Efficacy of topical silver against fungal burn wound pathogens

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Background: Fungal infections of burn wounds have become an important cause of burn-associated morbidity and mortality. The nature of fungal infections dictates aggressive treatment to minimize the morbidity associated with these infections. Persons with large total body surface area burns are particularly susceptible to fungal infections and are treated in such a manner as to minimize their risk of infection.

Methods: This study examined the in vitro fungicidal efficacy of a variety of different topical agents. By placing fungal inocula in contact with mafenide acetate, silver nitrate, silver sulfadiazine, and a nanocrystalline silver-coated dressing, we determined the kill kinetics of these topical agents against a spectrum of common burn wound fungal pathogens.

Results: The topical antimicrobials that were tested demonstrated varying degrees of efficacy against these pathogens.

Conclusion: The nanocrystalline silver-based dressing provided the fastest and broadest-spectrum fungicidal activity and may make it a good candidate for use to minimize the potential of fungal infection, thereby reducing complications that delay wound healing. (AJIC Am J Infect Control 1999;27:344-50)

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The rise in the frequency of fungal infections, particularly in burn wounds, is of great concern because fungal infections are associated with excessive lengths of hospitalization and a high mortality rate. Part of the reason for this association has been that a premortem diagnosis, made early enough to allow appropriate treatment, occurs in only 15% to 40% of the cases. This low rate, combined with a historical mortality rate for burn patients with fungal infections of more than 90%, makes fungal infections particularly worrisome. Fortunately, early and aggressive therapy is resulting in a better prognosis for these patients.

Infection, both bacterial and fungal, of burn wounds has prompted the development of a variety of different topical agents that may be applied to the wound to reduce the chances of wound infection. The most common topical agents used in the treatment of burn wounds include mafenide acetate (MA), silver sulfadiazine (SSD), and silver nitrate. Each of these agents, recognized as having good antimicrobial activity, has several potential drawbacks to its use. For example, MA is well known as being more painful than other treatments on application as well as having very limited cidal effects against fungi or bacteria. Silver nitrate causes a significant amount of staining of virtually any surface with which it comes into contact, in addition to possibly causing tissue irritation. SSD, although not demonstrating many of the negative aspects of silver nitrate, is also somewhat problematic because of its propensity to form pseudoeschar that must be removed on a regular basis; a cause of considerable discomfort for some patients.
To overcome some of the difficulties associated with many of the topical preparations used to combat wound infection, a novel silver-coated dressing recently has been developed and introduced to the North American market. The dressing is coated with nanocrystalline silver, a form of silver that has been demonstrated to be effective at rapidly killing a broad spectrum of bacteria. The current study was undertaken to determine the dressing’s in vitro efficacy against common fungal pathogens of burn wounds.

METHODS

Fungi

The fungal strains used in this study were derived from clinical isolates. The yeast isolates (Candida albicans, Candida glabrata, Candida tropicalis, and Saccharomyces cerevisiae) and Aspergillus fumigatus were obtained from the University of Calgary Faculty of Medicine. The Mucor isolate was obtained from the National Centre of Mycology at the University of Alberta (Edmonton, Alberta, Canada). All of the fungal species were stored on agar slants or as spore suspensions at –80°C. Before use, the fungi were revived by growth on an appropriate cultivation medium. The filamentous fungi were cultivated on Sabouraud Dextrose agar (Difco, Detroit, Mich) and the yeast on Yeast Dextrose agar. Yeast Dextrose agar was formulated in the laboratory as 10 g/L yeast extract (Difco), 10 g/L dextrose (Anachemia, Vancouver, British Columbia, Canada), and 15 g/L agar (Difco) in deionized water. For the purposes of these experiments, the revived yeast were harvested from plates and inoculated into tryptic soy broth (Difco) and allowed to grow until a dense culture was obtained. The yeast culture was diluted in fresh tryptic soy broth to yield a final optical density at a wavelength of 625 nm (OD$_{625}$) = 0.4 ± 0.05 for use in these experiments. These types of turbidimetric measurements provide a convenient method of standardizing the density of a microbial culture because, typically, the density is linearly related to the OD at a given wavelength.

For the A fumigatus experiments, the fungi were grown to produce 2 different types of suspensions. To recover mycelial cells, the fungi were grown in a shake flask of tryptic soy broth overnight. The culture was homogenized in a sterile blender flask (Fisher Scientific, Nepean, Ontario, Canada) for 3 minutes, with cooling. The dispersed mycelia were adjusted to an OD$_{625}$ = 0.8 ± 0.05. To obtain Aspergillus spores, a Sabouraud Dextrose plate was inoculated with fungus and allowed to grow for 2 days. The aerial spores were harvested by gently washing the fungal growth with 5 mL of a 0.85% saline solution and checked microscopically. The OD$_{625}$ of the resultant spore suspension was adjusted to 0.8 ± 0.05.

