In Vitro Toxicity and Activity of Dakin's Solution, Mafenide Acetate, and Amphotericin B on Filamentous Fungi and Human Cells

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Objectives: Posttraumatic invasive fungal infections threaten critically injured combat related injuries and require a combination of extensive surgery and systemic antifungal therapy, along with topical antimicro bials used adjunctively to control the infection. We evaluated the in vitro activity of topical agents in varying combinations and concentrations against molds from patients that were responsible for wound invasive fungal infections and the topical agents' toxicity to human cells.

Methods: Mafenide acetate solutions (2.5%, 5%, and 7.5%), amphotericin B solutions (2 μg/mL, 2 mg/mL, and 20 mg/mL), SMAT (5% mafenide acetate in combination with 2 μg/mL, 2 mg/mL, and 20 mg/mL amphotericin B), and Dakin's solutions (buffered sodium hypochlorite) (0.5%, 0.25%, and 0.125% and 10 fold serial dilutions of 0.25% 0.00000025%) were evaluated for antifungal activity against 4 molds using a time kill assay using standard conidial suspensions of 5×10^4 colony forming units per milliliter. To assess cellular toxicity, confluent monolayers of human keratinocytes, dermal fibroblasts, and osteoblasts were exposed to these topical agents. Based upon efficacy and toxicity ratios, an additional 10 molds were screened with selected concentrations of the topical agents for antifungal activity and toxicity.

Results: All the topical agents seemed to have a dose dependent killing with only mafenide acetate showing time killing associated with prolonged contact. There was overall evidence of dose dependent cytotoxicity of the various topical agents against the various cell lines tested, but there did not seem to be increased cell death with continued exposure to the agents over time. Dakin's solution exhibited dose dependent toxicity and efficacy with 0.00025% appearing to optimize those parameters.

Conclusions: Mafenide acetate and amphotericin B did not seem to persistently meet the toxicity and efficacy balance as consistently as Dakin's solution.

Key Words: mold, topical therapy, trauma, war, Afghanistan

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INTRODUCTION

Infections complicate approximately 25% of combat-related injuries, rising to nearly 50% if casualties are injured severely enough to be admitted to an ICU.[1,2] The majority of these infections are because of multidrug-resistant aerobic gram-positive and gram-negative bacteria.[1,2] However, over the last few years, the US military has noted increasing rates of wound infections with invasive fungal infections (IFIs) primarily in casualties that did not suffer severe burns, which has been the traditional population with IFIs.[3–5] The pathogens with the greatest impact have included molds such as *Aspergillus* and *Mucor* species. This experience is similar to the British military experience with IFIs during their deployments to Afghanistan especially in the Helmand province when casualties suffered severe lower extremity amputation with perineal/pelvic injury during dismounted patrols with injuries associated with high blood product support because of excessive blood loss.[6,7] Molds typically complicate the clinical care of noncombat trauma patients in settings associated with agriculture injuries, motor vehicle accidents, and blunt crush injuries when material from the environment is inoculated into the wound.[8,9] Recently, the Joplin tornado was associated with local invasive infections of a single mold species, *Apophysomyces trapeziformis* (family: Mucoraceae), resulting in a high rate of amputations (31%) and 25% mortality.[10]

Therapy for these pathogens has focused on repeated and extensive surgical debridement with systemic antifungal therapy, as there is a high complication rate including amputations and mortality.[9] Given the severity of these injuries and the poor outcomes, there has been interest in using adjunctive wound therapy such as topical antiseptic agents. Topical therapies have been used in combat-related injuries since antiquity.[11,12] However, topical therapies for extremity trauma have mostly fallen out of favor

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since the introduction of systemic antimicrobial therapy and aggressive surgical techniques.[11,12] Dakin's solution (0.5% sodium hypochlorite) was used frequently in World War I using the Cullen method of 5-minute instillation every 2 hours through small fenestrated tubes directly placed into the wound.[13,14] However, Dakin's solution does not seem to penetrate deeply into wounds and showed a negative impact on leukocytes, neutrophil migration, fibroblasts, and endothelial cells.[13–16] Original clinical or in vitro studies did not include molds, but Dakin's solution has shown activity against *Aspergillus flavus* and *Aspergillus niger* but less activity against *Aspergillus fumigatus*.[17] Other topical agents have been used to treat IFIs, including mafenide acetate and amphotericin B, primarily in burn patients at a 5% and 2 μg/mL combined solution, respectively.[18] It is notable that 100–1000 μg/mL of amphotericin B caused osteoblast cell death and 1–10 μg/mL amphotericin B caused sublethal toxicity to osteoblasts and fibroblasts.[19] In addition, mafenide acetate at a 1000-fold dilution of the clinical dose of mafenide showed significant inhibition of neutrophil function.[20]

Given the rising combat-related injury infections with IFIs, surgeons are using various adjunctive topical therapies in an effort to treat the fungal infection that leads to the hip disarticulation and even death.[21] Choosing the best antimicrobial is difficult because of our lack of knowledge of in vitro efficacy and limited toxicity data of amphotericin B, mafenide acetate, and Dakin's solution. Therefore, we designed time–kill studies and evaluated the potential cytotoxic effect of these agents on human cell lines relevant to healing to better characterize these topical agents' potential clinical utility.

