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A Preliminary Study on the Effectiveness of Glycerin Magnesia Dressing on Second Intension Healing of Wounds on the Distal Aspect of the Forelimb of Horses

Alaa Moustafa and Mohamed Marzok

Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, El Geish street, 33516, Egypt

Abstract: Second intention healing of full-thickness limb wounds in the horse is subject to numerous complications including excessive granulation. Various topical preparations are purported to avoid these complications, but beneficial effects of these medications seen in other species or *in vitro* have rarely been reproduced in equine limb wounds. Here we investigated whether application of Glycerin Magnesia (GM) dressing improves measures of wound healing compared with a non-adherent, semi-occlusive dressing applied to standard experimentally-created wounds on the distal aspect of the equine limb. A 2.5 X 2.5 cm wound was created on the dorsomedial aspect of the proximal metacarpus on each forelimb. A GM dressing was applied to one assigned limb as a treatment and a non-adherent, semi-occlusive dressing was applied to the contralateral limb as an internal control. Bandages were changed every 3 days for 39 days. Granulation tissue was scored and wound area measured every 3 days. GM treatment accelerated wound healing in each horse such that complete closure was achieved on average 6.2 ± 1.5 days sooner than in control wounds ($p < 0.05$). The GM wound dressing did not increase granulation relative to control at any time point. Although addition studies using the GM dressing are needed to characterize the cellular and temporal effects on wound healing and evaluate this dressing in a clinical equine environment, this preliminary study suggests GM dressing is a useful adjunct to accelerate equine wound healing.

Key words: Glycerin Magnesia · Wound · Horse · Wound healing

INTRODUCTION

Distal limb wounds are commonly encountered in equine clinical practice because of the horse's environment, its athletic usage and tendency toward flight behaviour when startled. Distal limb wounds in horses heal more slowly than wounds on other parts of the body because of a comparatively decreased blood supply, an increase in bone prominence and lack of deep supportive tissue, greater mobility over joints and predisposition for bacterial contamination because of proximity to the ground [1, 2]. Additionally, distal limb wounds in horses tend to have more skin retraction, exuberant granulation tissue formation, slower epithelialization and earlier cessation of wound contraction, partially attributed to an ineffective, but persistent inflammatory response [3, 4].

Several studies have attempted to identify strategies to improve management of distal limb wounds in horses. Whereas some treatments have been shown to improve wound healing [5-13] others have not [14-19] and no single preferred method to treat distal limb wounds exists. Wound healing in the equine distal limb may be improved by dressings that speed wound contraction rates without impairing motion, improve epithelialization, limit the formation of exuberant granulation tissue, be cost effective and easy to use and not cause pain on application or removal.

Glycerin is one of the most widely used ingredients in medical prescriptions [20]. It may be used on every part of the epidermis and mucous membranes [21]. When diluted to a concentration below 50%, it acts as an emollient and demulcent, finding important applications in

Corresponding Author: Alaa Moustafa, Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, El Geish street, 33516, Egypt
Tel: 0472231609 E-mail: alaa.moustafa@bristol.ac.uk.

ointments and lotions [18]. Antiseptic preparations for the. most sensitive areas of the body including vaginal, nasal, analgesic, dermatological and burn ointments and jelliesare commonly made of water soluble bases compounded with glycerin [20].

Glycerin scaffolds were introduced as another choice of wound dressing and their production process was simpler, more energy efficient and saves time and money compared to the freeze-dried scaffolds [21]. The glycerolized full-thickness skin harvested from body contouring procedures is clinically effective in burn and wound management in human being [22]. In the presence of regional coordination, it can serve as an abundant source for skin banking in where cadaveric skin use is not legalized [22]. Another study demonstrates that the use of glycerin hydrogel-based wound dressing significantly decreases the number of peristomal infections such that the frequency of dressing changes can be safely extended to 7 days during the first week, making it a less labor-intensive and more cost-effective option for wound management after percutaneous endoscopic gastrostomy [23]. A high glycerin econtent hydrogel mask dressing on post-laser resurfacing wounds is a better and suitable alternative to the open technique wound healing [24]. A glycerin based dressing with commercial honey can be used in the management of a recalcitrant diabetic foot ulcer [25]. In addition, glycerin can be used as a preservative for corneoscleral rim grafts used in the treatment of peripheral corneal disease [26]. Glycerin, vaseline and liquid paraffin cream (Dexeryl) is commonly used as topical hydrating agent for the treatment of skin xerosis [27].