The Mucor spores were harvested in a manner analogous to that used for the Aspergillus spores. As a result of the fragility of the coenocytic Mucor hyphae, it was
not possible to harvest *Mucor* mycelia to yield a homogeneous suspension that yielded reproducible numbers of colony-forming units (CFUs) after cultivation on agar. Therefore, the effects of the topical antimicrobial agents were examined only against the vegetative spores—the most likely source of wound inoculation; however, microscopic examination of the spore suspension showed the presence of some contaminating mycelia.

**Dressings**

The dressings and the amount of antimicrobial agent added to each dressing were chosen to reflect current clinical practices. Silver nitrate-moistened dressings were prepared by using a dressing composed of a rayon/polyester absorbent core sandwiched between 2 layers of high-density polyethylene mesh. The dressing was cut into squares measuring 2.5 cm × 2.5 cm. Silver nitrate (Fisher Scientific, Nepean, Ontario, Canada) was freshly prepared before each experiment and added to the dressing before the addition of the fungal test suspension. The final silver nitrate concentration on each dressing piece was 0.5%. The SSD dressing was prepared by using a dressing of the same configuration as for the silver nitrate. The SSD (Dermazin; Pharma Science, Montréal, Quebec, Canada), obtained as a 1% cream, was applied to the surface of the dressing pieces. Sufficient SSD was applied to each piece of dressing to yield a thin layer of SSD (approximately 0.56 g) on each piece of dressing. MA dressings were prepared in a manner similar to the silver nitrate dressings. The MA (obtained from the University of Alberta Hospital, Edmonton, Alberta, Canada) was freshly prepared in sterile water and applied to the dressing such that the final drug concentration was 5%. The 3 topical agents were applied to the dressing to maintain the fungal inoculum and antimicrobial agent in close juxtaposition and to facilitate sample handling. The silver-coated dressing (Acticoat Antimicrobial Barrier dressing, Westaim Biomedical Corp, Fort Saskatchewan, Alberta, Canada) was composed of the same materials as the silver nitrate dressing, except that the high-density polyethylene layers were coated with nanocrystalline silver. The control dressings for these experiments also were composed of the same materials as the silver nitrate dressings. However, for the control dressings, no antimicrobial agents were applied to the dressings.

**Design**

Control and test dressings were prepared in triplicate and placed individually on pieces of plastic sheet slightly larger than the test article. The dressings were inoculated with an aliquot of fungal suspension to yield approximate $5 \times 10^6$ CFUs per test article. Each dressing piece, except for the SSD dressing that did not absorb the liquid of the inoculum, was covered with an additional piece of plastic sheet and pressed down to ensure contact of the inoculum with the active components of the dressing. The dressings and inocula were incubated for the requisite length of time (see “Results”) at 37°C. After incubation, the dressings were
removed from the incubator and carefully immersed in a recovery solution (0.85% [wt/vol] NaCl, 1% [vol/vol] polysorbate 20, and 0.1% [wt/vol] sodium thioglycolate). The dressings were vigorously vortexed in the recovery solution and serially diluted in a phosphate buffered saline (pH 7.0) solution, containing 8.5 g/L NaCl, 0.61 g/L K\textsubscript{2}HPO\textsubscript{4}, and 0.96 g/L K\textsubscript{2}HPO\textsubscript{4}. The number of viable fungi remaining after exposure to the different dressings was determined by plating the serial dilutions on a medium appropriate for the cultivation of the fungi in question and enumerating the number of surviving CFUs. The yeast were cultured on yeast dextrose agar. *A. fumigatus* was cultured on Rose Bengal agar (Difco) and *Mucor* on Sabouraud Dextrose agar.

**RESULTS**

Figs 1-4 demonstrate the effect of the various antimicrobial agents on the viability of the yeast inocula. The results clearly demonstrate that the different yeast species demonstrate different sensitivities to these agents. In the case of *S. cerevisiae*, all of the silver-based antimicrobials demonstrated good antifungal activity, reducing the fungal inocula below the limit of accurate detection (10<sup>2</sup> CFU/dressing) within 60 minutes. *C. glabrata* and *C. albicans* responded similarly, although demonstrating a decreased sensitivity toward silver nitrate compared with SSD, taking 2 or more hours to be reduced to levels below detection on exposure to silver nitrate. *C. tropicalis* demonstrated the most resistance to silver nitrate, having a comparatively small reduction in the recoverable population after 2 hours of exposure to silver nitrate. Two other observations also are immediately obvious from these figures. First, MA was confirmed to be a very poor fungicidal agent for use against yeast. In all cases, the yeast were not killed by the presence of the MA. Second, the silver-coated dressing was demonstrated to be highly effective against the yeast isolates. In addition to demonstrating efficacy against these isolates, this dressing also demonstrated the ability to more rapidly kill these fungi than did the other 2 forms of silver.