METHODS

Fungal Time–Kill Study

Four clinical mold isolates [*Lichtheimia* spp. (previously *Absidia* spp.), *A. flavus*, *Exophiala* spp., and *Mucor circinelloides* group] from combat-related IFIs underwent screening to determine the most effective antifungal concentrations of several topical solutions at various dilutions. Mafenide acetate (UDL Laboratories, Rockford, IL) solutions (2.5%, 5%, and 7.5%), amphotericin B (Sigma, St Louis, MO) solutions (2 μg/mL, 2 mg/mL, and 20 mg/mL), SMAT (5% mafenide acetate in combination with 2 μg/mL, 2 mg/mL, and 20 mg/mL amphotericin B), and Dakin's (buffered sodium hypochlorite; Century Pharmaceuticals Inc., Indianapolis, IN) solutions (0.5%, 0.25%, and 0.125% and 10-fold serial dilutions of 0.25%–0.00000025%) (concentration-specific pH and temperature shown in Table 1) were evaluated for antifungal activity using a time–kill assay with water as a control. Fresh solutions of each agent tested were made before use. All mold isolates were subcultured onto potato flake agar slants (Remel, Lenexa, KS) before testing. Standard conidia suspensions of 5×10^4 colony-forming units per milliliter were prepared according to the Clinical and Laboratory Standards Institute guidelines.[22] Aliquots of the suspensions were exposed to the different topical solutions and dilutions for 24 hours. At different time points (0, 1, 3, 6, 12, and 24 hours), aliquots were plated onto potato flake agar plates to determine antifungal activity. The plates

TABLE 1. pH of Tested Dakin's Solutions

	pH	Temperature (°C)
Water	7.04	23.9
0.25%	9.80	23.9
0.025%	9.88	23.3
0.0025%	9.06	23.3
0.00025%	8.70	23.3
0.000025%	8.40	24.4
0.0000025%	8.06	24.3
0.00000025%	7.76	23.7

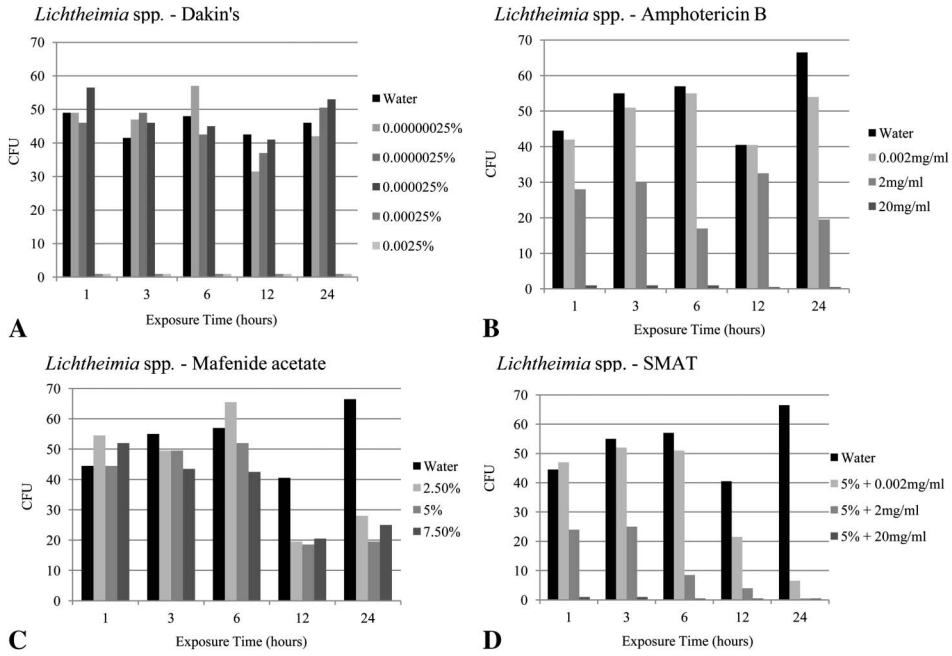

FIGURE 1. *Lichtheimia* spp. tested against various concentrations of Dakin's solution (A), amphotericin B (B), mafenide acetate (C), and SMAT (5% mafenide acetate + various concentrations of amphotericin B) (D) over time.

were incubated at 35°C and colony counts determined after 24 and 48 hours incubation. The assay was performed in duplicates for each isolate.

Based upon a combination of efficacy and toxicity results, 10 additional clinical mold isolates were chosen for testing with selected topical solutions. Using the same methods as described above, 5% mafenide acetate solution, 2 mg/mL amphotericin B solution, SMAT (5% mafenide acetate in combination with 2 mg/mL amphotericin B), and 4 Dakin's dilutions (0.0025%, 0.00025%, 0.000025%, and 0.0000025%) were evaluated for their antifungal activity against 1 *A. fumigatus*, 1 *Aspergillus terreus*, 2 *A. flavus*, 2 *Actinomucor elegans*, 1 *Apophysomyces* spp., 1 *Fusarium oxysporum*, 1 *Bipolaris* spp., and 1 *M. circinelloides* group using the time–kill assay described above. The 2 *A. flavus* isolates were obtained from one patient 45 days apart and treated with topical Dakin's solution (0.25% and 0.0125%), 5% mafenide acetate, and SMAT (5% mafenide acetate in combination with 2 μg/mL amphotericin B) as well as systemic courses of liposomal amphotericin B, caspofungin, and micafungin before the first culture or between cultures. The 2 *A. elegans* isolates were obtained from another patient 8 days apart with therapy before or between cultures of topical 0.25% Dakin's solution or 5% mafenide acetate along with systemic therapy with liposomal amphotericin B and voriconazole.

