Determining the Effect of an Oak Bark Formulation on Methicillin-resistant Staphylococcus aureus and Wound Healing in Porcine Wound Models

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Control of wound infections, especially those associated with methicillin-resistant Staphylococcus aureus, is necessary for the wound healing process. Selection of topical agents should be based not only on their ability to eliminate pathogenic bacteria, but also on whether they may be detrimental to tissue repair. Two randomized, controlled in vivo studies using different porcine models were conducted to evaluate the effect of a topical oak bark ointment (treatment) on 1) methicillin-resistant Staphylococcus aureus in partial-thickness wounds, and 2) healing of second-degree burn wounds. Silver sulfadiazine, oak bark ointment vehicle control (polyethylene glycol), and no treatment (untreated wounds) were used as controls in both studies. In the first study, 108 partial-thickness wounds in three animals were inoculated with a methicillin-resistant S. aureus suspension (average 6.96±0.4 log CFU/mL) and covered for 24 hours with a polyurethane film. After polyurethane film removal, treatments were applied twice daily and nine wounds per day (three per animal) from each treatment group were cultured after 24, 48, and 72 hours. Methicillin-resistant S. aureus colonization was lowest in the active treatment group at all three assessment times and after 72 hours ranged from (5.01±1.1 CFU/mL) in the treatment to (6.20±0.8 CFU/mL) in the vehicle control treated wounds.

In the second study, treatments were applied twice daily to second-degree burn wounds (n = 720) on eight animals. Daily epithelialization assessment (n = five wounds) was performed on day 7 through 10 after wounding. At every assessment time, the proportion of wounds healed was higher in the treatment than in the control treatment groups — days 8, 9, and 10 (active versus vehicle and untreated), P <0.01; days 9 and 10 (vehicle versus untreated), P <0.001. The oak bark formulation studied reduces methicillin-resistant S. aureus contamination and facilitates healing in vivo. Research to ascertain the importance of these findings for clinical practice is needed.

KEYWORDS: wound healing, antimicrobial, methicillin-resistant Staphylococcus aureus (MRSA), oak bark formulation, in vivo


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Wound infection, especially when associated with *Staphylococcus aureus*, is a major concern for healthcare providers. In a recent surveillance report, most methicillin-resistant *S. aureus* (MRSA) infections were nosocomial: 58.4% were community-onset infections, 26.6% were hospital-onset infections, 13.7% were community-associated infections, and 1.3% could not be classified. Community-acquired MRSA rates as high as 60% have been reported in patients with skin and soft-tissue infections. Additionally alarming is the fact that clinical studies have shown that antibiotics are often ineffective against MRSA infections. Although some controlled studies have shown that particular antibiotics have been fairly effective at eradicating MRSA in patients, the use of both topical and systemic antimicrobial agents may be necessary to control infection.

Antibiotic resistance is becoming ever more alarming in clinical practice. In a retrospective analysis of bacteria isolated from hospitalized dermatology patients at their facility, Colsky et al cultured 194 superficial skin wounds or leg ulcers in patients with a dermatological diagnosis of psoriasis, pemphigoid, atopic dermatitis, mycosis fungoides, pemphigus, and other dermatoses. The most common organism isolated was *S. aureus*, 50% of which was MRSA with one isolate also resistant to vancomycin. Mupirocin has been effective in the treatment of MRSA; however, some resistance has been reported.

Although *in vitro* studies are important to help determine initial appropriate dose of antimicrobials on particular bacteria, additional *in vivo* studies are necessary to take into account the effect of an antimicrobial agent in the presence of wound fluid, growth factors, and other considerations. Although only a few *in vivo* studies involving natural extracts have been conducted (many antimicrobial studies have been performed *in vitro*), use of the substances has been described anecdotally in wound healing for many years. Molochko et al demonstrated that *Quercus rubra* (oak bark) had the most anti-staphylococcal activity of eight chosen plant extracts. Tree bark extracts, including from *Quercus rubra*, traditionally have been used by the native elders of Vancouver Island to treat illnesses that range from digestive tract ailments to dermatological conditions.

