Topical Sulfamylon* Reduces Engraftment of Cultured Skin Substitutes on Athymic Mice

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Sulfamylon (mafenide acetate) remains extremely valuable for the control of the bacterial contamination of burn wounds, but it is cytotoxic to cultured keratinocytes used for wound closure. Because composite skin substitutes develop a partial epidermal barrier in vitro, they may hypothetically tolerate the use of topical Sulfamylon. To test this hypothesis, cultured skin substitutes were prepared from cultured human fibroblasts; keratinocytes were attached to these collagen-based substrates, which were grafted to full-thickness wounds in athymic mice (n = 8 per group). Wounds were irrigated twice daily with 5% (wt/vol) Sulfamylon solution or with a formulation of noncytotoxic antimicrobials (0% Sulfamylon). On day 9 after grafting, the wounds were treated with dry dressings and assessed at 4 weeks for expression of human leukocyte antigens-A, B, C and at 2, 3, and 4 weeks for percentage of original wound area and surface electrical capacitance in picofarads (pF). Data were analyzed for statistical significance ($P < .05$) by Fisher's exact test, Student's *t* test, and repeated measures analysis of variance:

Condition	% HLA-ABC positive	% Original wound area	SEC (pF)
0% Sulfamylon	75% (6/8)	46.4 ± 3.4	276 ± 189
5% Sulfamylon	13% (1/8)*	28.3 ± 5.2†	1363 ± 346‡

SEC, surface electrical capitance; *pF,* picofarad.
* Fisher's exact test.
† Student's *t* test.
‡ Repeated measure analysis of variance.

The data demonstrate that irrigation of cultured skin substitutes with a solution of 5% Sulfamylon results in smaller wound area, fewer wounds that contain human cells, and greater surface hydration (higher surface electrical capacitance) than irrigation with noncytotoxic antimicrobial agents. These results support the conclusion that cultured skin substitutes of this type do not tolerate the chemical toxicity of Sulfamylon as well as skin autografts. Further improvements in the properties of the epidermal barrier of cultured skin substitutes may facilitate the use of Sulfamylon or other potent antimicrobial agents for the management of microbial contamination during engraftment of transplanted skin cells. (J Burn Care Rehabil 1999;20:33-6)

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**Mafenide acetate; Dow Hickam Pharmaceuticals, Sugar Land, Tex.*

Sulfamylon (mafenide acetate) remains extremely valuable for the control of bacterial contamination of burn wounds, particularly for gram-negative organisms that may consume skin grafts during the period of vascularization. Sulfamylon is currently available for topical application on wounds as a cream, but it has also been studied as a topical solution or slurry for irrigation of meshed, split-

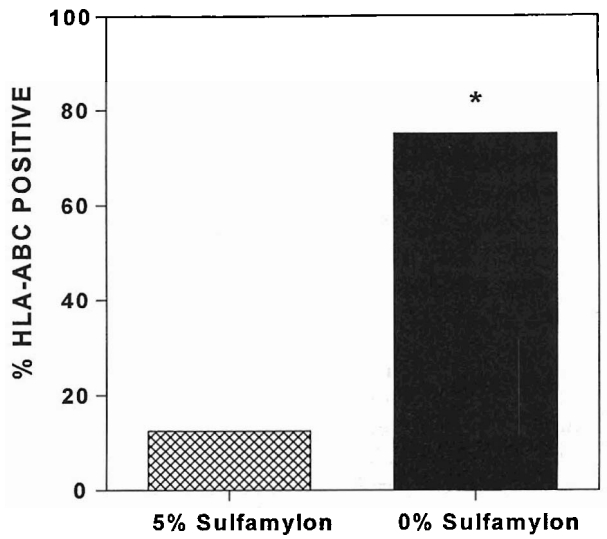

Figure 1. Irrigation of cultured skin substitutes with 5% Sulfamylon solution causes significant decrease in expression of HLA-ABC by epidermal keratinocytes at 4 weeks after grafting. CSS expressing HLA-ABC antigens was 12.5% (1 of 8) after irrigation with 5% Sulfamylon and 75% (6 of 8) after irrigation with noncytotoxic antimicrobials. $*P < .05$.

thickness skin grafts.[1,2] Although application as either a cream or solution may affect kinetic delivery and penetration of the drug to the wound, the effectiveness of mafenide acetate for control of burn wound contamination and sepsis is well-established.[3-6] Therefore, the use of Sulfamylon with alternative wound coverings, including cultured skin substitutes, would hypothetically be a reasonable extension of conventional wound care.

