Topical Use of Polaprezinc in Horses' Wounds: a Clinical Study

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SUMMARY

Traumatic wounds account for a large portion of the caseload of an equine practitioner. Any mechanism that accelerates the speed of wound healing would have a significant impact in horses' world. Polaprezinc (PZ) is a complex of L-carnosine and zinc, it has been proven to be very effective for the repairing process of the mucosa in many human's conditions such as gastric ulcers, ulcerative colitis and hemorrhoids. We conducted a randomized prospective study with the aim to evaluate the wound healing activity of Polaprezinc (PZ) in equine patients, applying the molecule topically on the wounds. Seventeen horses suffering from traumatized wounds were included in this study. Horses were randomly divided in two groups: horses treated with PZ, 11 animals, and horses treated with povidone iodine gel, 6 animals. The majority of wounds included in the study were located on limbs. Wounds were assessed on the basis of healing time, that was defined as time interval from first dressing to patient's discharge. The Mann-Whitney U Test was used to compare the results of both groups. Fisher's exact test was used to evaluate whether the onset of exuberant granulation tissue could have statistical significance between the two groups. The mean healing time was consistently reduced in treated horses leading to a faster resumption of the animal's activity; the reduced number of cases led to a low significance of EGT onset in treated group, while clinically a predisposition was noted among treated cases. The local treatment with PZ on skin wounds proved to be effective and safe and it represents an excellent alternative to the methods traditionally used for the dressing of lesions undergoing second intention repair. In particular, the molecule seems to be suitable to aid the healing process of large wounds with consistent loss of tissue, where the production of an important amount of granulation tissue is necessary.

KEY WORDS

Polaprezinc; Zinc-L-carnosine; wounds; equine; topical.

INTRODUCTION

Wound management in horses is a critical aspect of equine care, and it can be both financially and time-consuming, particularly when injuries involve structures beneath the skin [1]. Wound healing in horses, as in other animals, is a complex process that can take several weeks, with large wounds posing significant challenges. This extended healing period often necessitates prolonged rest for the injured horse, which can affect their value, especially in performance or working horses. As a result, any method or treatment that accelerates wound healing in horses is highly sought after in the equine industry [2]. The development of unwanted complications like exuberant granulation tissue (EGT), dense fibrogranuloma, or scars can further complicate the healing process and potentially impact the horse's long-term health and performance. Managing these complications effectively is an essential part of equine wound care [3].

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The use of zinc (Zn) in equine wounds is well known. Topical application of zinc accelerates the healing of small and acute skin wounds, as well as stimulates re-epithelialization, reduces inflammation and bacterial growth [4].

Polaprezinc (PZ), molecular formula $C_9H_{13}N_4O_3Zn^-$, is a complex of L-carnosine and Zn. Thus, the synthesis of Polaprezinc can be decomposed into L-carnosine synthesis and L-carnosine-Zn salt complexation. -Dihydro-1,3-thiazol-2,4-dione is synthesized from alanine and methyl o-ethyl xanthate, then acylated with L-histidine to obtain L-carnosine and finally complexed with zinc acetate to obtain Polaprezinc. It has been reported that this compound inhibits the induction of TNF- as well as cellular signaling of TNF- [5]. In human medicine PZ is commonly used in the clinical field for gastric ulcers, but it has recently been used to treat other types of diseases or issues [6]. The oral supplementation of Polaprezinc is also used to treat taste disorders [7], gastrointestinal diseases [8], skin conditions [9], liver disorders [10] and oral mucositis resulting from radiotherapy and chemotherapy treatments [6,11]. In addition, the efficacy of topical use of PZ has also been demonstrated in human hemorrhoidal disease [12]. All the aforementioned organs contain a consistent amount of epithelial cells, therefore PZ proves to be effective for the maintenance, prevention

and treatment of the mucosal lining and other epithelial tissues [13].
A large number of compounds has been clinically studied for wound healing in horses, the aim of this study is to test the efficacy of PZ on equine wounds.

MATERIALS AND METHODS

A randomized prospective clinical trial was performed. The study was conducted in a two years period, 2020 and 2021, in a Veterinary Teaching Hospital in Italy. Seventeen injured horses were enrolled in the study and randomly divided in two groups: Treated group (horses treated with PZ) and Control group (horses treated with povidone iodine gel). All wounds were considered similar in severity regarding the extent of tissue damage, and required second intention healing. On the day of admission (day zero), tetanus prophylaxis and flunixin meglumine (1.1 mg/ kg, IV) were administered to the animals. The wounds were carefully cleaned with povidone iodine soap and, after clipping, the skin was carefully shaved to remove short hairs. All wounds were debrided, the necrotic tissue was removed, cleaned with saline solution, and then assigned randomly to a group.
A daily topical application of the PZ composite ointment was administered to Treated group (11 horses) until complete healing. A daily topical application of povidone-iodine ointment was administered until complete healing to horses included in Control group (6 horses). If hyper granulation tissue occurred at some point of the study, it was surgically removed under sedation using xylazine hydrochloride at a dose of 1.1 mg/kg, IV. Whenever possible, the wounds were bandaged in both groups. Antibiotics were administered to the patients according to bacteriological investigations and response to treatment. The healing time was defined as the complete healing of the wound. Animals were discharged when judged to be in general good health condition and when the wound was completely epithelialized and healed.

