Collagenase Cream in Non-Stitched Skin Wounds after Punch Biopsy

ORIGINAL

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Abstract

Background: The use of debriding drugs is not common in a situation of acute wound healing, despite its widespread use in chronic wound healing, with satisfactory results. Collagenase is one of the most used debriding drugs, and its application after a non-stitched skin wound biopsy could lead to a better wound healing outcome and less complication.

Methods and Findings: Fifteen Wistar rats were used in this study; four excisional wounds were performed on the dorsum of each animal. Once in a day, collagenase cream was applied in the cranial wounds, while the caudal wounds were washed with isotonic saline. After 7, 14 and 21 postoperative days, 5 animals were randomly chosen for macroscopic and microscopic wound analysis. At the 7th postoperative day: collagenase cream induced greater monocytes invasion, fibroblast invasion, collagen deposit, angiogenesis and reepithelialization. 14th postoperative day: collagenase cream induced greater neutrophils invasion, fibroblasts invasion, collagen deposit, reepithelialization and higher proportion of type I collagen fibers. 21st postoperative day: collagenase cream led to greater angiogenesis and reepithelialization.

Conclusion: Collagenase cream led to a better wound healing outcome than simply saline rinse, induced earlier angiogenesis and inflammatory step; greater collagen deposit and reepithelialization.

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Introduction

Skin punch biopsy is usually indicated to diagnose a disease. [1] Inflammatory skin diseases and neoplastic skin lesions, that often are hard to diagnose through the clinical aspects, are the most common reasons for the punch biopsy procedure. Moreover, the subsequent anatomopathological study is very often useful to guide the follow up of the disease [1, 2].

The *Food and Drug Administration* (FDA) recommendations establish that skin biopsy should be done if there is any clinical need, and should work as a gold standard tool to exclude or confirm neoplastic, immunologic or infectious skin lesions [3].

The medical doctor that performs skin biopsies procedure should always have in mind its benefits, but also and not less important the harms that it might cause, such as aesthetic and functional deficits [4, 5].

3 to 4 mm punches are commonly used, since that size is adequate for most of the situations, showing a good imbalance for the tissue harvesting versus the aesthetic and functional harm. However, the medical doctor should have in mind that in some situations, small skin biopsies might cause difficult to the pathologist to establish a diagnose [6-9].

Following a punch biopsy, skin wounds are commonly stitched, such technique leads to a better aesthetic and functional outcome. However, in places where the stitches are not feasible for any reason, such as technically difficult areas to perform stitches, or in patients that do not agree with that, skin wounds are usually led to heal spontaneously [10].

Skin wound that involve at rate of 1.5 mm per week, usually heal through the secondary intention process without complications. [11] The wound care is usually restricted to daily cleaning, antiseptics, bandage replacement and sometimes antibiotic ointment [8, 9, 12].

The use of debriding drugs is not common in a situation of acute wound healing, despite its widespread use in chronic wound healing, with satisfactory results. Collagenase is one of the most used debriding drugs, and its application after a non-stitched skin wound biopsy could lead to a better wound healing outcome and less complication [12, 13].

Methods

Fifteen (8-10 weeks) male Wistar rats, weighting 250-300g were used in this study. The animals were kept in a vivarium of the Experimental Surgery Laboratory at the Para State University (LCE-UEPA) with a controlled temperature, light, humidity, and noise; water and food was provided ad libitum. The research followed the rules of Brazilian National Law for Animal Care (Law: 11.794/08). The project was previously approved by the Animal use and care committee at the Para State University (protocol 01/13).

Experimental Protocol

At day 0, the fifteen Wistar rats were anesthetized (ketamine hydrochloride and xylazine hydrochloride 60 mg/kg and 6 mg/kg, respectively, i.p.). Dorsal region was shaved using an electric shaver and then the antiseptics of region was done.

Four skin biopsies were performed on the dorsum of all animals, 2 each side of median line (2 cm in distance) and being divided in cranial and caudal wounds (4 cm in distance), using a 4 mm metallic punch. Once in a day, collagenase cream 0.6 UI/g was applied in the cranial wounds, while the caudal wounds were washed with isotonic saline solution (NaCl 0.9%).

Substances were applied using a sterile swab, and physical restraint was adopted. During the studied period, animals remained in individual cages and isolated for their bedding through a metallic net to avoid wound infection.

After 7, 14 and 21 postoperative days, five animals were randomly chosen for macroscopic and microscopic wound analysis.

Analyzed Parameters

Wound area

After euthanasia, rats were positioned and pictures of wounds were taken to measure wound area using the software Image J®.

Histological analysis

After euthanasia, wounds were harvested, fixed in 10% formaldehyde, embedded in paraffin, and stained using Hematoxyline-Eosine (HE) and PicroSirius-red (PS). In HE stained tissues; multiple sections were analyzed with regard to the presence of neutrophils, monocytes, angiogenesis, fibroblasts, collagen deposit and keratinocytes. These assessments were evaluated by a pathologist into: absent (score 0), mild (score 1), moderate (score 2), intense (score 3) [14]. In PS stained tissues, proportion of type I and III collagen fibers was measured.

Statistics

Analysis of variance (ANOVA), followed by Tukey post-hoc test correction, was performed for the area analysis and proportion of collagen fibers. Kruskal-Wallis test was used for the histological parameters. Statistical significance was assumed at $p < 0.05$.

Results

Wound area

There was no difference regarding the wound area in the analyzed periods. All skin wounds were healed on the 21st postoperative day

Histological analysis Hematoxyline-Eosine

The results in **table 1, 2** and **3**.