The results from Table 1 further emphasize the difference in susceptibilities of fungi to silver-based antimicrobials. Against *A. fumigatus*, SSD appears to be ineffective against the spores and very slow acting against mycelia. Silver nitrate demonstrates moderate fungicidal efficacy against the mycelia, taking at least 18 hours to effect a large decrease in the number of viable fungal CFUs. Silver nitrate also demonstrates some efficacy against the *Aspergillus* spores. The silver-coated dressing demonstrated rapid efficacy against the mycelia of *A. fumigatus*, effecting a reduction in the CFUs to levels below detection within 2 hours of exposure (shorter time frames were not tested). Like the other forms of silver, the nanocrystalline form of silver also demonstrated a reduction in the rate of killing of the *Aspergillus* spores. However, the dressing did demonstrate an ability to reduce to levels below detection the number of spores that could germinate and yield colonies on agar within a relatively short period of time.
The experiments with *Mucor* demonstrated that this phycymycete is also relatively insensitive to some forms of silver, similar to *A fumigatus*. The assay that was conducted examined the ability of the silver-based topical treatments to reduce the number of CFUs from an inoculum comprised of primarily vegetative spores, with some contaminating mycelia, inoculated onto the 3 types of silver-based products. The results presented in Table 2 demonstrate the efficacy of these agents against *Mucor* within a short period of time.

In this case, silver nitrate is less effective against *Mucor* than SSD. However, the nanocrystalline form of silver demonstrated rapid elimination of the viable fungal spores and any contaminating mycelia.

**DISCUSSION**

Silver, a well-known antimicrobial agent, has been used in clinical settings for more than a century. During this period, the safety of this agent has been well established. Typically, the only side effect to the use of silver is a transient discoloration of the treated area as a result of silver interaction with proteins and chlorides present on the body. In addition, in some cases of prolonged or heavy contact with silver, a permanent, benign discoloration (argyria) may develop. To further establish the safety of the silver-coated dressing described herein, a comparison between the dressing and silver nitrate in dermal sensitization and irritation studies was performed. Neither of the silver-based antimicrobial test subjects was found to elicit an adverse reaction. Comparative in vitro cytotoxicity tests also were conducted, and these tests indicated that the silver-coated dressing was markedly less cytotoxic than silver nitrate. Another benefit of topical silver applications is that concurrent emergence of resistance to antibiotics and noble metals, particularly silver, in clinical isolates is rare. Furthermore, documented cross-resistance between silver and antibiotics in clinically isolated organisms is also rare.

Similar to other heavy metals, silver exerts its antimicrobial effects by poisoning respiratory enzymes and components of the microbial electron transport system as well as interfering with some DNA functions. However, a number of factors make conventional silver-based topicals less than ideal. Depending on the method of application, these factors include irritating and astringent effects on tissues, poor penetration of eschar, formation of pseudoeschars that need to be removed, wound maceration, and retardation of epithelialization.

To overcome many of these negative aspects of silver usage in topical wound care and to provide an effective and long-lasting concentration of silver at the wound site, the silver-coated dressing was developed. These dressings were made by using a patented physical vapor deposition method to sputter silver onto the high-density polyethylene that constitutes the outermost layers of the dressing. The coating was demonstrated in the present study to be effective and fast-acting against a broad spectrum of common fungal wound pathogens.
In addition to being effective against fungi, this method of silver application also has been demonstrated to be efficacious against a broad spectrum of bacteria, including antibiotic-resistant strains.13

One of the characteristics of silver ions that makes them so effective against microorganisms is their rapid reactivity. However, this reactivity also results in the silver being relatively quickly inactivated by the presence of physiologic concentrations of chloride as well as protein. The nanocrystalline form of silver present in the silver-coated dressing seems to be less susceptible to this type of inactivation. The dressing was tested during the present studies for its ability to kill fungi in the presence of physiologic concentrations of chloride (results not shown) with no apparent negative impact on efficacy. Similarly, other studies also have failed to demonstrate a negative impact of serum proteins on in vitro dressing efficacy13 conducted in a manner similar to that described previously in the “Methods” section.

The results of the current study demonstrate the excellent in vitro performance of silver, particularly the nanocrystalline form, against a variety of common fungal pathogens. The most remarkable aspect of the fungicidal experiments is that nanocrystalline silver appears to be effective against the resistant spores produced by some of these organisms.

The insidious nature of fungal burn infections combined with their comparatively high mortality rate and the aggressive nature of treatment required to combat these infections make them particularly serious complications of burn wounds. Therefore, demonstration of an effective prophylactic measure to protect burn wounds at risk of fungal infection is particularly important. Silver, particularly in the nanocrystalline form, appears to be an effective means of prophylaxis given its rapid and broad-spectrum efficacy. These characteristics suggest that the use of nanocrystalline silver dressings may decrease the incidence of fungal infections that delay wound healing when they occur.

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References

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