

ENHANCED WOUND HEALING USING COLLAGENASE IN GUINEA PIG

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SUMMARY :

Purpose : The effect of *Clostridium histolyticum* collagenase on wound healing was investigated and compared to commonly used 0.9% sodium chloride, 10% povidon-iodine and chlorhexidine acetate gauze. **Methods :** Four 1cm² full-thickness skin defects created surgically in guinea pigs were treated daily with topical 10% povidon-iodine, chlorhexidine acetate gauze, collagenase, or 0.9% sodium chloride as for the control. Follow-up was made with standard photo-documentation until complete wound healing, and wound healing periods were figured out by computerized digital analysis. On the 9th postoperative day, wounds from 10 animals were scored histologically according to their epithelization, cell content, granulation tissue, collagen deposition, and vascularity. **Results :** The earliest wound healing was seen in the collagenase - applied wounds (16.2±0.3 days) which was statistically significant ($p<0.05$). However, histological scores failed to show any considerable difference between the groups ($p>0.05$) for all comparisons. **Conclusion :** Daily topical collagenase application to full thickness skin defects in guinea pig accelerated wound healing, but the mechanism that underlies this enhanced wound healing process still needs further investigations.

Key Words: Wound Healing, Enzymatic Debridement, Collagenase

INTRODUCTION

Including the local use of "honey" in ancient Egypt, different kinds of topically applied agents have been tried to enhance wound healing. Recently, therapeutics causing enzymatic debridement have found their places in this wide spectrum. Proteolytic enzymes are a family of proteins that serve to degrade necrotic debris. Collagenase, which belongs to this group, is the only enzyme known to cleave native collagens at physiological pH and temperature (1). Therefore, collagenase has been assumed to play an important role in wound healing; and especially in wound

contraction (2). Besides, endogen collagenases derived from neutrophils and fibroblasts, there are some commercially available exogen collagenases, such as the product of *Clostridium histolyticum* (3). In the present study, the effect of *Clostridium histolyticum* collagenase on wound healing period was tested in comparison to commonly used 0.9% sodium chloride, 10% povidon-iodine, or chlorhexidine acetate gauze.

MATERIALS AND METHODS

Animals and Surgical Technique

Twenty male guinea pigs weighing about 500 g

(500±10) were used. After intramuscular ketamine (35 mg/kg) and xylazine (2.5 mg/kg), four 1cm² full-thickness skin defects including the panniculus carnosus were created on the back of the animals, two on each side and equidistant from the midline. The nearest edges of the wounds were separated by a margin of 3 cm of unwounded skin.

Wound Dressings

After wounding, 10% povidon-iodine, chlorhexidine acetate gauze, collagenase (Novuxol, Knoll, Nordmark Arzneimittel GmbH D-25430 Uetersen/Germany), or 0.9% sodium chloride as for the control were applied on different wounds of the same animal, and then the wounds were covered with sterile gauze and fixed with circular adhesive bands. The animals had free access to water and standard laboratory pellet and were housed individually to prevent them from tampering with the others' wounds. The dressings were changed every day until complete wound healing.

Photodocumentation and computerized digital analysis

Starting from immediately after surgery, each wound was photographed at a fixed focal distance every other day. A millimeter ruler was included into the photographs for calibration of the digital analysis system. Photographs were transferred to the computer with a 600 dpi, 256 colour scanner and after revealing the color codes, the wound areas were described by four colours. This enabled to follow the wound areas and to figure out the healing time.

Histological Analysis

On the 9th postoperative day, 10 animals were sacrificed, whereas the remaining 10 animals were continued on their treatment protocol in order to determine the wound healing time. The wounds were excised down to the muscle layer, including a margin of 0.5 cm of unwounded skin. Specimens were fixed in 10 % formalin solution, embedded in paraffin, stained with hematoxylin-eosin and Mason's trichrome dyes. After light-microscopy examination, the wounds were given a histological score ranging from 1 to 15 with 1 corresponding to no healing and 15 corresponding to a scar with organized collagen fibers. The scoring scale was based on epithelization, cell content, granulation tissue, collagen deposition, vascularity, and was

used by previous investigators (4-6).

Statistical Analysis

The values of the histological scores and wound healing times were expressed as the mean±standard error of the mean. The data were compared using one-way ANOVA, and Student Newman Keul multiple comparison was used for specific differences. An alpha level of 0.05 was used to figure out significance.