Magnesium sulphate (epsom salt) has been used for many years in treatment of superficial equine wounds and infections effectively by establishing drainage, soaking the foot in an Epsom salt solution, poulticing the foot until drainage has ceased and protecting the foot until the hoof capsule defect has healed [28]. Another study has shown that the use of warm water/Epsom salt soaks for one week reduces the magnitude of infection and cause the swelling to subside in Asian elephant [29].

It is possible to combine the glycerin with magnesia. Glycerin magnesia (Magnesium sulphate and glycerine, GM) enhances healing in human diabetic foot ulcer by reducing the bacterial loads in the area [30]. A comparison between the effectiveness of fresh alovera and GM application based on the level of severity of phlebitis in children has shown that GM is significantly more effective in treatment of mild and moderate phlebitis when compared to fresh aloevera [31].A retrospective study on the effect of GM on treatment of necrosis after Chinese cobra bite envenomation shows that its external application can promote wound healing [32].

In the current study we aimed to determine if application of GM dressing alters wound healing in the distal limb of horses. Since magnesium sulphate is hygroscopic, it pulls out the fluid from the edematous tissue across the skin or the mucosa, which acts as a semipermeable membrane and the mixture is exothermic producing heat that cause vasodilatation which facilitates reabsorption of the fluid from edematous tissue and thereby reducing edema, whereas glycerin has a soothing action on the tissue [33].

Our hypothesis was that application of GM dressing would improve wound healing variables (decrease wound area, decrease granulation tissue formation, resulting in faster healing times) when compared with a non-adherent, absorbent dressing on surgically created wounds on the distal aspect of the forelimb (medial aspect of the proximal metacarpal region) of horses.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Animal Care Committee, Faculty of Veterinary Medicine, Kafrelsheikh University, in accordance with Egyptian ethical codes for studies on experimental animals.

Chemicals and Drugs

Magnesium Sulphate: Epsom salt was obtained from El-Mansorah Pharmaceutical Chemicals Co. (El-Mansorah, Egypt).

Glycerin: Pure glycerine was obtained from El-Gomhouria Pharmaceutical Chemicals Co (Ameria, Cairo, Egypt).

Glycerin Magnesia (33%): The mixture of 33% solution of GM was prepared by dissolving 330 g of Magnesium Sulphate in 100 ml distilled water with heat and thoroughly mixing. After completely dissolving the Magnesium sulphate, glycerin was added to the mixture slowly with heat and continued stirring up to a final volume of one litre.

Animals: Clinically healthy adult horses (n = 5), free of scars or blemishes on the forelimb metacarpal region were selected for this study. There were all non-castrated stallions aged 13–18 years (mean, 14.8 years), weighing 360–520 kg. Animals were housed in indoor stalls and fed on a balanced ration of mixed grain with hay and ad lib water.

Study Protocol: Horses were sedated intravenously with detomidine hydrochloride (0.01– 0.02 mg/kg) and butorphanol tartrate (0.01 mg/kg) (Dechra Pharmaceuticals, Shropshire, Uk). A 5 cm × 5 cm area was clipped of hair on the medial aspect of both forelimbs over both proximal metacarpal regions in each horse. The area was cleaned once with povidone-iodine solution and 100% ethyl alcohol. At the edge of the clipped region, 7 mg/kg 2% mepivacaine hydrochloride (Alexandria pharmaceutical company, Egypt) was infiltrated locally. A 2.5 cm × 2.5 cm template made by using a metal square was centred in the clipped regions and a full thickness wound was created by incising the skin within the metal square template using a #22 scalpel blade. Subcutaneous tissue was sharply removed down. A gauze was applied with pressure to the wound until haemostasis was achieved. Wounds were left open without treatment nor bandaging for 3 days. From day 3, bandages were applied cleanly with the investigators wearing non-sterile examination gloves. A 5X5 cm width of non-adherent semi-occlusive dressing (El Mahalla Co., El Mahalla, Egypt) was immersed in GM. The dressing was centred over the wound and secured with soft, elastic roll gauze (El Mahalla Co., El Mahalla, Egypt). A standard bandage of absorbent cotton (El Mahalla Co., El Mahalla, Egypt) was then applied to the distal limb (REF). For the control limb, the same non-adherent semi-occlusive dressing was applied to the wound, secured with soft, elastic roll gauze and covered with the standard bandage. Horses were fed phenylbutazone (3gm) (Dechra Dechra Pharmaceuticals, Shropshire, Uk) on the day of wound creation and 2 additional days. No peri- or postoperative antibiotics were administered. Bandages on both forelimbs were changed every 3 days until the wounds were healed.