Statistical analysis

The study population was subjected to descriptive statistical analysis regarding sex and age. Wounds were assessed on the basis of time of healing, intended as the time interval from first dressing to patient's discharge. A Mann-Whitney U Test was used to compare the results of the Treated group with the Control group.
To evaluate whether the onset of EGT could have statistical significance between the two groups, a Fisher's exact test was performed.
A Kruskal-Wallis test was performed to examine the potential differences in healing time based on gender and age.

RESULTS

The animals were divided according to age as follows: foals, horses from birth up to 1 year, with a total of eight animals (six in the Treated group and two in the Control group); adults, from 2 to 15 years, four animals, (two in the Treated group and two in the Control group); geriatrics with > 15 years of age total of five animals (three in the Treated group and two in the Control group). The males included in the study were seven (four in the Treated group and three in the Control group), while there were a total of 10 females (seven in the Treated group and three in the Control group) (Table 1).

Table 1 - Sex and age of animals included in the clinical study.

	Treated	Control	Tot
Male	4 (36.4%)	3 (50%)	7 (41.2%)
FEMALE	7 (63.6%)	3 (50%)	10 (58.8%)
Foal (10 d-1 y)	6 (54.5%)	2 (33.3%)	8 (47.1%)
Adult (2-15 y)	2 (18.2%)	2 (33.3%)	4 (23.5%)
Geriatric (16-31 y)	3 (27.3%)	2 (33.3%)	5 (29.4%)

The majority of wounds included in the study were located on limbs (76.5%), especially in the distal area, both in Treated and Control groups. The remaining 23.5% of the population presented a wound located on the body (chest, flank or croup). In the Control group, two out of six subjects presented exuberant granulation tissue, which corresponds to a percentage of 33.3%; in the Treated group three out of 11 subjects presented the problem, equal to a percentage of 27.3% (Table 2 and Table 3).

Table 2 - The table represents the healing time, location and development of exuberant granulation tissue for wounds included in Treated group.

Patient	Healing Time	Wound Location	Egt
Treat 1	13 days	distal limb	YES
Treat 2	9 days	distal limb	no
Treat 3	26 days	distal limb	YES
Treat 4	14 days	body	no
Treat 5	17 days	distal limb	no
Treat 6	27 days	body	no
Treat 7	12 days	proximal limb	YES
Treat 8	16 days	distal limb	no
Treat 9	33 days	distal limb	no
Treat 10	11 days	body	no
Treat 11	48 days	proximal limb	no

Table 3 - The table represents the healing time, location and development of exuberant granulation tissue for wounds included in Control group.

Patient	Healing Time	Wound Location	Egt
Control 1	150 days	distal limb	no
Control 2	90 days	distal limb	YES
Control 3	150 days	body	no
Control 4	30 days	distal limb	no
Control 5	180 days	distal limb	YES
Control 6	Not healed	distal limb	no

The Mann-Whitney test resulted in a value of U = 2. The continuous value of U for a P value <0.05 is 13, therefore it is highly significant. Fisher's exact test is not significant with a P value = 0.1618 for P <0.05.
There are no significant differences in healing time based on gender and age.

DISCUSSION

Wounds commonly occur in horses and treatment can be frustrating for owners and veterinarians. Complications and delays in healing process are frequent and often require changes in the treatment plan. However, if damage to synovial structures or tendons is not present, the prognosis for affected patients is generally favorable. The goal of treatment is to return an equine patient to soundness, but also to minimize the patient's down time and the associated costs of extensive after care and bandaging [14].

This study described the usefulness of application of PZ on horses' wounds. This compound based on Zinc-L-Carnosine has shown a significant ability to protect mucous membranes and to promote tissue repair. Zinc is a mineral with many important biological roles such as: antimicrobial action against bacteria and fungi [15], antinflammatory function [16], induction of endothelial growth factor and angiogenesis [17] and regulation of collagen and keratin expression [18], which are mechanisms involved in wound healing. Zinc deficiency, regardless of the cause, can easily lead to growth defects, skin conditions and taste disorders [19]. Past studies demonstrated that the bioavailability of Zinc, and therefore its efficacy, is strictly related to which molecule it is linked to; for example zinc oxide proved to be more effective than zinc solfate on skin wounds of porcine models [20]. L-Carnosine (-alanil-l-istidine) is a dipeptide that can chelate metal ions; it plays a role in promoting skin's healing process and the correct functioning of immune system and it can protect from diabetes development and sight loss, thanks to its antioxidant and barrier action [21]. Specifically, it seems that insulin-like growth factor-I (IGF-I) could be one of the main factors involved in the healing effect of zinc on gastric ulcers [22]. Zinc, in association with L-Carnosine, creates a great aid for mucosal lesions repair, especially in the gastrointestinal tract [23]. Some studies, both in vitro and on animal models, showed the ability of PZ to reduce the production of the nuclear factor k-light-chain-enhancer of activated B cells (NF-kB) which is one of the main transcription factors regulating the expression of genes involved in inflammatory and immune response [5,24,25]. The association of Zinc and L-Carnosine shows to be more effective than using the two elements separately as L-Carnosine enhances Zinc's absorption thanks to its solubility and ability to release Zinc in a delayed and protracted way [26]. In the anorectal field this compound has proven to be very effective for the treatment of radiation proctitis and ulcerative colitis [27,28], in fact it induced a significant clinical and endoscopic remission [29]. Recently PZ has also been tested topically for the treatment of hemorrhoids in humans providing reduction of pain and symptoms such as bleeding and thrombosis [12].

