Wound Debridement by Continuous Streaming of Proteolytic Enzyme Solutions: Effects on Experimental Chronic Wound Model in Porcine

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Abstract: Wound debridement for the removal of necrotic tissue is a crucial step in wound management. Enzymatic wound debridement is one example of a method currently used that removes necrotic tissue with proteases and offers selectivity without affecting healthy adjacent tissue. Proteolytic enzymes for wound debridement are commercially available as ointments. The authors previously proposed and demonstrated feasibility on small lab animals—an alternative mode of delivery of proteolytic enzymes for wound debridement with continuous streaming of protease solutions. The present study describes the impact of streaming of papain solutions, fortified by the incorporation of hypertonic agents, onto an experimental larger chronic wound model in pigs. Debridement of approximately half of the necrotic tissue mass was achieved within 6 to 11 h of streaming of papain solutions onto these experimental wounds. No adverse effects or noticeable morphological changes to the wound surface or its immediate surroundings were noted, indicating enzyme selectivity and preference for attacking necrotic tissue. The mechanism of enzymatic attack on the necrotic tissue is also discussed. In the control group, streaming of the basic solution formula (devoid of papain) was performed—no debridement of necrotic tissue was noticed in this case. The results indicate that the streaming delivery mode for enzymatic debridement is a highly effective tool designed to be completed in a few sessions, thus paving the way for extension of its application in clinical trials on humans.

Chronic limb wounds are associated with tissue ischemia and are typical in patients with diabetes. Such wounds can lead to serious infection, gangrene, and limb loss. Chronic wounds are characterized by the presence of devitalized tissue that enhances bacterial growth, reduces host resistance to infection, and inhibits the formation of granulation tissue.1-3 Debridement of necrotic tissue is therefore, an essential and crucial step in chronic wound management.4-7 The main approaches to wound debridement are based on surgical, mechanical, enzymatic, or biological methods.8-10

Enzymatic removal of necrotic tissue from chronic wounds and burns by
proteases primarily offers selectivity of debridement without impairing adjacent healthy tissue. Proteolytic enzymes for wound debridement are commercially available as ointments. The most frequently used products that contain papain are Accuzyme (Healthpoint, Fort Worth, Tex) and Panafil (Healthpoint, Fort Worth, Tex). Comparative preclinical and clinical studies on the efficacy of papain versus other enzyme ointments indicated that papain was the more effective enzyme from this group.

Papain is a proteolytic enzyme derived from the fruit of Carica papaya, and has been employed for decades in the food and pharmaceutical industries. Furthermore, it was used for many years for wound healing by natives in tropical countries. Papain is the best studied and most applied protease from the thiol protease family (proteases that have a thiol group playing an essential role in their catalytic mechanism). Therefore, the efficacy of papain's proteolytic activity is dependent on maintaining its active site's thiol group in its active form (protection from oxidation, complexation with certain metal ions, or regeneration and pH adjustment to 6–8 are commonly employed means). In many cases, thiol-containing compounds may restore reduced papain activity due to reactions involving its thiol group.

Wound debridement using papain-containing ointments is currently practiced with repeated topical applications over a period of 1–3 weeks. The procedure is both labor intensive and time consuming. A potential explanation for the reported low efficacy of this mode of papain application for wound debridement is that the environmental conditions created within wounds treated this way are suboptimal or inhibitory to full expression of its potential activity. These conditions include acidic pH values, competitive metal ions, proteases secreted in wound exudate, as well as diffusion limitations imposing limited access of papain to its targeted substrate sites.

The authors previously proposed and demonstrated feasibility of an alternative mode of delivery and application of proteolytic enzymes for wound debridement and cleansing: continuous controlled streaming of protease solutions onto a targeted treated area, providing optimal and controlled environmental conditions for full exploitation of the protease's potential debridement activity. The efficacy of the streaming approach was successfully demonstrated in removal of coagulated blood and debridement of experimental burn wounds in small lab animals by a series of proteases.

The present study takes the continuous streaming approach one step further, from small lab animal tests to feasibility demonstration of streaming a selected protease solution onto a large (approximately 3.5 cm x 3.5 cm) experimental necrotic wound model in pigs with a clinically-applicable system. The primary objective of this study is to demonstrate the feasibility of selective necrotic tissue removal by a single continuous streaming treatment applied onto wounds for 6 to 11 h to serve as a starting point to evaluate the potential of this method, and subsequently complete the evaluation with a short series of repetitive debridement treatments for clinical use. Another objective of this study is to demonstrate that hypertonic agents (eg, 3%–5% saline or 10%–15% glycerol) may be incorporated into the streamed protease solution with compliance to its debridging capability, adding reduction of bacterial load and enhancement of fluid circulation. Prior to the incorporation of these agents into a debridement experiment their possible impact on papain's specific proteolytic activity will be evaluated.

