

Exuberant granulation tissue in a horse: successful treatment by the intralesional injection of 4% formaldehyde solution

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Summary

The healing of wounds located on the equine distal limbs can often be impaired, which can result in exuberant granulation tissue and its associated aesthetic alteration and functional failures. Although a number of therapies have been developed and assessed, the treatment of these wounds still presents challenges. This report describes the treatment of exuberant granulation tissue in a horse using an intra-lesional injection of 4% formaldehyde solution. The successful outcome of this treatment suggests that further investigations are required in order to better evaluate its efficacy, as well as the incidence and the severity of any adverse reaction.

Tessuto ipergranuleggiante nel cavallo: trattamento riuscito con un'iniezione intra-lesionale di soluzione di formaldeide al 4%

Parole chiave

Cavallo,
Cicatrizzazione,
Tessuto
ipergranuleggiante,
Ablazione chimica,
Formaldeide.

Riassunto

La guarigione delle ferite del tratto distale degli arti degli equini può essere spesso compromessa e può provocare tessuto ipergranuleggiante con alterazioni estetiche rilevanti e perdite funzionali. Sono state sviluppate e valutate diverse terapie ma la ricerca di una soluzione soddisfacente è ancora una sfida impegnativa. In questa relazione si descrive il trattamento del tessuto ipergranuleggiante con un'iniezione intra-lesionale di soluzione di formaldeide al 4%. Ulteriori indagini per valutare l'efficacia, l'incidenza e la gravità di eventuali reazioni avverse potrebbero supportare il risultato positivo di questo studio.

Introduction

Equine practitioners commonly observe skin injuries in horses. These injuries have a negative impact on the economy of the equine industry (Perkins *et al.* 2005, USDA 2006). In equine clinical practice, skin wound treatment is often impaired because of factors including excessive skin tension, massive tissue loss, and delayed and/or inappropriate treatment (Theoret and Wilmink 2013, Theoret *et al.* 2013). As a consequence, wounds tend to repair as granulation tissue develops ('second intention healing'), which is subject to several complications. Complications most frequently occur in wounds located on equine limbs, where some anatomic features (e.g. poor wound contraction due to the absence of the panniculus carnosus) further impair the healing process (Bertone

et al. 1985, Theoret and Wilmink 2013). Complications can lead to chronic non-healing wounds on one side, or to fibroproliferative disorders (so called 'proud flesh', which consists of exuberant granulation tissue, or EGT), on the other, with relevant aesthetic and functional failure (Theoret and Wilmink 2013, Theoret *et al.* 2013).

Although a number of therapies have been developed and assessed (Theoret *et al.* 2008), and several reports focusing on the pathogenesis of EGT in horses are available (Theoret *et al.* 2008, Theoret and Wilmink 2013, Theoret *et al.* 2013), the treatment of wounds in horses remains challenging. On the basis of the above, this report aims to describe the successful treatment of a proud flesh wound by the intralesional injection of formaldehyde solution.

Case history

A female, 8-year-old Quarter Horse was admitted to the Veterinary Teaching Hospital at the University of Teramo (Italy) for a skin lesion affecting the lateral plantar region of the left hock. The owner reported a traumatic injury, which occurred about a month before and was treated with a single layer, non-absorbable suture and covered with unsuitable bandages.

Clinical findings and outcome

Upon presentation, the left hock was swollen and showed an itchy, bleeding, cauliflower-like skin

lesion that measured 3 cm in diameter and was located on the lateral plantar surface (Figure 1a). Apart from that, the horse was in good health, alert, responsive, and no lameness was observed.

The skin lesion was sharply excised on the standing, sedated horse. The standing chemical restraint was achieved with a combination of acepromazine (0.03 mg/Kg bwt i.v.) and xylazine (0.5 mg/Kg bwt i.v.). To improve the analgesia, butorphanol (0.02 mg/Kg bwt i.v.) was also administered. Local anaesthesia was obtained through the perilesional, subcutaneous infiltration of 5 ml of 2% lidocaine. The surgical wound was not sutured. Instead it was dressed with a triple layer bandage and carefully



Figure 1. Horse. Left hock. At first clinic visit, the skin lesion was about 3 cm in diameter, it protruded on the skin surface and was bleeding (a). Two weeks after surgery, the horse showed relapse of EGT, which rapidly enlarged up to 7 cm in diameter (b). Few days after the third infiltration with formaldehyde solution, the lesion was dramatically reduced, dry, crusty and brown to black in color (c). At 120 days' follow-up, a discrete scarring alopecia only remains (d).

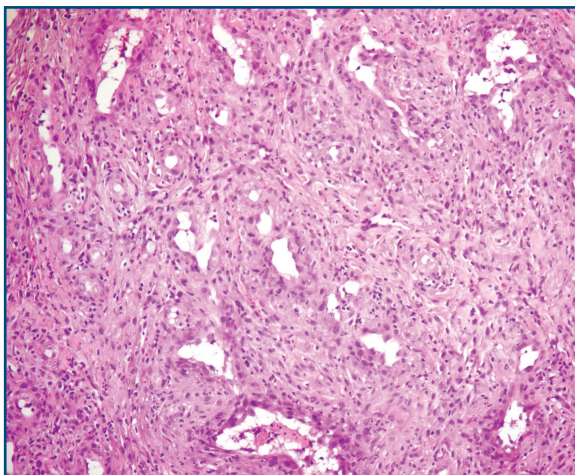


Figure 2. Histopathological section of the skin. The lesion mainly consists of newly formed blood vessels embedded within the connective tissue. A prominent infiltration of inflammatory cells is also observed. H&E stain, final magnification x100.

monitored for 1 week. The bandage was changed at 3-day intervals in order to monitor skin healing. Subsequently, the bandage was removed and the wound was treated with ketansarin (2.5 mg/g gel for horses, twice a day (q.12 h) for another week.