Cellular Viability Assays

Human dermal keratinocytes (HEK-001; ATCC CRL-2404; American Tissue Type Collection, Manassas, VA) were grown in keratinocyte serum-free medium (GIBCO, Grand Island, NY) with 5 ng/mL human recombinant Epidermal Growth Factor and 2 mM L-glutamine. Human dermal fibroblasts and osteoblasts (PromoCell, Heidelberg, Germany) were grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 10 U/mL penicillin, and 10 μg/mL streptomycin. All cell lines were maintained at 37°C in 5% CO_2. Confluent monolayers of cells seeded into 96-well black plates with clear bottoms (Fisher, Pittsburgh, PA) were exposed to mafenide acetate, amphotericin B, SMAT, and Dakin's solutions diluted in saline (0.9% NaCl, pH 7.4) for 0.5, 1, 3, and 24 hours at concentrations used for initial time–kill studies described above. After exposure, cells were washed and resuspended in 1× phosphate-buffered saline, pH 7.4 (Sigma). Toxicity of topical solutions was measured using the CellTiter Flour assay (Promega, Madison, WI) as recommended by the manufacturer. As a negative control, cells were exposed to saline at the designated time points, with the exception for the Dakin's solution assay in which saline adjusted to pH 9.0 was used. Cell viability was reported as a percentage of the nontreated control group. Assays were performed in triplicate with a minimum of 3 technical replicates per test condition.

Fluorescence Microscopy

Cell morphology after treatment with select topical agents was also evaluated by fluorescence microscopy. Briefly, confluent monolayers of cells in 24-well plates (Fisher) were exposed to the various concentrations of the topical solutions for 3 hours. After treatment, cells were washed, fixed with 4% paraformaldehyde (15710-S; Electron Microscopy Sciences, Hatfield, PA), and permeabilized with 0.1% Triton-X 100. Cells were then stained and a fluorescent phallotoxin stain for F-actin (0. 2 μM) (Molecular Probes, Eugene, OR) for 30 minutes, washed then visualized at ×10 with a HeNE-G laser at 546 nm using an Olympus IX71 inverted microscope (Olympus

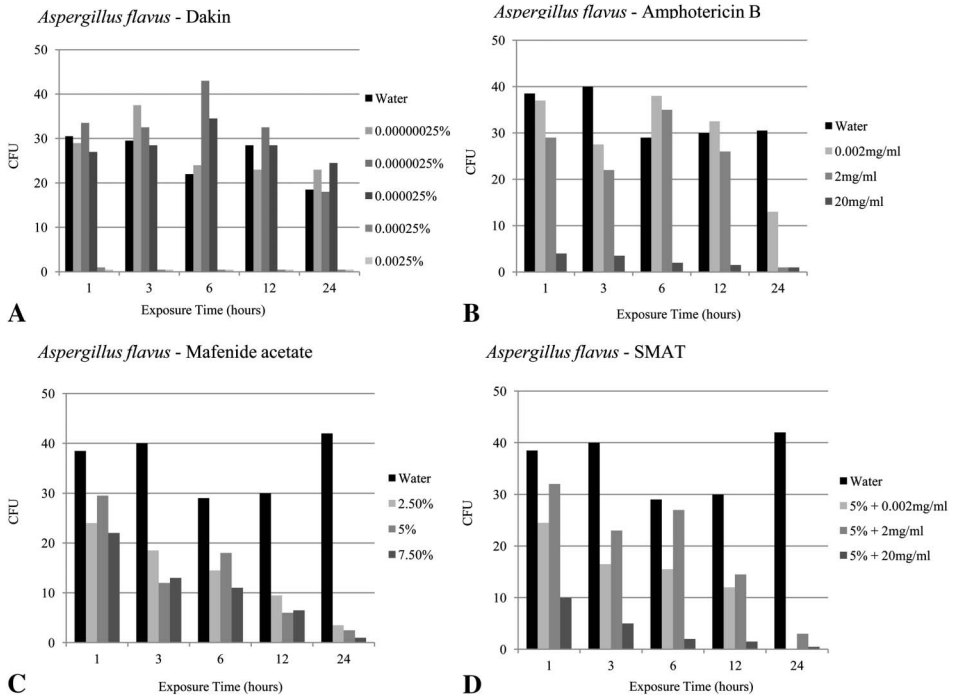

FIGURE 2. *Aspergillus flavus* tested against various concentrations of Dakin's solution (A), amphotericin B (B), mafenide acetate (C), and SMAT (5% mafenide acetate + various concentrations of amphotericin B) (D) over time.

