreated, infected burns produced positive cultures for the test organism throughout the study. For this reason, it was assumed that diffusion of the active ingredient from one burn to another did not occur and, therefore, did not have an inhibitory effect upon the test organism in untreated areas. Therefore, the use of four burns per animal, each treated with a different dosage form, appeared to be a reasonable study method.

To determine if cross-contamination might occur from an infected burn to an adjacent area of no colonization, the fourth burn on each animal was not seeded. This burned, uninfected, untreated control served to demonstrate that recontamination was occurring throughout the study, since six of these areas produced positive cultures at the end of the study. From this observation, it seemed probable that cross-contamination of the other burns was occurring at a similar rate.

However, since the burns treated with the medicated dry foam or medicated ointment produced no positive cultures, it is suggested that both medicated dosage forms were equally effective in inhibiting the growth of *Pseudomonas in vivo*. That is, both preparations eradicated supra-eschar colonization after 36 hr of therapy and subsequently prevented the expected cross-contamination due to the presence of *Pseudomonas* on the other burn areas. Both the *in vitro* and *in vivo* methods of evaluation suggest that the dry foam and ointment are equally effective dosage forms for mafenide.

To demonstrate visually the presence of *Ps. aeruginosa* on the burns, the methods of Ward *et al.* (5) and Caplan (8) were used. At the conclusion of the study, UV light was directed onto the burns to detect the characteristic fluorescence of the test bacteria. Unfortunately, mafenide fluoresced with a similar intensity and color to that of fluorescein; therefore, no differentiation could be made between the presence of *Pseudomonas* and the presence of mafenide.

The untreated, infected areas produced fluorescence characteristic of *Pseudomonas*. Of the 15 infected, untreated burns, all produced fluorescence on exposure to UV light. This indirect visual demonstration of the presence of the test organism tends to validate the swabbing technique used, because all infected, untreated burns produced positive cultures for *Pseudomonas*.

In addition, macroscopic examination of the burned, uninfected areas under UV light produced fluorescence with five burns. In contrast, six of these areas produced cultures positive for *Pseudomonas*. This discrepancy between the number of fluorescent areas and the number of areas with positive cultures may be explained by the limitation of the UV technique. According to Polk *et al.* (9), the fluorescence of *Pseudomonas* may be observed with the naked eye as the concentration approaches and exceeds 10^5 organisms/cm^2. Evidently, the contamination and proliferation of *Pseudomonas* on the initially uninfected areas were not of sufficient magnitude, so the fluorescence was not visible on the sixth area.

SUMMARY AND CONCLUSIONS

This report provides additional data regarding a medicated dry foam for local therapy. Using the burned, infected guinea pig, the efficacy of 8.5% mafenide dry foam was compared with that of the corresponding commercial ointment. Since both medicated dosage forms demonstrated equivalent efficacy in inhibiting *Ps. aeruginosa* on burn wounds, further study is indicated to assess the utility of a medicated dry foam as a useful approach to local therapy in human subjects.

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