The excised tissue was promptly fixed in 10% neutral buffered formalin (pH 7.2) and routinely processed for histopathological investigation (Hematoxylin and Eosin stain, H&E). Microscopically, the epithelial surface appeared ulcerated, while the dermal layer consisted of granulation tissue with prominent neo-angiogenesis (Figure 2). Small foci of necrosis, as well as of purulent exudate surrounding bacterial aggregates were also observed. On the basis of gross and microscopic findings, EGT was diagnosed.

The horse was then discharged from the Veterinary Teaching Hospital and rested in a small paddock. A twice daily topical administration of ketanserin was recommended. Two weeks later, the horse showed relapse of EGT, which was complicated by scratching. Under these conditions the wound rapidly reached the size of a mandarin (Figure 1b).

Because of the poor results obtained after surgery, the EGT was treated with the intralesional infiltration of a 4% formaldehyde solution (i.e. 10% neutral buffered formalin, pH 7.2). The treatment was administered to the standing, sedated horse. Sedation and local anesthesia were achieved as described above. The site of the lesion was surgically prepared and 10 minutes after sedation, 1 ml of formaldehyde solution was injected into the EGT in a fan-shaped manner. The treatment was repeated 3 times at 3-day intervals. The skin lesion was carefully monitored in order to promptly detect any adverse reaction.

A few days following the last infiltration, the EGT was

greatly reduced (by about 70%) and had assumed a crusty appearance (Figure 1c). Moreover, the bleeding was markedly reduced and the itching was observed to have stopped. At 180 days' follow-up, the lesion did not relapse and appeared as a small, scarring alopecia (Figure 1d).

Discussion

Impaired wound healing is a major concern in veterinary clinical practice. In particular, skin wounds in horses often undergo "secondary" healing and can develop a number of severe complications. This includes EGT, which shares some features with human keloids and represents a potential animal model in comparative pathology (Theoret *et al.* 2013).

The predisposing factors and pathogenesis of impaired wound healing in horses are only partially known and have been recently reviewed by Theoret and colleagues (Theoret and Wilmink 2013). Some evidences suggest that the relative deficiency of inflammatory mediators (Wilmink *et al.* 1999), the protracted expression of profibrotic cytokines such as TGF- β 1 (Theoret *et al.* 2001, van den Boom *et al.* 2002), the poor contractile activity of myofibroblasts (Wilmink *et al.* 2001), the dysregulation of apoptosis, and the excessive accumulation of the extracellular matrix (Ladin *et al.* 1998, Luo *et al.* 2001, Lepault *et al.* 2005) might induce EGT. In addition, low oxygen tension, associated with newly formed microvessels, might further stimulate angiogenesis and encourage fibroproliferative disorders (Zhang *et al.* 2006, Celeste *et al.* 2011, Deschene *et al.* 2012). Noteworthy, bandages can promote the formation of EGT in different ways, including the reduction in oxygen tension in the wound tissue. By contrast, the use of bandages can also promote healing (Theoret *et al.* 2008). The decision to use bandages should therefore always be assessed and monitored carefully.

The intricate etio-pathogenesis of EGT goes hand-in-hand with its complicated therapeutic issues. Although a number of innovative therapies have been attempted over time, including pluripotent stem cells, healing may still remain elusive in some limb wounds (Theoret 2009, Theoret and Wilmink 2013). Alternative therapeutic tools that are not only effective, but safe, cheap, and easy to apply under field conditions are therefore highly sought after. Intralesional infiltration of formaldehyde meets these requirements.

Formaldehyde is a natural chemical compound, which has been widely used in many industrial fields (e.g. insulation materials) and is still the most common fixative agent in medical laboratories. The short-term health effects of formaldehyde are well-known and include severe irritation of mucous

membranes and skin, coughing, wheezing, and nausea. In addition, formaldehyde has been named as a known human carcinogen (U.S. Department of Health and Human Services 2011). Formaldehyde should therefore be carefully handled and exposure minimised. In this respect, the subcutaneous administration of very small amounts of formaldehyde represents a 'negligible risk' for the horse, as well as for the veterinary practitioner.

In vitro studies clearly indicate that the biological actions of formaldehyde are dose-dependent. At high concentrations, formaldehyde results in necrosis, enhanced apoptosis, and reduced mitotic activity; low concentrations, however,

stimulate cell proliferation and impair apoptotic activity. Such data point to the possible use of 'formaldehydogenic compounds' for the therapy of proliferative disorders (Szende and Tyihák 2010). To date, formaldehyde solution has been successfully used to treat different disease conditions in humans and animals, including progressive ethmoidal hematomas in horses (Schumacher *et al.* 1998, Conti *et al.* 2003). To the best of our knowledge, this is the first report describing the chemical ablation of proud flesh by local injection of formaldehyde. The positive outcome observed here suggests the need for further investigations in order to better evaluate its efficacy, as well as the incidence and the severity of any adverse reactions.

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