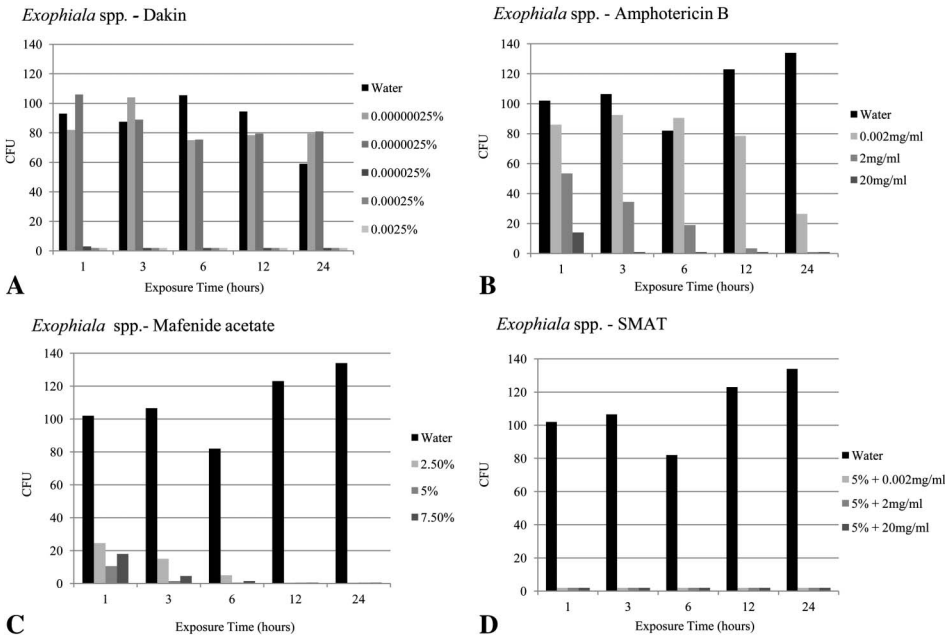

FIGURE 3. *Exophiala* spp. tested against various concentrations of Dakin's solution (A), amphotericin B (B), mafenide acetate (C), and SMAT (5% mafenide acetate + various concentrations of amphotericin B) (D) over time.

America, Center Valley, PA). Representative images for each treatment in human keratinocytes and fibroblasts were selected.

RESULTS

For the 4 molds screened initially with varying concentrations and combinations of topical agents, there was overall evidence of varying dose and time killing aspects of the different agents against those molds (Figs. 1–4). Dakin's solution was active against all molds screened at concentrations equal to or greater than 0.00025% except for *A. flavus*, which showed growth in 1 of the 2 runs at the 0.0025% and 0.00025% concentrations at the 1-hour time point. The 20-mg/mL amphotericin B solution was rapidly fungicidal at the 1-hour time point. The 2-mg/mL amphotericin B dose showed less efficacy with no significant enhanced killing over time. The 2-μg/mL amphotericin B solution had even less activity than higher concentrations. Mafenide acetate at the 3 concentrations seemed to be effective with less dose–response; however, longer durations of exposure were associated with more killing, in contrast to Dakin's solution or amphotericin B. The addition of varying

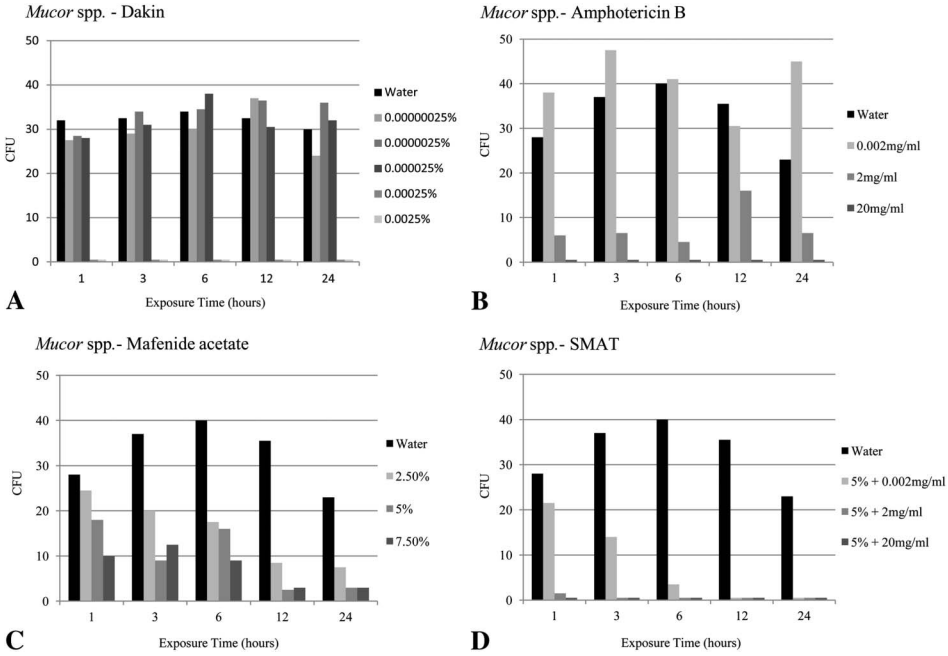

FIGURE 4. *Mucor* spp. tested against various concentrations of Dakin's solution (A), amphotericin B (B), mafenide acetate (C), and SMAT (5% mafenide acetate + various concentrations of amphotericin B) (D) over time.

doses of amphotericin B with standard 5% mafenide acetate seemed to provide some enhanced activity to mafenide acetate alone especially at the 20-mg dose and seemed to retain some killing activity over time. Overall, the combination of mafenide acetate and amphotericin B had more effect than using the same doses of amphotericin B alone.