A novel therapeutic agent is available that contains benzoic acid (6%), salicylic acid (3%), and a proprietary extract of oak bark (QRB-7: 3%) — Bensal HP® (HS Pharmaceuticals, LLC, Greenville, SC). Benzoic acid is slightly soluble in water, soluble in ethanol, very slightly soluble in benzene and acetone, and occurs naturally in many plants and resins. It can be converted to its salts and/or esters; in these forms, it is used as a preservative in food, drugs, and personal care products. Information in the literature on the antimicrobial properties of benzoic acid alone is limited but in an *in vitro* study, Ivanova et al found that isolated components of benzoic acid were able to inhibit the growth of *S. aureus* and *Escherichia coli*, two clinically important wound pathogens.

Salicylic acid is soluble in acetone, ether, and alcohol and slightly soluble in water. It is an important component in the preparation of other pharmaceutical products, dyes, flavors, and preservatives. The sodium salt (sodium salicylate) is used for antiseptic preparations and as a preservative. The hydroxyl group reacts with acetic acid to form

**KEY POINTS**

- Concerns about the prevention and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections are increasing in all healthcare environments.
- Researchers conducted two animal studies to assess the effectiveness of a topical oak bark formulation (treatment) in reducing MRSA colonization and evaluate its effect on healing second-degree burn wounds.
- Compared to control and untreated wounds, colonization was lower and more wounds healed in the treatment group.
- Preclinical studies to confirm these findings and investigate the mechanism of action of this formulation as well as clinical studies to ascertain its potential benefits are needed.
acetylsalicylic acid, the major in vivo metabolite of aspirin. Topical salicylic acid has long demonstrated wide-ranging beneficial properties: it has been shown in vitro to reduce adhesion to biomatrices and effect formation of biofilm—ie, the complex structure of microbial-associated cells embedded in a self-produced extracellular matrix of hydrated extrapolymeric substances (EPS), irreversibly attached to a biological or nonbiological surface. In vitro, salicylic acid also has been reported to down-modulate two important virulent phenotypes in strains of S. aureus (α-hemolysin and fibronectin binding). Based on this interference at the transcriptional level, Kupferwasser et al reasoned that salicylic acid might exert a direct effect on bacterial adhesions necessary for bacterial colonization and propagation in host tissues. Herrmann expanded on the topic of bacterial adhesion and proposed new therapeutic targets by reporting the multiple effects of salicylic acid proteinaceous and nonproteinaceous cell wall and cell surface components. An additional benefit of salicylic acid treatment is its reported capacity as an ultraviolet absorbent; it has been shown to suppress skin tumor development in mice and to stimulate nitric oxide synthesis when delivered via aspirin. Clinically, topical salicylic acid remains an effective topical acne treatment. Interestingly, Imayama et al showed an increase in the number of epidermal basal cells and thickening of the cornified layer following peeling with salicylic acid, suggesting a possible positive effect on re-epithelialization.

The combination of benzoic and salicylic acids was shown in a prospective study of 100 children to partially inhibit the proliferation of Streptococcus in partial-thickness burn wounds. Additionally, another formulation containing both ingredients has suggested a positive effect on the healing of skin transplantation patients. A comparative study conducted by de Kock et al suggested no appreciable bactericidal effect of a similar formulation in burn patients; however, a slight improvement in healing time was noted.

Two controlled in vivo studies using a porcine model were conducted to evaluate the effect of a topical oak bark ointment on 1) MRSA in partial-thickness wounds, and 2) healing of second-degree burns.

Materials and Methods

General study methods.

Experimental animals. The study was conducted in two phases and both studies were submitted to and approved by the University of Miami’s Animal Use Committee. Pigs were chosen for research because of the morphological similarity between swine and human skin. A total of 11 young female specific-pathogen-free pigs weighing 25 to 30 kg were kept in house for 2 weeks before initiation of the experiment (three pigs for the MRSA evaluations and eight for the wound healing assessments). The animals were fed a basal diet ad libitum and were housed individually in the university’s accredited animal facilities (American Association for Accreditation of Laboratory Animal Care [AAALAC]) under controlled temperature (19–21°C) and lighting (12h/12h LD).