Histological analysis PicrosiriusRed staining

The results in **Table 4**.

There was no difference regarding the proportion of type I and type III collagen fibers on the other analyzed periods. (**Figure 1**)

Table 1. Histological findings in Hematoxyline-Eosin staining on the 7th postoperative day.

	Neutrophil	Monocyte *	Angiogenesis *	Fibroblasts *	Collagen *	Reepithelialization *
Collagenase	1.8 ± 0.421	2.6 ± 0.516	2.8 ± 0.421	1.9 ± 0.316	1.9 ± 0.316	1.4 ± 0.516
Saline	1.4 ± 0.516	1.8 ± 0.421	1.6 ± 0.699	1 ± 0.666	1 ± 0.666	0 ± 0.00

* $p < 0.05$. Kruskal Wallis test. Mean and standard deviation

Table 2. Histological findings in Hematoxyline-Eosin staining on the 14th postoperative day.

	Neutrophil	Monocyte *	Angiogenesis *	Fibroblasts *	Collagen *	Reepithelialization *
Collagenase	0.6 ± 0.843	1.7 ± 0.483	1.4 ± 0.516	2.2 ± 0.421	2.2 ± 0.421	2.6 ± 0.516
Saline	1.7 ± 0.462	1.8 ± 0.353	1.7 ± 0.462	1.5 ± 0.534	1.5 ± 0.534	0.125 ± 0.333

* $p < 0.05$. Kruskal Wallis test. Mean and standard deviation

Table 3. Histological findings in Hematoxyline-Eosin staining on the 21st postoperative day.

	Neutrophil	Monocyte *	Angiogenesis *	Fibroblasts *	Collagen *	Reepithelialization *
Collagenase	0 ± 0.000	1.2 ± 0.421	1 ± 0.000	2.9 ± 0.316	2.9 ± 0.316	3 ± 0.0
Saline	0.4 ± 0.516	1.4 ± 0.516	2.6 ± 0.516	2.4 ± 0.516	2.4 ± 0.516	1.8 ± 0.4

* $p < 0.05$. Kruskal Wallis test. Mean and standard deviation

Table 4. Type I and type III collagen fibers on the 14th postoperative day.

Collagen	Collagenase *	Saline *
Type I fiber	64.333 ± 11.079	36.2 ± 18.972
Type III fiber	34.666 ± 11.079	62.8 ± 18.972
* p< 0,05 (ANOVA)		

Figure 1: Wound treated with collagenase cream.

Figure shows deposit of collagen fibers type I and type III on the 14th postoperative day. Red: Type I fibers. Green/blue: type III fiber.

Discussion

Wound contraction is the main mechanism of wound closure in rodents [15]. Contraction occurs when miofibroblastos connect to the type I collagen fibers, generating a concentric force that ultimately leads to wound closure [16]. No difference in wound closure was noted when collagenase cream or saline was used. This fact demonstrates that there was no impairment of fibroblastic function.

Skin wounds that were treated with collagenase cream had less neutrophil invasion on the 14th and 21st postoperative days. Since the neutrophils are the main responsible for the wound debridement, it is reasonable to infer that the debriding effect of collagenase cream diminished the requirement of neutrophils. This fact can result in less oxidative burst and local inflammatory reaction [17].

Collagenase cream induced to greater monocytes invasion at the 7th postoperative day. However, on the subsequent analyzed periods monocytes invasion was similar. Monocytes are the responsible for the sequel of healing process; they attract fibroblasts and stimulate the angiogenesis [17]. Greater presence of monocytes after 7 days, elicit that collagenase cream induced to a more rapid wound healing sequence.

The angiogenesis is a crucial step, since the newly formed vessels promote the washout of toxins and the income of necessary nutrients for the tissue repair [10]. Collagenase cream induced to an intense angiogenesis on the 7th postoperative day. This fact strengthens that those wounds had a quicker wound healing process. Saline also showed intense angiogenesis, however, this was observed only at the 21st postoperative day.

Fibroblastic cells are the main responsible for the formation of the consolidated scar and are directly related to the collagen deposition [18]. In this setting, collagenase cream induced to greater fibroblastic cell invasion at the 7th and 14th postoperative days. Collagen deposition followed the same pattern observed for the fibroblastic cells.

Such finding demonstrates that collagenase cream leads to a quicker wound healing, where the early wound is richer in collagen deposit, which may lead to more resistant wounds. On the other hand, collagenase cream does not enhance the total amount of collagen in the consolidated scar. The same amount of collagen was observed in wound treated with saline or collagenase at the 21st postoperative day.

Initially the wound is rich in type III collagen fibers, which is constantly being substituted for type I collagen fibers during the wound healing sequence. Type I fibers are thicker and more efficient to cause wound contraction [18]. Collagenase cream showed greater proportion of type I collagen fibers at the 7th day and no difference on the other analyzed periods; however, such finding was not translated to a smaller wound area.

Reepithelialization is the final and the most important step in the wound healing process of humans [19]. Collagenase cream induced to a more intense reepithelialization in all analyzed periods. It is remarkable that at the 7th postoperative day, no signs of reepithelialization were detected in the wound treated with saline, and that process was sorely occurring when collagenase cream was used.

Early reepithelialization is very important to the skin wound healing process. It creates a barrier between the skin and the environment, reduces the dryness of the wound and the probability of wound infection, diminishes the pain and discomfort and accelerate the wound healing.

Conclusion

Daily topical application of collagenase cream led to a better wound healing process than simply washing with saline. It induced to a shorter inflammatory step, earlier angiogenesis, collagen deposit and reepithelialization.

Conflict of interest

None.

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