There was overall evidence of dose-dependent toxicity of the various topical agents against the various cell lines tested, but there did not seem to be increased cell death with continued exposure of the agents over time (Table 2). Dakin's solution showed a dose-dependent decrease in toxicity with overall concentrations less than or equal to 0.00025% being safe among all cell lines tested, with some toxicity at concentrations of 0.0025%. Overall, 20 and 2 mg/mL of amphotericin B showed significant toxicity with more than 90% and 50% cell death as early as 30 minutes postexposure. No substantial cytotoxicity was observed for amphotericin B at concentrations less than 2 μg/mL in the various cell types at all time points tested. There were mild differences in toxicity of mafenide acetate with less toxicity at lower concentrations of the agent. Notably, SMAT seemed to show cell death at higher concentrations in combination than the agents had alone for fibroblasts. In contrast, the toxicity for the keratinocytes and osteoblasts paralleled the toxicity of the individual agents alone. Visual inspection of cells by immunofluoresence confirmed our in vitro results and demonstrated the negative effects of topical agents, including amphotericin B (2 mg/mL), mafenide acetate (5.0%), and SMAT, on cell viability and morphology at concentrations shown to be effective for molds. In contrast, Dakin's solution at concentrations equal to or less than 0.00025%, consistent with the above results, showed minimal effect on cell morphology. Immunofluorescent images of human keratinocytes and dermal fibroblasts demonstrating the effect of Dakin's solution are shown in Figure 5 and the impact of amphotericin B, mafenide acetate, and SMAT in Figure 6.

The results for the 10 clinical isolates using a more tailored screening of topical agents were consistent to those described above; however, there was variability of growth between molds but overall activity remained (Tables 3 and 4). Dakin's solution showed activity at 0.00025% for all but the *Aspergillus* isolates. *Aspergillus fumigatus* and *A. terreus* showed minimal growth at 1 hour at 0.0025% and 0.00025% but no growth at those concentrations at 3 hours or later time periods. SMAT demonstrated excellent activity against *F. oxysporum* and *M. circinelloides*. However, SMAT was less active for other species such as *Bipolaris* spp., *A. fumigatus*, *A. terreus* and *Apophysomyces* spp. It seems that mafenide acetate retains increasing killing activity over time as shown in the screening of the initial 4 pathogens. With regard to the 2 patients with isolates over time (Table 4), there

TABLE 2. Viability Profiles of Fibroblasts, Keratinocytes, and Osteoblasts With Varying Concentrations of Dakin's Solution, Mafenide Acetate, and Amphotericin B

	Fibroblasts				Keratinocytes				Osteoblasts			
	30 min	1 h	3 h	24 h	30 min	1 h	3 h	24 h	30 min	1 h	3 h	24 h
Dakin's solution (%)												
0.0000025	nd	nd	nd	99	99	98	101	100	98	101	97	101
0.000025	98	93	92	93	99	95	97	99	101	99	86	89
0.00025	89	82	78	80	105	94	92	97	95	78	72	71
0.0025	42	10	2	4	25	17	10	4	13	41	36	29
0.025	7	1	1	2	3	2	2	4	2	2	2	2
0.125	1	0	2	2	1	1	2	2	2	2	2	2
0.25	1	1	3	2	1	1	1	2	2	2	2	1
0.5	1	1	4	2	1	1	1	2	2	2	2	2
Amphotericin B												
0.002 μg/mL	100	108	80	93	93	96	96	86	96	97	92	84
0.02 μg/mL	111	106	82	99	94	89	100	88	93	87	85	84
0.2 μg/mL	115	106	84	86	92	92	98	81	91	85	86	84
2 μg/mL	101	102	90	94	96	93	89	76	86	81	82	73
2 mg/mL	54	27	8	12	75	61	43	42	43	36	34	28
20 mg/mL	7	2	9	2	3	2	3	2	2	2	2	1
Mafenide acetate (%)												
2.5	50	78	72	55	77	72	75	70	81	70	63	29
5	59	48	84	37	61	55	51	48	62	50	41	46
7.5	35	48	73	30	53	45	33	31	56	41	22	68
Mafenide acetate + amphotericin B												
5% + 2 μg/mL	78	58	1	35	76	70	64	58	72	70	68	77
5% + 2 mg/mL	14	12	1	9	61	52	45	41	51	49	46	50
5% + 20 mg/mL	1	2	2	2	2	2	3	2	2	2	2	2