Previous studies have shown that Sulfamylon in solution is highly cytotoxic to cultured keratinocytes used for wound closure.[7,8] Conversely, formulations of noncytotoxic antimicrobial agents do not inhibit cell growth in vitro.[7,9] However, most cytotoxicity studies have been performed on cultured cells in monolayer culture, not on keratinized epidermis as found in meshed autografts. Recent models of cultured skin substitutes (CSS) that consist of cultured human keratinocytes and fibroblasts attached to collagen-based substrates develop partial barrier in vitro.[10,11] Destruction of CSS by *Pseudomonas aeruginosa* has been controlled by irrigation with noncytotoxic antimicrobial agents in full-thickness skin wounds with no inhibition of wound healing.[12] Therefore, it was hypothesized that keratinized epithelium of CSS may protect proliferative keratinocytes from the toxicity of mafenide acetate. The purpose of this study was to test whether partially keratinized skin substitutes may better tolerate potent antimicrobial agents such as Sulfamylon.

MATERIALS AND METHODS

CSS were prepared as previously described from human keratinocytes and fibroblasts inoculated onto collagen-glycosaminoglycan substrates.[13,14] On culture day 3, CSS were lifted to the air-liquid interface in saturated humidity at 37°C in 5% CO_2 with daily medium changes until grafting on day 20 of incubation. Animal subjects were acquired, housed, and studied experimentally under a protocol approved by the University of Cincinnati Institutional Animal Care and Use Committee. CSS were grafted orthotopically to full-thickness wounds (2 × 2 cm) in athymic mice. On days 0 to 4 postgrafting, grafted wounds in both groups ($n = 8$ per group) received irrigation (1 cc/graft/day) with ciprofloxacin (20 µg/ml) and nystatin (100 units/ml) in nutrient medium as previously described.[13] On days 5 to 8 postgrafting, grafted wounds received 4 irrigations (1 cc/graft) at 3-hour intervals. The control group received only the nutrient and antimicrobial solution, and the experimental group received alternating treatments with aqueous solution of 5% (wt/vol) Sulfamylon (mafenide acetate; Dow Hickam Pharmaceuticals, Sugar Land, Tex) and the nutrient and antimicrobial solution. On postgrafting day 9, animals were changed to dry dressings and evaluated at 2, 3, and 4 weeks after grafting.

Engraftment of CSS with human cells was assessed at 4 weeks after surgery by scoring immunopositive epidermis from healed wounds after preparation of cryostat sections and staining with a monoclonal antibody against a common hapten of the HLA-ABC histocompatibility antigens.[15,16] Wounds were assessed at 2, 3, and 4 weeks for percentage of original wound area by direct tracing of perimeters onto frosted mylar followed by computer-assisted planimetry, and surface electrical capacitance (SEC) was measured with a Dermal Phase Meter (DPM-9003; Nova Technology Corporation, Portsmouth, NH) as a noninvasive index of epidermal barrier formation.[10] Data were analyzed for statistical significance ($P < .05$) by Student's t test, Fisher's exact test, and repeated measures analysis of variance.

RESULTS

Irrigation of cultured skin substitutes with 5% Sulfamylon causes significant decrease in the expression of HLA-ABC by epidermal keratinocytes at 4

weeks postgrafting, as shown in Figure 1. Human epidermal cells were detected in 1 of 8 wounds treated with CSS and irrigated with Sulfamylon. The majority of control CSS grafts (6 of 8) were healed with human epidermis at the end of the observation period. This result demonstrates a loss of CSS grafts as a function of irrigation with 5% Sulfamylon solution.

Wound area at 4 weeks after grafting with CSS was significantly reduced by irrigation with 5% Sulfamylon (Figure 2). At this time point control wounds were closed with stable epidermis, but some wounds treated with CSS and Sulfamylon remained open. Typically, wounds that had not healed completely were open in the center with epithelium migrating from the wound perimeter. Reduced wound area during healing of CSS was interpreted as increased wound contraction and secondary healing by murine skin.

Measurement of hydration at the wound surface, as measured by SEC, confirmed that fully keratinized epidermis had not formed by 4 weeks postgrafting on wounds irrigated with Sulfamylon. Figure 3 shows that SEC in control wounds decreased steadily and was comparable to native human skin (32 ± 5 pF) by 4 weeks after grafting. Wounds irrigated with Sulfamylon had statistically higher wound hydration (wetter surface) at all time points after grafting. This result demonstrates that stable epithelium had not formed after treatment of CSS with 5% Sulfamylon solution.

DISCUSSION

This study demonstrates that irrigation with 5% Sulfamylon solution results in CSS with fewer healed wounds that contain human cells, reduced wound area, and greater surface hydration as compared with irrigation with noncytotoxic antimicrobial solutions. These results are consistent with in vitro studies that show the cytotoxicity of mafenide acetate to keratinocytes in monolayer culture.[8] Although the mechanism of toxicity of Sulfamylon to CSS during wound healing is not understood, the toxicity may result from factors of drug exposure to transplanted cells including time, concentration, and penetration into CSS.