Therefore, based on its characteristics and the good results obtained from the use of Zinc-L-Carnosine in tissue repair on the human mucosa, it is possible to hypothesize an effective therapeutic use in the context of other pathologies. In human patients PZ oral supplementation is administered to improve wound healing [15]; we decided to test it for topical use, since the gastrointestinal tract of horses widely differs from humans' one and therefore the amount of PZ absorbed could not be predicted.

The results obtained in the present study appear promising, although the examined population is little. Based on the data collected during our clinical study, a clear increase in healing speed is found in patients treated with PZ compared to the control group; no significant difference was revealed in healing time associated to gender and age. The mean healing time in this study was approximately one month in the Treated group and approximately four months in the Control group. This could be a great result but should be supported by a larger number of cases or by experimentally induced lesions permitting to collect data on more homogeneous wounds. There are no other clinical studies in literature regarding the use of PZ as a topical treatment in veterinary medicine; equally, there are no studies regarding the use of this molecule in equines, neither as oral supplementation nor as topical application. This makes the present clinical study particularly innovative, but, at the present day, the obtained results cannot be compared with those of other studies.

Although the small size of the sample makes the statistics slightly significant, it was clinically noticed that the presence of hyper granulation was a concrete possibility for patients undergoing PZ treatment. PZ induces a fast growth of granulation tissue that, if not adequately managed, may evolve in exuberant granulation tissue. Therefore, particular attention may be required when using the product in the distal portion of limbs which are the most frequent site of EGT onset, as well as the most frequent site of traumatic wounds in equine patients. In fact, the fibroblasts present in these high-tension areas produce greater quantities of TGF- 1 than those in other body regions, less prone to develop EGT [30]. Humans and horses appear to be the only mammals with a physiological tendency for excessive granulation tissue. In wounds with EGT, cell proliferation remains active, wound contraction is delayed, and excess tissue protruding from wound edges prevents epithelial migration and keratinocyte growth [31]. Several factors predispose to EGT formation, including species, site, bone sequestration, but the major predisposing factor is the establishment of a chronic local inflammatory state that is often sustained by bacterial populations present on the wound surface in the form of biofilm, which eludes the patient's immune defense and exhibits characteristics of resistance to antibiotics [32]. The EGT, in fact, microscopically differs from keloids in the presence of increased myofibroblasts, small vessels, and acute inflammatory cells [33]. Another factor that predispose to the occurrence of EGT is the use of bandages. Wounds in the distal portions of equine limbs are more likely to undergo EGT formation when bandaged than in wounds of the same type where bandaging is not applied [34]. The explanation may be that the pressure exerted by the bandage causes the oxygen gradient between the tissues and the wound surface to increase, resulting in a strong stimulus for angiogenesis; the resulting reduction of oxygen tension promotes fibroblast proliferation [35]. Wounds on the body seem to be much less prone to this issue, although it should be kept in mind that these are unlikely to be bandaged in everyday veterinary practice [36].

As there are many factors involved in EGT formation, it is difficult to be able to assert that polaprezinc treatment effectively increases the incidence of EGT occurrence. Further studies may clarify this aspect as well.

CONCLUSION

In conclusion, the local application of zinc l-carnosine on skin wounds in horses seems to be effective and safe, representing

an excellent alternative to the conventional treatments used for the dressing of lesions that must undergo repair by second intention. The molecule seems to be particularly suitable to aid the healing process of large wounds with consistent tissue loss, where the production of an important amount of granulation tissue is necessary. The reduction of healing time and consequently the faster resumption of the animal's activity and prompt discharge from hospital are factors of great importance that can lead to a decrease in management costs and economic loss for the owner.

Ethical Approval

Ethical review and approval were not required for the study in accordance with the local legislation and institutional requirements.

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Author Contributions

Conceptualization, R.R., A.S.; methodology, R.R., A.L.; validation, A.L.; formal analysis, M.V.R.; resources, M.V.R., G.F.; data curation, M.V.R.; investigation, M.V.R, A.L.; writing-original draft preparation, R.R.; writing-review and editing, R.R., M.V.R., G.F.; visualization, A.L., G.F.; supervision, A.S.; project administration, R.R., A.S.

Conflicts of Interest Statement

The authors declare no conflict of interest.

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