



## ARAŞTIRMA

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### Comperative Evaluation of Collagenase and Silver Sulfadiazine on Burned Wound Healing in Rats

This experiment was conducted to compare collagenase the effects of ointment and silver sulfadiazine (SSD) on burned wounds for healing in rats. Sixty male Wistar albino rats were divided into three equal groups. A burned model was constituted on the back of all rats. The burned areas in the first, second and third groups were covered daily with collagenase, SSD skin cream and cold cream (control), respectively. Ten and 21 days later the rats were anaesthetized and the burned skin tissue samples of ten cases of each group were collected for histopathological examinations. In conclusion, application of collagenase ointment is significantly effective in healing of burned skin wounds in rat model ( $P < 0.0001$ ).

**Keywords:** Collagenase, silver sulfadiazine, burn, rat.

#### Ratlarda Yanık Yaralarının İyileşmesi Üzerine Kollagenaz ve Silver Sulfadiazine'in Etkilerinin Karşılaştırılması

Bu çalışma ratlarda yanık yaralarının iyileşmesinde kollagenaz ve silver sulfadiazine'in (SSD) karşılaştırmasını yapmak için gerçekleştirildi. Altmış Wistar albino rat üç eşit gruba bölündü. Bütün ratların sırtlarında yanık modeli oluşturuldu. Yanık bölgeler günlük olarak, birinci, ikinci ve üçüncü gruplarda sırasıyla kollagenaz, SSD deri kremi ve cold cream (kontrol) ile kaplandılar. On ve 21 gün sonra histopatolojik muayeneler için ratlar anestezi altına alınarak her gruptan 10 hayvandan yanık uygulanan deri örnekleri alındı. Sonuç olarak, kollagenaz merhem uygulamasının rat modelinde deri yanıklarının iyileşmesi üzerinde anlamlı derecede etkili olduğu belirlenmiştir ( $P < 0.0001$ ).

**Anahtar kelimeler:** Kollagenaz, silver sulfadiazine, yanık, rat.

#### Introduction

One of the important characteristics of burns is the formation of an eschar, resulting from burned and traumatized tissue (1). Necrotic tissue covering the burned wound has a negative influence on wound healing and provides a medium for the growth of microorganisms and is therefore a source of infection, contamination and sepsis (2). Wounds do not heal as long as dead tissue remained on its via natural collagen fibers (3). The first aim in the initial treatment of burned wounds is the complete removal of necrotic tissue and other contaminants (4-8). An ideal surgical technique should remove all necrotic tissue in a single procedure leaving behind viable undamaged tissue (7,9).

Enzymatic debridement of necrotic tissue has gained interest in the recent years providing an efficient alternative treatment to replace the standard of care surgical debridement. (5, 10-13).

Enzymatic agents are also relatively safe and effective (12, 14). Preparations including enzymes such as collagenases, fibrinolysin, deoxyribonuclease, or streptokinase are seen as alternatives to surgical debridement (2, 3). For example, Mekkes et al. (6), reported that krill enzyme preparation, applied twice daily in a concentration of 3.0 or more casein units per ml, appears to be an effective product for necrotic wound debridement in the pig model, its debriding properties.

One of the exogeneous enzymes used for burn wound debridement is collagenase clostridipeptidase A (CCA), derived from *Clostridium histolyticum* (2, 3). Collagenase has a high affinity for all major collagen types and is useful in debridement, increase of granulation tissue formation, and prevention of abnormal scar formation (3, 15).

Collagenase is best used for digesting collagen and elastin, but it does not decrease fibrin. This agent should be applied only to the nonviable tissue within the wound and not to the surrounding normal tissue (12, 16).

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Silver sulfadiazine (SSD) is the topical agent of choice in severe burns and is used almost universally today in preference to compounds such as silver nitrate and mafenide acetate. SSD cream, while being effective, causes some systemic complications which include neutropenia, erythema sultiforme, crystalluria and methaemoglobinemia (17-21).

The purpose of this study was to compare healing rates of wounds treated with collagenase dressing and with silver sulfadiazine clinically and histologically in rat model to produce likely an alternative for the treatment of thermal wound in domestic animals, i.e. dog, cat, cattle and horse

## Materials and Methods

**Animals:** This study was carried out in sixty male Wistar albino rats weighing between 250 and 300 g. Animals were housed at 21°C with a day/night cycle of 12 h. During the study these animals were fed *ad libitum* standard rodent feed.