Because Sulfamylon solution can be used effectively to support engraftment of meshed split-thickness autografts, it is possible that drug exposure is excessive or that the anatomy and physiology of CSS does not provide protection against the drug. In this study, CSS were irrigated for 4 days with Sulfamylon, which corresponds to the amount of time for clinical irrigation of skin grafts. However,

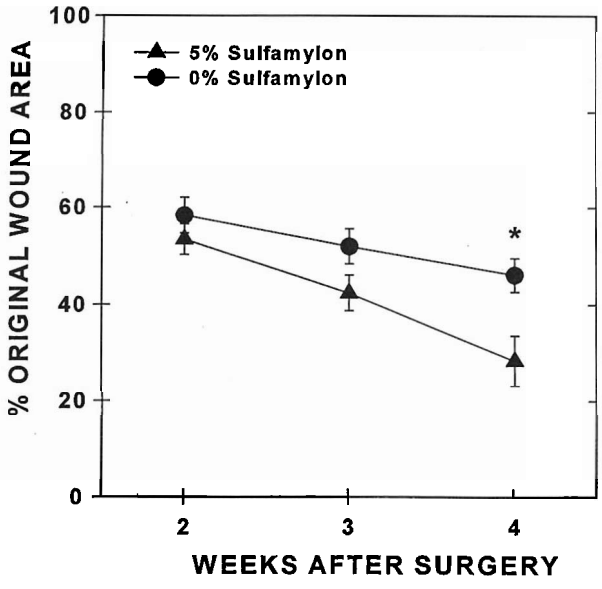

Figure 2. Irrigation of cultured skin substitutes with 5% (wt/vol) Sulfamylon solution causes significant reduction in wound area at 4 weeks after grafting. Healed wounds treated with CSS and irrigated with 5% Sulfamylon were significantly smaller (▲) than CSS irrigated with noncytotoxic antimicrobials (●). *$P < .05$.

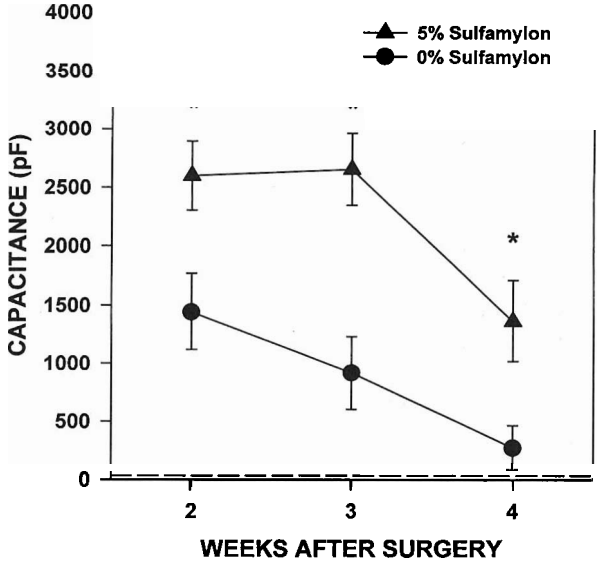

Figure 3. Irrigation of cultured skin substitutes with 5% Sulfamylon solution causes significant increases of SEC at all time points in this study. At all time points tested, SEC was significantly higher (wetter) after irrigation with 5% Sulfamylon solution (▲) than with noncytotoxic antimicrobials (●). The reference line indicates the SEC value (32 ± 5 pF) for native human skin (NHS). *$P < .05$.

engraftment of CSS may increase if the irrigation period were reduced. Application of topical antimicrobial agents is required mostly during the period of graft vascularization. For autografts, vascularization usually requires less than 5 days. Recent modifications of CSS have reduced the time of vascularization to approximately 1 week. Therefore, as the time for vascularization of CSS approaches that of autograft, it may be expected that the time required for application of topical antimicrobial agents will decrease correspondingly. As the period of drug exposure decreases, the total drug toxicity may also decrease. Similarly, reduction of the concentration of Sulfamylon solution may increase the survival of CSS grafts if the antimicrobial activity can be maintained. It is possible that these modifications to the doses of topical Sulfamylon may increase its utility in combination with CSS.

However, the greatest and most obvious differences between CSS and autograft are anatomic and physiologic. Historically CSS have formed only partial epidermal barriers and have required longer times to become perfused because they are avascular. In this study, both of these factors may have contributed to the reduced engraftment of CSS by irrigation with 5% Sulfamylon. In more recent studies, a complete epidermal barrier has been formed in this model of CSS.[11] A complete barrier may provide better protection to proliferating keratinocytes in CSS as it does in autografts. Therefore, additional studies can test whether the development of a complete epidermal barrier in CSS decreases susceptibility to the toxicity of topical Sulfamylon solution.

Engraftment and survival of CSS are expected to increase as functions of improved anatomy and physiology. Conversely, engraftment may also be improved if the chemical toxicity of topical antimicrobial agents is reduced. Wound healing with CSS that is comparable to that of autograft depends on both of these clinical parameters. Findings of this study provide insights into improved efficacy for closure of burn wounds with cultured skin substitutes.

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