Effects of topical application of antimicrobials and bandaging on healing and granulation tissue formation in wounds of the distal aspect of the limbs in horses

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Objective—To determine whether povidone iodine ointment or 2 forms of silver sulfadiazine applied topically to wounds of the distal aspect of the limbs in horses affect the rate of second intention healing and to evaluate the additional influence of bandaging with these antimicrobials on granulation tissue formation.

Animals—6 healthy adult horses.

Procedure—Six standardized 2.5-cm² skin wounds/horse were distributed between the dorsomedial surfaces of the metacarpi and metatarsi. One of the following 6 treatments was applied to each wound: 1% silver sulfadiazine cream with bandage, 1% silver sulfadiazine slow-release matrix with bandage, 1% silver sulfadiazine slow-release matrix without bandage, povidone-iodine ointment with bandage, untreated control with bandage, and untreated control without bandage. Wound area, granulation tissue area, and perimeter were measured by use of planimetry software applied to digital images. Exuberant granulation tissue was excised when present. Days until healing, rate of healing parameter, rate of contraction, and epithelialization were compared among wound treatment groups.

Results—Healing parameters and mean days to healing did not differ significantly among any of the wound treatment groups. Percentage wound contraction and rate of epithelialization were similar among wound treatments. All bandaged wounds produced exuberant granulation tissue, which was surgically excised; none of the unbandaged wounds produced exuberant granulation tissue.

Conclusions and Clinical Relevance—When exuberant granulation tissue is removed, rates of epithelialization and wound contraction were not different among wound treatment groups, whether bandaged or unbandaged. Topical application of 1% silver sulfadiazine slow-release matrix on unbandaged wounds induced the same result as medications applied beneath bandages, but without exuberant granulation tissue formation. (Am J Vet Res 2003;64:88–92)
iodine ointment and 1% silver sulfadiazine cream. Topical application of either antimicrobial provides broad spectrum of antibacterial and antifungal activity in vitro. Results of in vivo studies in human burn patients, mice, and pigs indicate that silver sulfadiazine cream increases the rate of epithelialization, compared with other medications applied topically including povidone-iodine. A 1% silver sulfadiazine slow-release matrix has recently been developed that adheres to the wound bed when dry and can remain in place on unbandaged wounds, therefore avoiding the need for a bandage. This formulation has not been examined for effects on wound healing. To our knowledge, the assessment of these treatments in wound management in horses has not been reported. Controlled trials to examine the effect of commonly applied antimicrobials in wounds of horses would be beneficial.

The objective of the study presented here was to evaluate rates of contraction, epithelialization, and days to healing of wounds of the distal aspect of the limbs in horses treated with 1% silver sulfadiazine cream, 1% silver sulfadiazine slow-release matrix, or 10% povidone iodine ointment. The additional influence of bandaging with these antimicrobials on granulation tissue formation was evaluated.

Materials and Methods

Animals—The Institutional Animal Care and Use Committee approved the protocol used in our study. Six adult geldings (4 Thoroughbreds, 1 warmblood horse, and 1 Quarter Horse) ranging in age from 5 to 11 years (mean, 9.2 years) and weighing 527 to 645 kg (mean, 593.5 kg) were used. Horses were vaccinated routinely and received phenylbutazone (4.4 mg/kg, PO) once daily for 72 hours. Following the withholding of food for 12 hours, each horse was premedicated with xylazine hydrochloride (0.5 mg/kg, IV) and guaifenesin (5% solution, IV to effect). Anesthesia was induced with ketamine hydrochloride (2.2 mg/kg, IV) and guaifenesin (500 mg, 2,000 mg, and 50 g, respectively) in 1 L of sterile saline (0.9% NaCl) solution administered IV to effect. Horses were placed in pairs and housed in covered stalls with an adjoining small dirt paddock. All horses were allowed free choice timothy-orchard grass hay and 1 kg of concentrate/day.