Preparation for wounding. Each animal was anesthetized intramuscularly with ketamine HCl (15 mg/kg), xylazine (0.2 mg/kg), and atropine (0.05 mg/kg), followed by mask inhalation of an isoflurane and oxygen combination. The skin on the back and both sides of each animal was prepared for wounding by washing with a nonantibiotic soap and sterile water. Antiseptics were not used due to their potential effect on the healing process. Hair on the backs of the pigs was clipped with standard animal clippers.

MRSA study. Three animals each received 39 partial-thickness wounds (10 mm x 7 mm x 0.3 mm) made with a specialized electrokeratome; 36 were treated and three wounds provided a baseline quantity of colony forming units per mL (CFU/mL) before treatment began. A fresh culture of pathogenic isolate (MRSA ATCC 33593) was obtained from the American Type Culture Collection (ATCC), Rockville, Md for these studies. The freeze-dried bacteria culture was recovered per ATCC standard protocol. Inoculum suspensions were prepared by scraping the overnight growth from a culture plate into 5 mL of normal saline until the turbidity of the suspension was consistent with the MacFarland #8 Turbidity Standard. This resulted in a suspension concentration of approximately 10⁸ CFU/mL that was serially diluted to a concentration of 10⁶ CFU/mL.
of 10^6 CFU/mL. A small amount of the inoculum suspension was plated onto culture media to quantify the concentration of viable organisms. After incubating 24 hours, the inoculum suspension was determined to contain 6.96±0.4 log CFU/mL. This suspension was used to inoculate each site directly. A 0.025 mL (25 µl) aliquot of the suspension was deposited into a sterile glass cylinder (22 mm diameter) in the center of each wound. The suspension was lightly scrubbed into the test site for 10 seconds using a sterile spatula and allowed to dry for 3 minutes. Within 10 minutes of inoculation, all inoculated wounds (n = 36) were covered for 24 hours with a polyurethane film dressing, allowing the bacteria to colonize the wound and develop a biofilm.[30]

**Treatment.** After the polyurethane film was removed, wounds were randomly assigned to one of the following treatment groups: untreated control, oak bark formulation (active), vehicle control (polyethylene glycol), and silver sulfadiazine (SSD). Silver sulfadiazine was selected because it is commonly used in many burn units. Each treatment was applied to nine wounds per animal and re-applied twice a day using sufficient amounts of ointment to cover the entire wound. Once a wound was cultured, it was not assessed further.

**Recovery methods.** A total of nine wounds per day (three per animal) were cultured from each treatment group at 24, 48, and 72 hours post treatment. At each sampling time, sites were cultured quantitatively in a blinded fashion. Each site was cultured only once. The wound site was encircled by a sterile glass cylinder (22 mm outside diameter) held in place by two handles. One (1) mL of scrub solution (containing an appropriate neutralizer) was pipetted into the glass cylinder and the site was scrubbed with a sterile spatula for 30 seconds. Serial dilutions were made and scrub solutions were quantified using the Spiral Plater System (Spiral Systems Inc., Bethesda, Md.), which deposits 50 µL of suspension over the surface of a rotating agar plate. Meticillin-resistant *S. aureus* was grown on selective media with ORSAB selective supplement (OXOID, Inc., Ogdensburg, NY). The plates were incubated for 24 to 48 hours. The geometric mean and Standard Deviation of the log CFU/mL were calculated for each time and treatment. Statistical analysis was performed using a student *t*-test to determine whether differences in MRSA numbers existed between treatment groups.