Wound Healing Assessment: Wound measures were collected throughout the 39-day study. Time of wound healing was noted for those wounds not healed by 39 days. At every bandage change, assessment of granulation tissue was graded using a previously-reported scale [13] (1 = below skin edge; 2 = level with skin edge; 3 = above skin edge but not overlapping; 4 = overlapping skin edge). Assessment of granulation tissue was performed by 2 surgeons (A.M., M.M.) throughout the study. Every 3 days, as the bandages were changed, the wounds were digitally photographed directly in line with the wound. Wound area was measured using imaging software (Image J 1.46s, National Institutes of Health, Bethesda, Maryland, USA), using the wound template to calibrate the program and correct for magnification, by outlining the granulation tissue-epithelial margin surrounding the wound, which was converted to cm by the software program. Wound area was measured 3 times and the mean wound area was used for statistical analysis. Wound measurements were performed by A.M. Complete wound healing was defined as convergence of the epithelial margin without any evidence of granulation tissue.

Statistical Analysis: A mixed model multivariate analysis in SPSS Statistics (IBMS, v.21) was used to compare the percentage difference in wound surface area between GM-treated and paired control wounds relative to 3 day (set at 100% for each wound). The model evaluated the main effects of treatment (GM or control), time (6 days to 39) and treatment by time interaction. A post-hoc Bonferroni correction was applied to compare treatments at each time point. Horse ID was included as a random effect, accounting for pairs of wounds being from the same horse.

A Wilcoxon signed rank test was used to compare granulation scores across all time points. Paired t-tests were used to compare the time taken to complete wound closure and the time to maximum granulation between GM-treated and control wounds.

$P < 0.05$ was considered statistically significant. Data is presented as the mean ± standard deviation (St Dev) unless otherwise stated.

RESULTS

GM Accelerates Equine Wound Healing: The surface areas of each control and paired GM-treated wound from each horse were normalised to baseline (day 3, set at 100%). As a proportion of their original size, GM-treated wounds were significantly smaller than control at each time point between 15 day and 39 day (Figure 1a, b). The greatest difference between GM and control wounds was observed on day 24 (GM: 75.9% ± 2.8% reduction in wound size, Control: 55.5 ± 3.6% reduction in wound size, $p < 0.001$).

The GM-treated wound reached complete closure before the control wound in each horse (Figure 1c). GM-treated wounds achieved complete closure on average 6.2 ± 1.5 days sooner than control wounds (Figure 1c).

GM Does Not Result in Excessive Granulation During Equine Wound Healing: Granulation of each wound was scored every three days. All wounds received a maximum granulation score of 3 at least once during the study, but none of the experimentally-created wounds investigated