**Anaesthesia:** The rats were anaesthetized with single intramuscular injection of 6 mg/kg xylazine hydrochloride (Rompun, Bayer, 23.32 mg/ml) and 85 mg/kg ketamine hydrochlorure (Ketalar, Parke-Davis, 50 mg/ml).

**Test drugs:** Collagenase ointment (Novuxol, Abbott, Collagenase SF, clostridiopeptidase A, 1.2 IU/1g), silver sulfadiazine (Silverdin, Deva, Silver sulfadiazine, 10mg/g) skin cream and cold cream (Botafarma, 12.5 % spermaceti + 12 % white wax + 56 % liquid paraffin + 0.5 % borate of soda + 19 % distilled water) were used in this study.

**Thermal injury:** The backs of the rats were shaved and prepared with 10 % antiseptic povidone-iodine solution (Kim-Pa, Poviiodeks, % 10 povidone-iodine) and burns of 1 cm in diameter were established.

Skin burns were made as described by Hoşnüter et al. (22). Animals were subjected to a 1 cm diameter surface area of full-thickness second-degree skin burns by brass probe. The brass probe, was immersed in boiling (100 °C) water until thermal equilibrium was achieved, then it was placed without pressure for 20 s on the back of the rats. All animals were resuscitated immediately with lactated Ringer's solution (2 ml/100 g body weight) intraperitoneally. Following the burning, each animal was placed in a separate cage.

**Experimental protocol:** The animals were divided randomly into three equal groups. Immediately after burn, the burned areas were cleaned by sterile saline solution, and these areas in the first ( $n = 20$ ), second ( $n = 20$ ) and third groups ( $n = 20$ ) were covered with 2 mm thickness collagenase ointment, SSD skin cream and cold cream (control) respectively. These applications were repeated every day. Any topical antibacterial agent was not combined with these applications. The wounds in all groups were observed clinically every day.

**Histopathological examination:** Ten and 21 days following injury, ten rats in each group were

anaesthetized and the burned skin tissue samples were collected for histopathological examinations. This tissue samples were fixed in 10% neutral-buffered formalin solution and embedded in paraffin wax, and were cut into 5 µm-thick sections and stained with hematoxylin and eosin (H&E) and Mason's trichrome, and examined by light microscopy.

**Statistical analyses:** The thickness of granulation tissue at the center of each wound was examined and recorded. Statistically, all data are expressed in millimeters as mean  $\pm$  standard error of the mean. The differences between 10 and 21<sup>th</sup> days were compared using the Mann-Whitney U test. The differences between groups were compared by analysis of Kruskal-Wallis and Mann-Whitney test. *P* values of less than 0.05 were considered as statistically significant. These analyses were accomplished by using statistical analysis system configured for computer (SPSS, Release 10.0, SPSS. Inc).

## Results

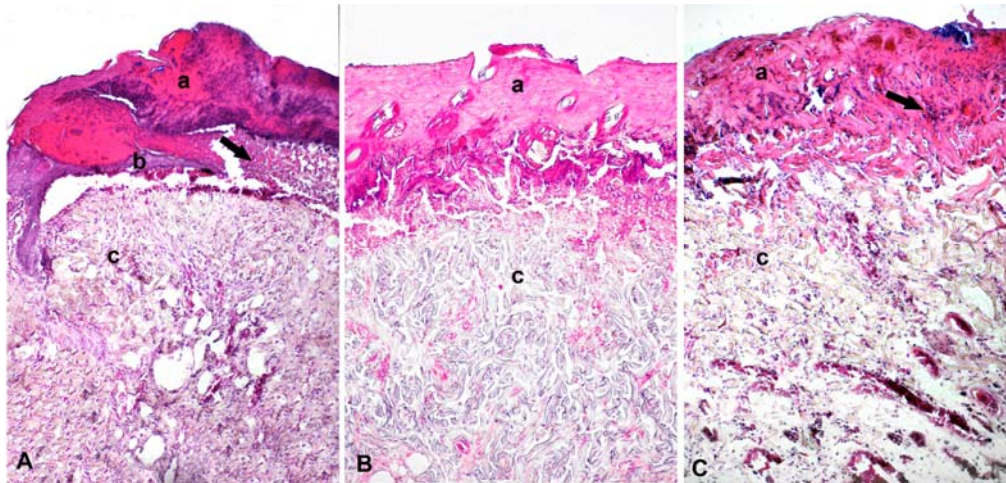
No mortality was seen in the animals during the study. Burn wounds treated with SSD appeared to display a greater degree of inflammation as notable by the three clinical signs of the inflammatory process such as heat, redness, and swelling which appeared to be lessened in wounds treated with collagenase.