Wounds—Each horse received penicillin G procaine (22,000 U/kg, IM) preoperatively and then twice daily and phenylbutazone (4.4 mg/kg, PO) once daily for 72 hours. Following the withholding of food for 12 hours, each horse was premedicated with xylazine hydrochloride (0.5 mg/kg, IV) and guaifenesin (5% solution, IV to effect). Anesthesia was induced with ketamine hydrochloride (2.2 mg/kg, IV) and maintained with the combination of xylazine, ketamine, and guaifenesin (500 mg, 2,000 mg, and 50 g, respectively) in 1 L of sterile saline (0.9% NaCl) solution administered IV to effect.

Dorsomedial surfaces of metacarpi and metatarsi were aseptically prepared with chlorhexidine gluconate and water. A 6.25-cm² full-thickness skin wound was created on the proximal and distal aspects of each metacarpus approximately 12 cm apart and on the proximal aspect of each metatarsus, avoiding the underlying long or common digital extensor tendons for a total of 6 wounds/horse (Fig 1). Each standardized wound was created by outlining a 2.5-cm, flexible, square, stainless steel template with a No. 10 scalpel blade. The tissue within the template was excised with scissors to the level of the periosteum. Permanent tattoo ink was injected intradermally through predrilled holes in the template 10 mm from and parallel to the margin of the wound. These markers were to be a secondary measurement for assessing total wound expansion and contraction.

Treatments—One of 6 treatments was administered to each of the wounds on each horse. Wound treatment groups included the following: 1% silver sulfadiazine cream bandaged (SSD-B), 1% silver sulfadiazine slow-release matrix bandaged (SDX-B), 1% silver sulfadiazine slow-release matrix not bandaged (SDX-NB), 10% povidone-iodine ointment bandaged (PI-B), uncontrolled bandaged (C-B), and untreated control not bandaged (C-NB). The 1% silver sulfadiazine cream and 10% povidone-iodine ointment formulations were not examined unbandaged because they do not have prolonged adherent properties to wounds. Treatments were continued until complete epithelialization of the wound had occurred. Bandaged wounds were covered with a rayon-polyethylene nonadherent dressing and elastic adhesive tape. The elastic tape was wrapped until pressure was firm. Bandages were changed, and each wound was photographed on the first postoperative day, every other day for 17 days, and then every third day thereafter until the wound had healed.

Wound treatments consisted of 500 mg total weight of each medication applied to the nonadherent pad. Wounds in the SDX-NB treatment group had equal amount of preparation applied with a clean tongue depressor, and the preparation was massaged into the wound bed or eschar. Wounds in the SDX-NB treatment group were further evaluated on ability of the medication to adhere to the wound bed.

Assessment of wound healing—Wounds were considered to have healed when visible epithelium covered the wound. Wounds were considered to have exuberant granulation tissue when the periphery of the granulation bed was above the surrounding level of the skin or the advancing epithelium. When exuberant granulation tissue was present, it was managed by excision to the level of the surrounding skin or migrating epithelium with a No. 10 scalpel blade. Careful excision allowed removal of the tissue without damage to the surrounding migrating epithelium.

Photographs were taken with a digital camera through a macro lens at a 22-cm focal distance after wiping the wound clean of any exude and clipping any hair interfering with identification of the wound margin and prior to any exuberant granulation tissue excision. A horizontal metric scale was attached just proximal to the wound to provide a calibration reference. A label was placed below the wound to identify wound location, date, and horse. The total wound area,
Mean days to complete healing, healing rate parameter, number of times granulation tissue was excised, and percent wound healing attributable to contraction for all wound treatment groups were determined (Table 1). Mean days to complete healing for wound treatment groups ranged from 83 days for C-B and PI-B treatments to 101 days for SSD-B treatments, with a combined mean for all wound treatment groups of 92 days. Significant differences were not detected in healing, healing parameter, and percent wound contraction for all wound treatment groups. Additionally, no significant difference was detected when comparing unbanded and bandaged wound treatment groups separately. All the wounds of horse 3 healed significantly faster (mean 57 days) than the remaining 5 horses (P = 0.01). Horse 3 was the smallest (ie, 527 kg vs a group mean of 593.5 kg).

All bandaged wounds eventually produced exuberant granulation tissue that required excision. The unbanded wounds became covered with a dry eschar.