**Second degree burn study.** Using a second-degree burn model,39 eight animals received approximately 100 burn wounds each. Wounds were produced with six specially designed cylindrical brass rods each weighing 358 g. The rods were heated in a boiling water bath to 100° C, dried to prevent steam burns, and held vertically on the skin for 6 seconds. Pressure was applied by gravity alone, resulting in a burn wound 8.5 mm deep. Immediately after the burn was created, the roof of the blister was removed with a sterile spatula before treatment. Wounds were assigned using block randomization to one of the following groups: oak bark formulation (active), vehicle only, or untreated control. Treatments were blinded —wounds were harvested from each treatment groups and labeled A, B, or C and a particular investigator separated the skins and evaluated the wounds for complete epithelialization. After all data were tabulated, treatments A, B, and C were unblinded. Wounds received applications twice a day for the first 5 days. A total of five wounds per treatment group for each animal were assessed each day.

Using a well-published salt-split technique,31 wounds were evaluated for complete re-epithelialization. Briefly, beginning on Day 7 after wounding (Day 0), five burn wounds and the surrounding normal skin from each treatment area in each animal were excised using an electrokeratome. This was repeated until all wounds were judged to be 100% epithelialized. Any specimens not excised intact were discarded. The excised wounds and the surrounding normal skin were incubated in 0.5 M sodium bromide (NaBr) for 24 hours at 37° C. After incubation, the specimens were separated into epidermal and dermal sheets. The epidermis was examined macroscopically for defects in the area of the burn wounds. Epithelialization was considered complete (healed) if no defects were present; any defect in the wound area indicated that healing was incomplete. The number of wounds healed (completely re-epithelialized) was divided by the total
number of wounds sampled per day, (n = 280 per treatment group, 840 total) and multiplied by 100 to produce a percentage of wounds healed (data were combined from eight animals). The percentage then was plotted against days after wounding. Statistical analysis was performed on all data using the chi-square test with four-fold tables.

**Results**

**MRSA study.** Average baseline bacterial colonization was 7.3 +/- 0.3 log CFU/mL. After 24 hours, wounds treated with active oak bark formulation, vehicle, and SSD had MRSA counts of 5.33 +/- 0.9 log CFU/mL, 6.81 +/- 0.4 log CFU/mL, and 6.83 +/- 0.3 log CFU/mL, respectively. The untreated air-exposed wounds had MRSA counts of 6.94 +/- 0.4 log CFU/mL (see Table 1). Wounds treated with active oak bark had a significant reduction at 24 hours as compared to vehicle and untreated (P < 0.005). At the 48-hour assessment, wounds treated with the active oak bark formulation, vehicle, and SSD had MRSA counts of 5.65 +/- 0.2 log CFU/mL, 6.56 +/- 0.2 log CFU/mL, and 6.53 +/- 0.2 log CFU/mL, respectively. The untreated air-exposed wounds had MRSA counts of 6.83 +/- 0.4 log CFU/mL. Wounds treated with active oak bark had a significant reduction at 48 hours as compared to vehicle and untreated (P < 0.002). At the 72-hour assessment, wounds treated with the active oak bark formulation, vehicle, and SSD had MRSA counts of 5.01 +/- 1.1 log CFU/mL, 6.20 +/- 0.8 log CFU/mL, and 5.94 +/- 0.5 log CFU/mL, respectively. The untreated air-exposed wounds had MRSA counts of 6.17 +/- 0.4 log CFU/mL. Wounds treated with active oak bark had a significant reduction of MRSA at 72 hours compared to vehicle and untreated (P < 0.009). In addition, wounds treated with SSD had a significant reduction compared to untreated wounds (P < 0.04).

**Second-degree burn wound healing.** On days 8 and 9, respectively, 33% and 88% of wounds treated with the active agent, 5% and 43% of wounds treated with vehicle control, and 0% and 0% of untreated wounds were completely epithelialized. On day 10, all wounds covered with the active agent had healed compared to 75% of vehicle and 50% of untreated wounds (see Figure 1). Statistical differences were noted on days 8, 9, and 10 (active versus vehicle and untreated [P < 0.01]); and days 9 and 10 (vehicle versus untreated [P < 0.001]). The authors observed temporary vasodilation in one of the treatment groups, which after unblinding, was found to be in the active (treatment) group. No major clinical differences were observed in terms of erythema, infection, or crust formation between the treatment, vehicle control, and untreated group.