Materials and Methods

Continuous streaming of proteolytic enzyme solution. The streaming system was comprised of a feeding reservoir (standard infusion bag, Teva Medical Ltd, Israel), connective tubing (Teva Medical Ltd, Israel), and an occlusive dressing designed for wound sealing with effective internal streaming and washing (DermaStream, Enzysurge Ltd, Shoham, Israel). The device outlet was connected to a standard fluid collection bag.

Experimental wound model. Pig wound models are considered to be the closest model to humans available in terms of skin, and anatomical and physiological characteristics. The necrotic porcine wound model used in the study was selected based on the literature. The study was conducted on large white female pigs (supplied by Lahav Institute of Animal Research, Israel) that were 3–4 months old and weighed 28–30 kg. Upon arrival, the pigs were allowed to undergo an acclimatization period of 4 days. Prior to wound induction, pigs were sedated and anesthetized intramuscularly (IM) with 100 mg/mL ketamine, 20 mg/kg body weight (BW); IM xylazine (10%) 2 mg/kg BW (100 mg/mL), and IM atropine 0.02 mg/kg BW (1 mg/mL), intubated with assistance (if required) by intravenous (IV) midazolam (5 mg/mL), and inhalated by isoflurane (1.5%–3%) in pure oxygen.
Animals were shaved at the posterior lateral aspects of their backs. Hartman’s solution was IV infused at a constant rate of 30–50 drops/min throughout the experiment.

Five myocutaneous (double flap) wounds (9 cm x 3.5 cm) were induced on the dorsal plane of pigs’ backs in the para-vertebral regions according to a known protocol (Figure 1). Wounds were spaced 10 cm apart in order to leave enough skin space to attach the debridement device surrounding each wound.

**Papain debriding solutions.** The papain debriding solution contained 2 mg/mL papain dissolved in 0.9% sodium chloride (saline) including 0.1 M tris buffer (pH 7.3), 5 mM cysteine, and 2 mM EDTA (all solutions manufactured and distributed by Merck & Co., Whitehouse Station, NJ).

**In-vitro determination of incorporated hyperosmolar agents’ impact on the enzymatic activity of papain.** The effect of the incorporation of 0.9% sodium chloride, 3% sodium chloride, 5% sodium chloride, 10% glycerol, 15% glycerol, and combinations of 5% sodium chloride/10% glycerol, 0.9% sodium chloride/10% glycerol into a 2 mg/mL papain solution (in a 0.1 M tris buffer, pH 7.3, containing 5 mM cysteine and 2 mM EDTA) was determined spectrophotometrically employing BAPNA (3-N-alpha-benzoyl-DL-arginine p-nitroanilide) as a low molecular weight synthetic substrate at 30 °C.

**Debridement protocol.** Seven days post wound induction, the 2 flaps of each wound of the sedated animal were removed, exposing 3.5-cm x 3.5-cm openings of necrotic wounds (Figure 2A). Histoacryl® (Tissue Seal, LLC, Ann Arbor, Mich) was used for hemostasis. A thin layer of Vaseline® was applied on intact skin at wound boundaries to prevent contact with the debridng solution. The occlusive dressings were placed over the wounds (Figure 1D) and their inlets and outlets were connected to a 500-mL solution reservoir (infusion bag) and fluid collecting bag, respectively.

Freshly prepared enzyme or control solutions were
injected into the infusion bag connected to the inlet of each device, and controlled streaming at a flow rate of 1 mL/min was affected for a period of 6-11 h at room temperature.

**Wound assessment. Photography.** Digital photographs (at maximum resolution) of the wound surfaces were taken before and immediately after debridement or control streaming periods. The photos were taken 40 cm from the wound surface at an angle of 80 degrees. Scanned area included the entire wound area and its margins.

**Histology.** Following the 6- to 11-hour treatment, pigs were sacrificed by injection of Pental (200 mg/mL, 1 mL/1.5 kg BW) and full-thickness, cross section of 1-cm x 4-cm biopsies were removed from all wounds for histological analysis providing a deep cross-section of the wounds with or without treatment to compare treated and non-treated areas on the same slide. Tissue samples were immediately fixed in 4% phosphate buffered formaldehyde for 48 h and embedded in paraffin. Serial sections perpendicular to the skin surface were cut at 8-μm thickness and later stained with trichrome stain.

**Results**

**Establishment of working system.** The experimental chronic wound double flap model in

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**Figure 2.** Overview (A) and histological section (cut along the line between the arrows). Observations (B) of double flap experimental wound 7 days post wound induction.