Table 1—Mean (± SD) values for wound healing in the 6 wound treatment groups

<table>
<thead>
<tr>
<th>Wound healing variables</th>
<th>SDX-NB</th>
<th>SSD-B</th>
<th>SDX-B</th>
<th>PI-B</th>
<th>C-NB</th>
<th>C-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of days until healing</td>
<td>95 ± 25</td>
<td>101 ± 32</td>
<td>96 ± 35</td>
<td>83 ± 18</td>
<td>95 ± 31</td>
<td>83 ± 23</td>
</tr>
<tr>
<td>Initial size (cm²)</td>
<td>7.51 ± 0.96</td>
<td>7.39 ± 0.60</td>
<td>7.23 ± 0.96</td>
<td>7.04 ± 0.47</td>
<td>7.27 ± 0.61</td>
<td>7.11 ± 0.65</td>
</tr>
<tr>
<td>Maximum size attained (cm²)</td>
<td>11.37 ± 1.21</td>
<td>11.80 ± 2.56</td>
<td>11.63 ± 2.86</td>
<td>10.42 ± 1.86</td>
<td>11.34 ± 1.40</td>
<td>10.88 ± 2.27</td>
</tr>
<tr>
<td>Final size (cm²)</td>
<td>2.87 ± 0.63</td>
<td>2.88 ± 0.53</td>
<td>2.74 ± 0.44</td>
<td>2.80 ± 0.80</td>
<td>2.63 ± 0.74</td>
<td>2.68 ± 0.67</td>
</tr>
<tr>
<td>Healing parameter (mm)</td>
<td>5.5 ± 1.24</td>
<td>5.3 ± 1.47</td>
<td>5.2 ± 1.34</td>
<td>5.0 ± 1.67</td>
<td>5.7 ± 1.47</td>
<td>5.2 ± 1.47</td>
</tr>
<tr>
<td>Percentage contraction (%)</td>
<td>74.7 ± 6.11</td>
<td>74.0 ± 5.34</td>
<td>73.7 ± 3.99</td>
<td>75.3 ± 5.26</td>
<td>76.3 ± 6.90</td>
<td>75.2 ± 5.23</td>
</tr>
<tr>
<td>No. of granulation tissue excisions</td>
<td>0</td>
<td>5.5 ± 2.97</td>
<td>4.0 ± 1.91</td>
<td>4.8 ± 2.80</td>
<td>0</td>
<td>4.5 ± 2.14</td>
</tr>
<tr>
<td>Rate of epithelialization (mm²/d)</td>
<td>4.21 ± 0.52</td>
<td>3.48 ± 0.52</td>
<td>3.21 ± 0.52</td>
<td>2.96 ± 0.52</td>
<td>3.06 ± 0.52</td>
<td>4.22 ± 0.52</td>
</tr>
<tr>
<td>Rate constant for contraction (log area)</td>
<td>−0.0271</td>
<td>−0.0296</td>
<td>−0.0235</td>
<td>−0.0228</td>
<td>−0.0305</td>
<td>−0.0274</td>
</tr>
</tbody>
</table>

beginning 14 to 21 days after wounding and did not produce exuberant granulation tissue. The eschar remained in place and reduced in size as contraction and epithelialization progressed until it fell off when epithelialization was visibly complete. Among the bandaged wounds, the mean number of times the exuberant granulation tissue was excised was 5 (range, 1 to 9 times). These wounds would remain moist then progress to purulent exudate prior to becoming exuberant, regardless of treatment. Following exuberant granulation tissue excision, wound surface appearance returned to moist and clean at the next bandage change.

Epithelium was first present in 15 of the 36 wounds by day 7 following wounding. No differences were found among wound treatment groups for initiation of epithelialization. Mean (± SD) rate of epithelialization ranged from 2.96 ± 0.30 mm/d for Pi-B treatment to 4.21 ± 0.30 mm/d for C-NB treatment, but differences were not significant.

Maximal wound size was attained during the second week after which the wounds in each treatment group contracted to 75% of their maximal size when healed. Significant differences were not detected in any measurement of wound areas among any treatment groups. The slope from the exponential regression model from the maximal wound size through the rapid phase of contraction ending at 62 days after wounding ranged from −0.023 for Pi-B treatment to −0.030 for C-NB treatment.