The results clearly indicated that early removal of the injured tissues with collagenase ointment accelerated the rate of burn wound healing compared the other groups. Treatment with collagenase resulted in significantly shorter time to achieve a clean wound bed than SSD and control groups. Collagenase debridement had promoting effect compared to SSD treated and control group.

Histopatologic examinations, on the 10<sup>th</sup> day showed that burn healing was better in collagenase than the other groups. Regenerative and reparative attempts were started in epidermal layer. No epithelium was present in the SSD and control groups, however inflammatory cell infiltration was observed beneath the scab in the all groups. Also, few capillaries and sparse collagen deposition were noticable especially at wound centers. The capillaries were hyperemic in the dermis with no hair follicularis, sebace and sweat glands in the all groups (Figure 1).

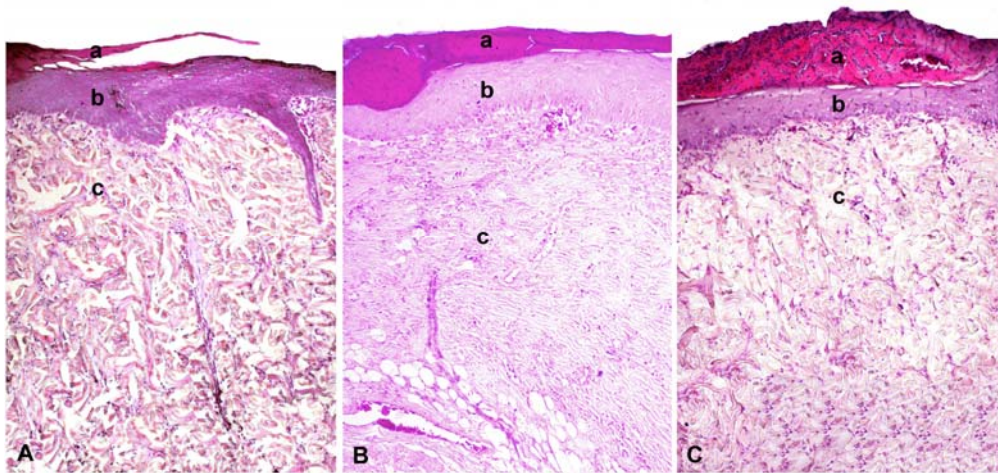
On the 21<sup>th</sup> day, development of the epidermis was observed in all groups. But, this development was better in collagenase group. While wound healing was completed in this group it remained incomplete in the SSD and control groups. Also, in the SSD and control groups the scab is clearly vissible on the 21<sup>th</sup> day (Figure 2).

Wound healing was significantly different in each groups at 10 and 21<sup>th</sup> days ( $p < 0.0001$ ). Thickness of granulation tissue was significantly different between each groups ( $p < 0.0001$ ). The mean values of thickness of granulation tissue in the center of the wounds for collagenase cream, SSD and control groups are shown in Table 1.



**Figure 1.** Microscopic appearance of burned skin on the 10<sup>th</sup> day.

**A.** Collagenase group a) Scab b) Epidermis c) Dermis, and infiltration of mononuclear cells (arrow)  
**B.** SSD group a) Scab c) Dermis  
**C.** Control group a) Scab c) Dermis, and infiltration of mononuclear cells (arrow), (H E x 50).



**Figure 2.** Microscopic appearance of burned skin on the 21<sup>th</sup> day.

**A.** Collagenase group a) Scab b) Epidermis c) Dermis  
**B.** SSD group a) Scab b) Epidermis c) Dermis  
**C.** Control group a) Scab b) Epidermis c) Dermis, (H E x 50).