Data normalized to % of control.
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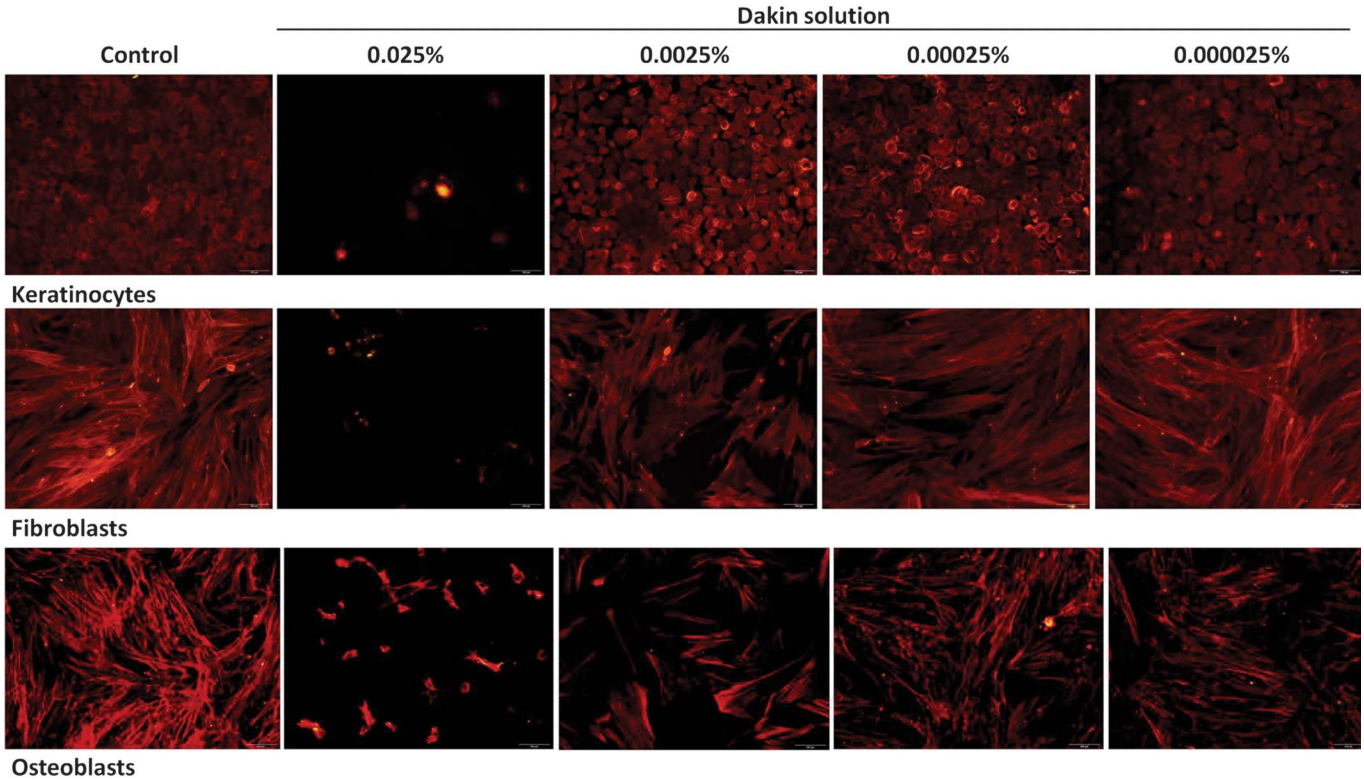

FIGURE 5. Representative immunofluorescent images of human keratinocytes, dermal fibroblasts, and osteoblasts demonstrating the effect of efficacious antimicrobial concentrations of Dakin's solution on cell viability and morphology after exposure for 3 hours at 37°C. Images were captured at ×10 using an Olympus IX71 inverted microscope.

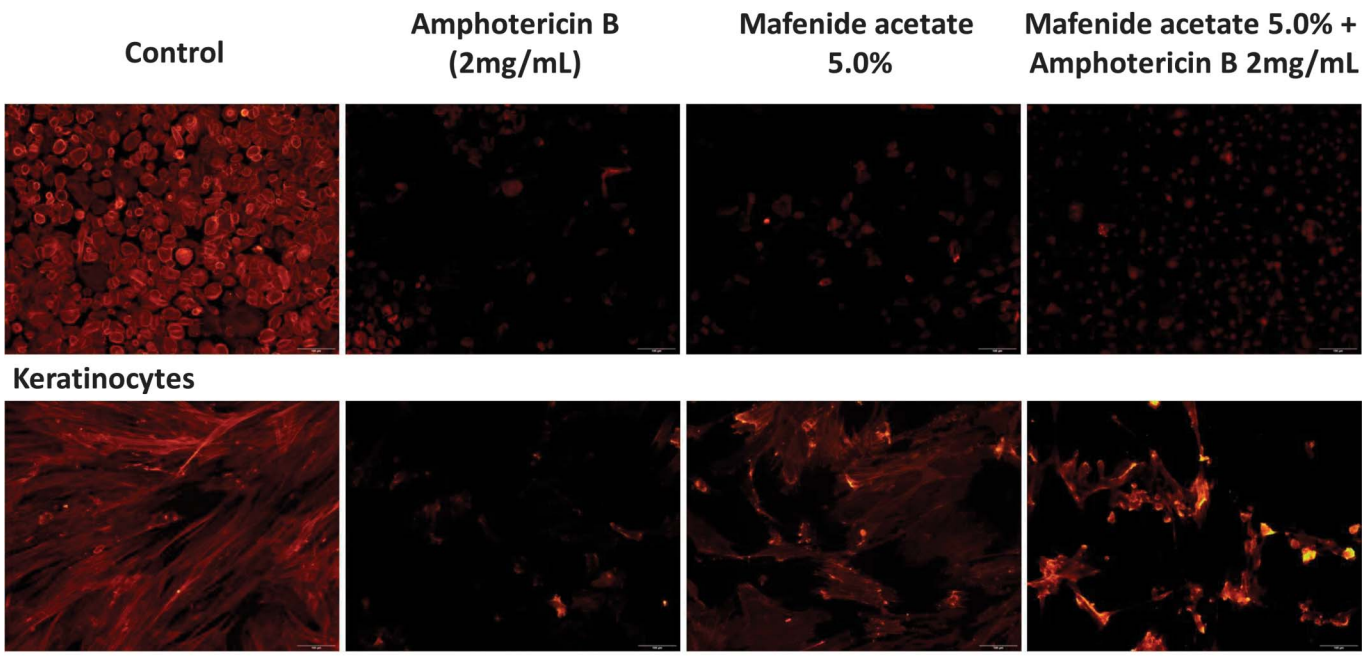

FIGURE 6. Representative immunofluorescent images of human keratinocytes and dermal fibroblasts demonstrating the effect of various topical agents, including amphotericin B (2 mg/mL), mafenide acetate (5.0%), or SMAT (mafenide acetate 5.0% + amphotericin B 2 mg/mL) on cell viability and morphology after exposure for 3 hours at 37°C. Images were captured at ×10 using an Olympus IX71 inverted microscope.