Discussion
In our study, topical application of medication when compared with control treatment did not affect the rate of healing of wounds of the distal aspect of the limbs in horses when exuberant granulation tissue is managed by excision. The increased rate of epithelialization or decreased rate of wound contraction with 1% silver sulfadiazine found in other species was not observed in the horses of our study.

Our results are interpreted with the knowledge that resection of exuberant granulation tissue was necessary to ensure complete epithelialization of the wounds, and in doing so, each bandaged wound treatment group would have had a substantially longer healing time. Because the objective of our study was to examine whether topical application of either of the antimicrobials encouraged or inhibited wound contraction and epithelialization, the presence of exuberant granulation tissue was intended to be an observation and not to interfere with assessment of healing. Potential methods to manage exuberant granulation tissue in the clinical setting are by excision or topical application of corticosteroids, which could not be used in our study. Therefore, we elected to carefully remove the exuberant granulation tissue as not to damage the migrating epithelium. Excision was performed before application of the next treatment to ensure the wound would be exposed to treatment for a maximal amount of time. When the rates of contraction, epithelialization, and healing are examined with the frequency of exuberant granulation tissue excision, the conclusion should be that the bandaged wounds would have required a longer time to heal, if at all. This conclusion is based on the knowledge that migrating epithelium cannot advance over exuberant granulation tissue, and without excision, our study could not be performed to conclusion.

The bandages were applied with uniform pressure without adherence to the wound sites in attempt to reduce exuberant granulation tissue formation while providing protection from environmental contamination and secondary trauma. The protocol in our study was similar to previous reports and closely mimics typical clinical management of granulating wounds. The protocol was not designed to maximize the antimicrobial effects of the individual preparations tested, as each has a proposed application frequency of 12 hours for burns. Subsequently, the bandage change every third day could have permitted enough exudate accumulation to affect the environment and facilitate exuberant granulation tissue formation. Further studies designed with daily bandage changes could facilitate differences in wound healing of similar wounds. Conversely, once the granulation bed was established, superficial contamination may have played a minimal role in the course of healing. The absence of overt infection characterized by reddened granulation tissue and noticeable purulent exudate is supportive.

Recurrent exuberant granulation tissue production specific to bandaging has been reported, however, the reason for the exuberant granulation tissue formation in our study was not definitively determined. We believe the production of exuberant granulation tissue in wound treatment groups that were bandaged (both control and treated) suggests that the use of bandage alone promoted its formation in our study. Bandaging decreases the ambient oxygen tension, thereby stimulating angiogenesis and fibroplasia, which lead to exuberant granulation tissue. This may occur when the 3 preparations are placed under a bandage simulating a semiocclusive-type dressing. A second hypothesis is the accumulated exudate under these bandages causes persistent inflammation that encourages the development of granulation tissue. This was considered when developing our study design and led to the implementation of the SDX-NB and C-NB treatments.

The rationale for topical application of antimicrobials is prophylaxis against and treatment of infection in compromised skin. These preparations allow high local antimicrobial efficacy while avoiding systemic toxic effects and are most effective in the earlier stages of healing prior to a solid granulation bed. Because a negative effect of medication was not found, we believe the use of 1% silver sulfadiazine or 10% povidone-iodine ointment is advisable in the clinical situation. However, prolonged application of a bandage or either antimicrobial tested under a bandage was associated with exuberant granulation tissue formation, and prolonged bandaging appears detrimental to the goal of healing. The lack of benefit from bandaging found in our study herein cannot be universally applied to naturally occurring wounds in horses. Larger wounds will have variables not present in these small controlled wounds. Further, larger granulating areas will have a greater tendency to become exuberant.
Therefore, if a bandage is not necessary for protection of deeper vital structures, reduction of edema, or other mechanical stabilization of the wound on the basis of location, the topical use of the silver sulfadiazine slow-release matrix without bandage would provide antimicrobial protection without promoting exuberant granulation tissue formation.

Further studies should continue to focus on the efficacy of other antimicrobial preparations for healing of wounds of the distal aspect of the limbs in horses. The use of silver-containing dressings continues to promise antibacterial properties, and silver-chloride nylon wound dressings may provide antibacterial activity without the deleterious effects of bandaging previously outlined.25

References