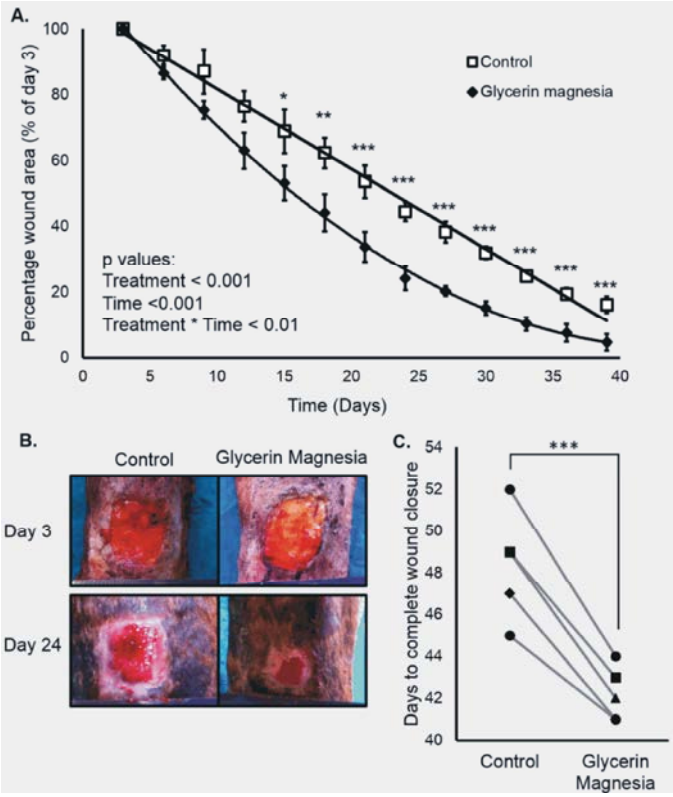

Fig. 1: Glycerin magnesia accelerates equine wound healing. A) Wound area was measured every three days and normalised as a percentage of wound area at the start of the study (day 3, set at 100%). P values relate to mixed model comparison of absolute wound area on each day with Bonfrerroni post-hoc correction. Points represent mean percentage of initial wound area ± St Dev. Trend lines represent linear (control) and second order polynomial (GM) lines of best fit. B) Representative images of control and GM-treated wounds on days 0 and 24. C) The time to complete wound closure of experimentally-created wound was recorded for each horse. Time to closure was compared between control and GM-treated by paired t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ control versus GM-treated

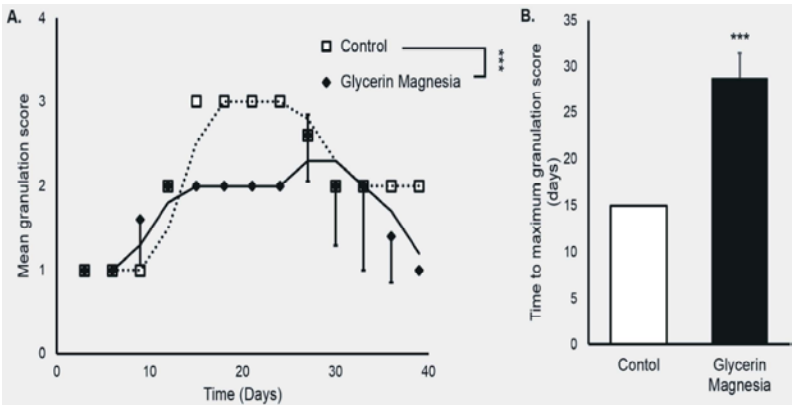

Fig. 2: Glycerin magnesia reduces overall mean granulation score and delays peak granulation. A) Granulation of each control and paired glycerin magnesia treated wound was scored every three days. Points represent mean ± St Dev granulation scores. Trend lines represent two-period moving average lines of best fit. B) The time point at which each wound reached the maximum granulation score was recorded and compared between control and GM-treated wounds. All wounds reached a peak granulation score of 3 and all control wounds first received a granulation score of 3 on day 15. *** $p < 0.001$

in this study showed excessive granulation overlapping with the wound margins (score 4) at any time point. Control wounds produced increasing quantities of granulation tissue up to a maximum at day 15, which was maintained for approximately nine days before partly decreasing towards baseline (Figure 2a). In contrast, GM-treated wounds reached the same level of granulation 13.8 ± 2.7 days later than control wounds (Figure 2b) and fully returned to baseline by the end of the study (Figure 2a).

DISCUSSION

The influence of GM dressing on second intention healing of surgically created wounds on the dorsomedial aspect of the proximal metacarpus in adult horses was evaluated. The GM dressing resulted in significantly decreased wound areas and reduced granulation tissue scores compared with the control dressing. In addition to the significant effects of GM on wound area and granulation tissue scores, complete wound healing times were significantly lower in GM-treated wounds that healed faster than the control wounds.

Studies investigating the effects of semi-occlusive dressings on healing time, granulation tissue formation and effects on inflammatory cells have produced mixed results [7, 14-19, 34]. Howard *et al.* [7] reported an average healing time of 71 days using a synthetic semi-occlusive dressing on 6.25 cm^2 wounds created similar to our study, which was longer than non-adherent control dressing, but significantly shorter than a synthetic occlusive dressing. The conclusion from Howard *et al*.[7] study was that synthetic semi- and fully- occlusive dressing resulted in prolonged healing compared with the control dressing. Our results differ from Howard *et al*. [7] in that granulation tissue production was not increased with the GM dressing compared with control wounds. However, the control dressing we used was different from the control dressing used by Howard *et al.* [7] which limits direct comparisons between studies.