RESULTS

Macroscopically, the wounds treated with 0.9 % sodium chloride and 10 % povidon-iodine appeared to have marked fibrinous exudate and crust formation which caused adherence to the gauze. Therefore, they required greater tearing force for removal of the dressings. On the other hand, collagenase and to a lesser degree, chlorhexidine acetate gauze - applied wounds showed less exudate. Moreover, collagenase-treated ones had almost no crust with few necrotic debris.

Histological Scores

During the histological examination, all wounds showed similar healing patterns. In general, the wounds had partial epithelization, inflammatory cell predominance with few fibroblasts, and a thin layer of granulation. Also, few capillaries and sparse collagen deposition were noticeable especially at wound centers. There were no significant differences between histological

Wound treated with	Histologic score
Collagenase	4.6±1.09
Chlorhexidine acetate gauze	3.9±0.16
10% Povidon-iodine	4.6±0.54
0.9% Sodium Chloride	4.7±0.39

* No statistical difference between groups, $p>0.05$ for all comparisons

Table - 1 : Histological scores (Mean ±SEM).

scores of the wounds ($p>0.05$) (Table 1).

Wound Healing Time

Wound healing was accepted as complete when the wound area was totally covered by epithelization and contraction. All wounds healed between the 14th and 20th days (Table 2). The

Wound treated with	Healing time (day)
Collagenase	16.2±0.36*
Clorhexidine acetate gauze	17.0±0.45
10% Povidon-iodine	17.4±0.43
0.9% Sodium Chloride	18.2±0.63

* The difference was significant, $p < 0.05$

Table - 2 : Wound healing times (Mean ±SEM).

wound healing time with collagenase was reduced significantly when compared with other three dressings ($p < 0.05$). However, there was no difference between 0.9% sodium chloride, 10% povidon-iodine, and clorhexidine acetate gauze-treated wounds ($p > 0.05$).

DISCUSSION

Independent of the therapeutic agent used, the comparability of wounds plays a great role in the assessment of the wound healing process (7). In addition, for intraindividually performed comparisons, the wounds must be separated by a sufficient skin bridge which will avoid not only the interference of different therapeutics, but also the effects of adjacent wound contraction. Therefore, a model with four wounds on the same animal separated by a 3 cm of unwounded skin was used in this study. To standardize the histological assessments, a histological scoring system was applied (5).

The results of this study suggest that daily applied collagenase enhanced wound healing when compared to the other three wounds on the same animal. In addition, the application of antiseptics (10 % povidon-iodine or clorhexidine acetate) did not adversely alter the wound healing process. In this study, either povidone-iodine or clorhexidine acetate gauze did not affect the rate of wound healing when compared with sodium chloride treated controls. Although current recommendation in the literature is to avoid using antiseptics in open wounds, Niedner and Schopf found no significant effect on the formation of granulation tissue in full-thickness wounds of guinea pigs after treatment with povidone-iodine (8). Also, it was shown that povidone-iodine did not affect the rate of epithelization in partial-thickness wounds in pigs, as compared with saline solution-applied wounds (9). On the other hand, Kjolseth et al. mentioned the impairment of epithelialization in addition to increased vascularization in wounds

treated with povidone-iodine (10).

Although histological analysis showed no significant difference, collagenase-treated wounds healed earlier than the povidone-iodine, clorhexidine acetate gauze or sodium chloride-treated ones. The dissolution of fibrin, necrotic tissue and debris occurs naturally in wounds through the action of local enzymes causing autolytic debridement. This action is essential for proper wound healing process. Collagenases released in the wound both aid in necrotic tissue digestion and are associated with angiogenesis (11) Apart from human-derived collagenases, there are commercially available ones, such as collagenases from *Clostridium histolyticum*. The debridement of collagenase is quite specific as it recognizes amino acid sequences with great specificity, cleaves glycine and is not active on viable tissues (12). This results in protection of newly formed epithelialization that is quite susceptible to trauma which can be caused by other means of debridement. Moreover, enzymatic debridement decreases the need for surgical interventions to remove the slough and debris. It has been suggested that the decreased wound contraction is, at least, partly due to reduced collagenase level (13). Collagen fragments resulting from collagenase cleavage have been shown to act chemotactically on fibroblasts and macrophages, and this may contribute to diminished contraction in the presence of insufficient collagenase.

In conclusion, wound healing is improved by using collagenase in full-thickness wounds in guinea pigs. Specific enzymatic debridement increased neovascularization, and increased chemotaxis for fibroblasts and macrophages, and effective contraction are the attributable causes for this finding. However, this study failed to reveal any histological evidence or differences. Therefore, further ultrastructural investigations are needed to elucidate the exact mechanisms of the effect of exogen collagenases on wound healing process.

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