did not seem to be decreased efficacy associated with exposure to topical or systemic agents before or between recovery of the infecting pathogens. *Aspergillus terreus* was not completely eradicated at 1 hour using 0.0025% Dakin's solution, but at 3 hours or subsequent time periods, 0.00025% Dakin's solution was completely active. SMAT seemed to have some activity against *A. elegans* but minimal activity against *A. flavus*, although there was increased killing during prolonged exposure to SMAT for the *A. flavus* isolates.

DISCUSSION

Complex extremity injuries in casualties from Afghanistan have been noted to have increased rates of wound IFIs.[21] Although primary management is still surgical debridement, the nature of these injuries does not always allow for initial surgical control of all compromised tissues because of a myriad of issues. Therefore, patients are increasingly being treated with systemic antifungal therapy in hopes of controlling the infection along with implementation of adjunctive therapy with topical agents. This study suggests that Dakin's solution might retain efficacy when diluted to a strength that it retains its antifungal activity while at the same time minimizing toxicity in an in vitro model of human cell lines. Although mafenide acetate and amphotericin B showed activity across a range of molds, these did not perform as consistently as Dakin's solution.

The most often proposed use of Dakin's solution employed the Cullen method in which Dakin's solution was instilled for 5 minutes every 2 hours for 2–10 days through small fenestrated tubes directly placed into the wound.[13,14] It was only instilled for a short period of time as the

TABLE 3. Time Kill Study of 6 Single-Patient Clinical Isolates of Molds With Varying Concentrations of Dakin's Solution, Mafenide Acetate, and Amphotericin B

Exposure Time (h)	Topical Solution	*Fusarium oxysporum*	*Bipolaris* spp.	*Mucor circinelloides*	*Aspergillus fumigatus*	*Aspergillus terreus*	*Apophysomyces* spp.
		Mean colony-forming units					
0	Water	140	68	52	70	82	12
1	0.0025% Dakin's solution*	0	0	0	17	35	0
	0.00025% Dakin's solution	0	0	0	1	4	1
	0.000025% Dakin's solution	9	69	55	48	72	11
	0.0000025% Dakin's solution	132	88	56	71	66	11
	2 mg/mL Amphotericin B	91	51	15	50	64	7
	5% Mafenide acetate	26	69	35	16	27	5
	SMAT	0	69	1	29	48	3
3	0.00025% Dakin's solution	0	0	0	0	0	0
	0.000025% Dakin's solution	2	81	58	65	56	9
	0.0000025% Dakin's solution	128	78	54	48	57	9
	2 mg/mL Amphotericin B	27	63	22	62	60	5
	5% Mafenide acetate	12	64	27	6	12	2
	SMAT	0	41	0	32	30	3
6	0.00025% Dakin's solution	0	0	0	0	0	0
	0.000025% Dakin's solution	6	101	49	63	67	11
	0.0000025% Dakin's solution	120	110	55	77	94	9
	2 mg/mL Amphotericin B	4	57	16	73	67	6
	5% Mafenide acetate	1	65	18	7	11	2
	SMAT	0	16	0	29	39	2
12	0.00025% Dakin's solution	0	0	0	0	0	0
	0.000025% Dakin's solution	2	69	54	73	60	8
	0.0000025% Dakin's solution	117	75	56	48	62	11
	2 mg/mL Amphotericin B	1	51	10	66	55	3
	5% Mafenide acetate	0	63	16	2	4	2
	SMAT	1	6	0	10	29	1
24	0.00025% Dakin's solution	0	0	0	0	0	0
	0.000025% Dakin's solution	5	92	40	52	95	8
	0.0000025% Dakin's solution	107	88	69	62	70	9
	2 mg/mL Amphotericin B	0	46	32	57	67	6
	5% Mafenide acetate	0	44	6	0	3	2
	SMAT	0	0	0	1	26	0

A control (water) was also performed with each time point (data not shown).
*No growth at this concentration for any mold at other time points.
SMAT, 5% mafenide acetate + 2 mg/mL amphotericin B.

TABLE 4. Time Kill Study of Serial Clinical Mold Isolates From 2 Patients With Varying Concentrations of Dakin's Solution, Mafenide Acetate, and Amphotericin B