It has been shown that Semi-occlusive dressings have been noted to be most effective during the inflammatory and proliferative phases of wound repair [7, 13]. Our results confirm that the GM Semi-occlusive dressing is indicated during the early phases of wound healing, as the wound area and granulation scores were significantly different between groups primarily within the first 25 days.

Semi-occlusive dressings are thought to be advantageous because they allow oxygen exchange at the surface of the wound while maintaining a moist environment, which is proposed to permit autolytic debridement and promote epithelialization [34]. We did not evaluate the population of cells at the surface of the wounds microscopically at any point during the study to characterize the cell population and correlate the cellular effects of a moist environment with the observed changes in wound area or granulation score. In addition, different semi-occlusive dressings vary in their ability to absorb exudate and, when saturated, can exhibit characteristics of occlusive dressings, which have been shown to promote exuberant granulation tissue formation [7, 34]. Therefore, it is difficult to broadly compare semi-occlusive wound dressings between products or wounds, because there are intrinsic and extrinsic factors that may impact their effectiveness.

Furthermore, GM dressings were associated with slower increases in granulation tissue scores than control wounds, but the granulation tissue was more often scored as being level with the skin edge, which should be optimal for wound contraction and epithelialization. Although the cellular mechanisms were not characterized, it appears that the main GM treatment effects we observed could be attributed to reduced wound size, followed by inhibition of exuberant granulation tissue. It is known that excessive inflammation can impair wound healing. Glycerin in high concentrations has a slight but definite anti-microbial action [24], which may reduce inflammation. It also appears to have anti-inflammatory effects by influencing the inflammatory response to injury, at least in part because glycerin's strong negative charge binds to extracellular matrix molecules and modulates the inflammatory response [24, 35], which could conceivably impede exuberant granulation tissue formation. The dressing never sticks to the wound and does not dry out. By maintaining a moist environment, the chances of contamination decrease and initiate immediate pain relief [24].

Human full thickness allografts have used glycerin as a simple preservative, as it is cost-effective and possesses antibacterial and antiviral properties as well as suppressing allograft immunogenicity. A euro skin bank postal survey of 37 European burn centers found that 90% of responding burn centers used glycerin preserved allografts regularly [22, 36]. Further studies are needed to verify and elucidate the antimicrobial effects of GM dressings in wounds of the equine distal limb.

External application of magnesium sulphate is usually adopted to reduce local inflammation and swelling in clinical practice. Once the magnesium sulphate is mixed into glycerin, the glycerin can effectively prevent its evaporation and extend its duration of action [32].

Concentrated magnesium sulphate in glycerin is an absorptive dressing as the chemicals used are hypertonic/hygroscopic. They absorb water from the wound and thus maintain a moist environment congenial for autolytic debridement and wound healing [37]. Moreover GM has been used in concurrent with Autologous Blood Injection to enhance healing in diabetic Foot ulcers in Egyptian patients [30].

The differences in wound healing between the GM semi-occlusive and control dressing we measured were statistically significant. One of the main limitations of this study is the lack of histological examination to support our findings. Further studies are required to understand the mechanism underlining the effect of GM on wound healing in horses. The second limitation is that we did not culture bacteria from all wounds to check the effect of the GM treatment dressing on bacterial contamination compared with the control dressing. Further studies using the GM dressing to determine temporal microscopic effects on wound healing, such as cellular composition and bacterial quantification and to evaluate this dressing in a clinical environment should now be performed. Finally, the small number of horses used in this preliminary study precludes generalization of the results obtained.

We concluded that use of a GM dressing significantly decreases wound area, lowers granulation tissue scores and decreases time to complete wound healing in horses compared with a non-adherent, semi-occlusive dressing on surgically created wounds on the distal forelimb of adult horses allowed to heal by second intention. The dressing may be indicated for mild to moderately exudative equine wounds in the inflammatory and proliferative phases of repair.

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