**Discussion**

The emergence of methicillin-resistant clinical isolates has reduced the range of options available to clinicians to combat *Staphylococcus* infections. The results of this study suggest that the oak bark ointment may be an effective antimicrobial agent and facilitates healing in *in vivo* burn wounds. The use of topical antimicrobials in wounds is controversial because many of these agents can not only kill bacteria, but also impede the wound-healing process. Previously, a cadexomer iodine formulation was reported to reduce MRSA counts without impairing epithelialization of partial-thickness wounds. Controlling MRSA has become increasingly important, especially with increasingly fewer options for treatment of MRSA-infected wounds. The authors’ study data suggest that the oak bark formulation can decrease MRSA in colonized wounds. To understand the data, it is important to remember that a 1-log reduction from baseline value indicates a 90% MRSA kill. The oak bark

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after Treatment (hours)</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air-exposed</td>
<td>6.94 +/- 0.4</td>
<td>6.38 +/- 0.4</td>
<td>6.17 +/- 0.4</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>5.33 +/- 0.9</td>
<td>5.65 +/- 0.2</td>
<td>5.01 +/- 1.1</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.81 +/- 0.4</td>
<td>6.56 +/- 0.5</td>
<td>6.20 +/- 0.8</td>
<td></td>
</tr>
<tr>
<td>SSD</td>
<td>6.83 +/- 0.3</td>
<td>6.53 +/- 0.2</td>
<td>5.94 +/- 0.5</td>
<td></td>
</tr>
</tbody>
</table>

*CFU = colony forming units  SSD = silver sulfadiazine  Initial inoculum = 6.96 +/- 0.4 log CFU/mL  Baseline culture = 7.30 +/- 0.3 log CFU/mL
extract was able to reduce the MRSA organisms by almost 2 logs after 72 hours. This log reduction suggested a 99% kill — a significant decrease.

Additionally, the wounds in this study were heavily colonized with the MRSA strain (6.96±0.4 log CFU/mL) and the bacteria were allowed to form a bacterial biofilm, making them more difficult to eradicate. The authors previously demonstrated that pathogenic wound bacteria in vitro can form a mature biofilm within 10 hours. Results of an in vivo study have shown that occluding wounds following inoculating with pathogenic bacteria facilitates the development of biofilms after 24 hours. The authors believe that biofilms are likely to be present in chronic wounds and additional strategies to disturb or eliminate them are sorely needed.

It remains to be determined whether one of the active ingredients (benzoic acid, salicylic acid, or extract of oak bark) or the synergistic effect between the active compounds is responsible for the differences observed between the oak bark formulation and vehicle control. In the current model, only active agents in the ointment form were examined; it is unknown whether delivery of these agents in an impregnated dressing or other delivery system are more or less effective. Also, studies to determine if once-daily treatment is as effective as twice-daily treatments would be of great interest because this could reduce nursing and/or home health practitioners’ need for multiple treatments. The differences observed in the proportion of second-degree burn wounds healed were significant on days 8, 9, and 10 compared to both vehicle and untreated (air-exposed) approaches. The healing rate was similar to that of an experimental cream comprised of a hydrated polymeric complex, polyglyceryl methacrylate in an oil-in-water emulsion containing low concentrations (40 ppm) of fibronectin and the amino acids proline, arginine, and glycine examined in the same model.

**Conclusion**

In this study, the reduction in MRSA was higher in wounds treated with oak bark formulation than in control (treated and untreated) wounds. However, after 3 days of twice-daily treatment, large numbers of MRSA (1% of the original inoculum) remained in the wounds, suggesting additional treatment for a prolonged time may be needed to completely eliminate this organism or that complete elimination may not be possible. Of course, it is also possible that clinically reducing the number of organisms present to less than 1% may be sufficient for healing to occur. The results of the burn wound study suggest that the oak bark formulation can enhance the migration of epidermal cells to accelerate healing.

The potential clinical implications of this study are considerable, especially because the incidence of MRSA infections is on the increase. Additional studies, including well-controlled, prospective clinical studies, are warranted.

**Acknowledgment**

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References


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