Exposure time (h)	Topical Solution	*Aspergillus flavus* (1)	*Aspergillus flavus* (2)	*Actinomucor elegans* (1)	*Actinomucor elegans* (2)
		Mean colony-forming units			
0	Water	32	31	5	30
1	0.0025% Dakin's solution*	15	10	0	0
	0.00025% Dakin's solution	8	1.5	0	0
	0.000025% Dakin's solution	27	16	8	22
	0.0000025% Dakin's solution	34	23	4	22
	2 mg/mL Amphotericin B	30	27	5	20
	5% Mafenide acetate	25	11	2.5	13
	SMAT	31	22	1	4
3	0.00025% Dakin's solution	0	0	0	0
	0.000025% Dakin's solution	26	12	8	30
	0.0000025% Dakin's solution	29	24	9	18
	2 mg/mL Amphotericin B	18	26	9	14
	5% Mafenide acetate	15	10	5	7
	SMAT	27	21	1	2
6	0.00025% Dakin's solution	0	0	0	0
	0.000025% Dakin's solution	12	18	4	18
	0.0000025% Dakin's solution	17	23	7	23
	2 mg/mL Amphotericin B	41	31	7	14
	5% Mafenide acetate	14	8	1	7
	SMAT	32	24	0	0
12	0.00025% Dakin's solution	0	0	0	0
	0.000025% Dakin's solution	25	15	2.5	14
	0.0000025% Dakin's solution	23	25	2.5	7
	2 mg/mL Amphotericin B	39	31	8.5	22
	5% Mafenide acetate	5	6	2	7
	SMAT	24	23	0	0
24	0.00025% Dakin's solution	0	0	0	0
	0.000025% Dakin's solution	27	14	7	19
	0.0000025% Dakin's solution	14	19	6	23
	2 mg/mL Amphotericin B	20	10	3	15
	5% Mafenide acetate	3	2	3	2
	SMAT	8	7	0	0

A control (water) was also performed with each time point (data not shown).
*No growth at this concentration for any mold at any other time points.
SMAT, 5% mafenide acetate + 2 mg/mL amphotericin B.

antimicrobial effect only lasts at most 10 minutes.[13,14] It is notable that Cullen's description of the use of Dakin's solution was only after meticulous wound debridement, close microbiological wound monitoring with smears and culture after initial surgery, and a focus on delayed primary closure.[14] He also highlighted that Dakin's solution did not penetrate deep complex wounds or deeply into tissue. In addition, he described the destruction of leukocytes by Dakin's solution to the same degree that it destroyed the bacteria, so there was a net equivalent effect in the tissue; however, he proposed that because Dakin's solution did not penetrate deep tissues, phagocytosis continued where Dakin's solution was not active. Cullen's contemporary, Alexander Fleming, performed extensive studies and doubted the true effect of Dakin's solution on wounds and indicated that antiseptic solutions showed maximal activity in a watery medium and minimal activity in the presence of blood, purulent material, or deep into tissue.[13] Numerous other studies have supported the negative impact of Dakin's solution on neutrophil migration, fibroblasts, and endothelial cells; however, the concentrations evaluated are variable from study to study.[14–16] Because many of these IFIs are also associated with bacterial infections, an understanding of the role of Dakin's solution in bacteria, especially multidrug-resistant bacteria such as *Acinetobacter* and methicillin-resistant *Staphylococcus aureus*, is needed. However, studies have shown that 0.005% Dakin's solution has activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacteroides fragilis*, *Enterococcus* spp., and *S. aureus*.[23] It is notable that no in vivo animal- or human-controlled experiments have been performed with Dakin's solution, and our study did not assess the impact of Dakin's solution on the immune response especially on neutrophils, which has been a noted issue with this

agent. Further studies are needed in animal models to assess for safety and efficacy especially if lower concentrations of 0.0025% or less Dakin's solution are implemented. To maximize the agent's antimicrobial properties, the process of instillation of Dakin's solution into the wound must be considered, including the role of frequently placing soaked gauze into the wounds, placement of a fenestrated tube for frequent instillation as initially described by Cullen, or using the widely implemented negative pressure wound therapy (eg, V.A.C.; KCI, San Antonio, TX). Dakin's solution has been instilled using a specialized instillation device in conjunction with negative pressure wound therapy. However, this was used in venous stasis wounds before split-thickness skin graft placement, which does not adequately represent the high-risk wounds associated with wound IFIs.[24]

The use of mafenide acetate and amphotericin B seems less effective in this study. In addition, there have been limited clinical efficacy data in the literature with low-dose amphotericin B at 2 μg/mL with mafenide acetate.[18] It is notable that mafenide acetate did provide some prolonged killing activity against some molds, implying that it might be a suitable agent for some molds if a patient is unable to get periodic wound instillation of the other agents. The agent still needs to be used twice a day and is associated with metabolic acidosis and electrolyte disturbances. Mafenide acetate is indicated for patients who suffer severe burns as it rapidly penetrates full-thickness eschar making it effective in heavily colonized and infected wounds.[25] However, burn eschars have very different tissue parameters than these complex wounds. Amphotericin B does seem to have a toxic effect on human cell lines but has been used in a bead format with clinical improvement in an immunosuppressed patient with femoral mucor infection.[17,26] Given the presence of other antifungal agents, including voriconazole and posaconazole, with their differing antifungal spectrums of activity, other locally delivered antifungal agents should be considered as there are some data alluding to their ability to be used in implantable bone cement placed into wounds.[27,28]

In this study, we have shown that Dakin's solution exhibited dose-dependent toxicity and efficacy, whereas mafenide acetate and amphotericin B did not seem to persistently meet the toxicity and efficacy balance as consistently as Dakin's solution. One has to be cautious relying on antimicrobial therapy, systemically or locally, instead of aggressive surgical intervention, as was illustrated during World War I, World War II, and the Yom Kippur War.[13,29–31] Further studies are needed, including animal models of IFIs and human clinical evaluations of topical antifungal agents, to ensure that the risk–benefit ratio of toxicity and efficacy is adequately characterized.

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