**Table 1.** Thickness (mm) of granulation tissue in the center of the wound.

Groups	Days		P
	10(n=10)	21(n=10)	
Collagenase	1.468 ± 0.01 <sup>a</sup>	2.281 ± 0.03 <sup>ab</sup>	0.0001
SSD	1.014 ± 0.03 <sup>b</sup>	1.523 ± 0.02 <sup>bb</sup>	0.0001
Control	0.568 ± 0.03 <sup>c</sup>	1.075 ± 0.03 <sup>cb</sup>	0.0001
P	0.0001	0.0001	

<sup>abc</sup>Values in the same column with different lower cases are significantly different (P<0.05).

**Discussion**

Dead tissue covering the burn wound also serves as a medium for bacterial growth, reduces the host's resistance to infection, and delays the formation of granulation tissue and the re-epithelialization. Therefore, wound debridement is an imperative to the healing of burns. Surgical tangential excision of dead tissue, the repeated application of moistened dressings, hydrocolloid or semioclusive dressings, dextranomers, intracavity gels, hydrosurgery, or various enzyme

preparations can be used of burn debridement (4, 6, 7, 23).

While effective, surgical tangential excision has several major disadvantages. This method is non-selective and it is technically difficult to control the amount of tissue to be removed, often converting a partial thickness burn into a full thickness defect. In addition, tangential excision may be associated with pain and bleeding (1, 5, 6, 12).

Different test animals such as pig (6, 24), Guinea pig (5, 13) and rat (14, 19, 22) were used for various studies. We used rat model in the present study similar to some investigators (14, 19, 22). The model used here is simple and repeatable. The burned wound healing model provides an *in vivo* approach for studying the healing of burned wounds in domestic animals.

Topical agents with benefits only as antimicrobials include silver nitrate, sulfamylon and a combination of a sulfonamide and SSD. Sulfamylon has wide spectrum activity, but it is easily absorbed systemically and can result in toxic complications. SSD has become the standard topical treatment for burn wounds (17). Thus, we have chosen SSD in our study.

A wide range of enzymatic debridement have been used in the treatment of burn injury both clinically and experimentally. Among them; trypsin (5), bromelain (1, 5), collagenase (2, 3, 13), krill enzymes (6), and papain (11, 14) are advocated. These agents offer the advantage of easy application with additionally being relatively safe and effective (12).

Collagenase is an essential component in the wound healing process. Collagenase is best used for digesting collagen and elastin, but it does not degrade fibrin. This enzyme is responsible for the breakdown of collagen and its presence is vital to maintain the dynamic equilibrium between the breakdown of old collagen and new

collagen formation in the scar-remodeling phase (16). This agent should be applied only to the nonviable tissue within the wound and not to the surrounding normal skin or tissue (12). Therefore, in this study, collagenase ointment was applied only on the burned tissue.

Detergents, soaps, antiseptic solutions, and heavy metal ions are decreased efficacy of collagenase (8). When using enzymatic debridement, the main problem is infection as is reported by Klasen (15) and Özcan et al. (2). In the present study topical antiseptic solutions and antimicrobial agent were not used with collagenase, and no infections were observed.

Alaşam et al. (10) treated chronic wounds located between udder and hind extremities using collagenase in cows. They reported that collagenase had a beneficial effects on the healing of these wounds. The results of current study showed that collagenase ointment plays a role in healing of burned skin wounds possible via its enzymatic debridement effect.

The present study showed that early nonsurgical removal of injured tissues is an effective treatment for thermal burns. Collagenase debridement accelerated burn wound healing as determined by the rate of lesion area closure and histopathological evidence. Histopathological comparison of the three groups indicated that healing of burned skin wounds was best in the collagenase group ( $p < 0.0001$ ).

Consequently, the data and observations collected in this study indicated that collagenase could be applied to treatment of thermal burns and it may be viable and desirable alternative to the use of SSD. Acknowledging the limitations of rat model of this experimental animal study, we believe that further studies are warranted to investigate whether collagenase is useful in domestic animals with burn wounds.

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