**Figure 3.** Effect of combinations of hypertonic agents with papain on its *in-vitro* relative specific activity.
pigs\textsuperscript{20} was successfully adopted in this study. This model yielded necrotic wounds (3.5 cm x 3.5 cm) exhibiting a gradient ranging from necrotic material to healthy tissue, and allowed for simultaneous examination of streaming impact on either necrotic or healthy segments within a single frame of observation (photograph or histological cross-section, Figure 2). The wounds obtained were readily scaled by the application of the occlusive dressing to allow continuous streaming.

**Effect of added hypertonic agents on in-vitro enzymatic activity of papain.** The impact of incorporation of hypertonic agents (sodium chloride and glycerol) on the specific activity of papain was determined spectrophotometrically using the synthetic, low molecular weight substrate BAPNA. The results indicate that while added high salt concentration increased papain’s specific activity, the addition of glycerol reduced the recorded specific activity (Figure 3). Incorporation of salt mixtures and glycerol mixtures resulted in a “compensation” effect. Furthermore, salt-solvent combinations may allow either retention (eg, 0.9% saline/10% glycerol combination) or enhancement (eg, 5% saline/10% glycerol combination) of papain’s basic in-vitro proteolytic activity.

**Wound debridement by continuous streaming of papain solutions.** The effect of continuous streaming of papain debriding solution containing several combinations of hypertonic agents for 6 h on model necrotic wounds was investigated through observing their impact on 26 wound pairs. Comparisons were made between the effect of streaming 2 mg/mL papain solution in running buffered solution to the effect of running the same solution devoid of the enzyme. Removal of approximately half of the necrotic material developed in these model wounds was observed following a single run of 6 h continuous streaming of 2 mg/mL papain solution (at a flow rate of 1 mL/min) in all

**Figure 4.** Effect of streaming different papain/hypertonic agent combinations onto wounds: compare photographs taken prior to streaming (A, C, E) with photographs taken following 6 h of continuous streaming of papain in 5% saline (B), in 15% glycerol (D), or in 5% saline/10% glycerol mixture (F), respectively. Photos G and H are an enlargement of areas marked in photographs E and F, respectively.
cases, as illustrated by the 3 experiments described in Figure 4. Changing of the composition of the hypertonic agents added had a minor effect, indicating potential flexibility in choice of running solution composition.

In contrast to the data of Figure 4, streaming of running buffered solution devoid of the enzyme had no debridement effect (data not shown).

In an attempt to better understand the mechanism involved in debridement affected by the streamed papain solutions, streaming was arrested in one of the experiments after 3 h for examination. Results indicate that throughout the first 3 h of streaming the necrotic mass of the wound changed its morphology into a softened, swollen, and semi-liquefied gel (Figure 5). It appears that the process of wound debridement by the streamed papain solution starts with swelling of the targeted necrotic tissue, penetration and equilibration of the enzyme inside the targeted necrotic tissue, and finally, a gradual cleavage, dissolution, and washing of the fibrin based matrix.

Effect of streaming of papain debriding solution on vitalized tissue. In contrast to its impact on necrotic wounds, streaming of papain debriding solution for 6 h onto the surface of a freshly cut acute wound had no impact on its surface as shown in Figure 6. The data shown in Figure 6 indicate that debridement affected by streaming of the papain debriding solution is selective—substantial removal of necrotic, nonvitalized tissue was readily achieved, while vitalized healthy tissue was not impaired under same conditions.

Effect of extended debridement time. Elongation of wound treatment from 6 h to 11 h of streaming of papain debriding solution resulted in removal of a larger fraction of the necrotic tis-
sue. Comparison of details of histological sections taken from wounds treated by 11 h streaming (Figure 7) indicates that papain debriding solution containing 5% sodium chloride affected hyperemia while iso-osmolar (0.9% saline) papain debriding solution did not (Figure 7).

**Discussion**

Wound debridement for the removal of necrotic tissue is a crucial and essential step in wound management. The overall objective of this study was to demonstrate the feasibility of wound debridement by continuous streaming of a selected protease solution for the removal of necrotic tissue formed within a porcine wound model carried out by a working system, which may be subsequently applied to clinical trials and applications in humans. The technical feasibility of streaming the selected protease-papain solution was successfully demonstrated by a simple system comprised of an IV bag as solution input reservoir and feeding the occlusive dressing providing continuous wound washing with its outlet connected to a standard collecting bag.

The feasibility of this system to substantially affect necrotic wound debridement in a single run of continuous streaming of a papain solution onto a pig wound model several square centimeters in size in a treatment period of 6 to 11 h was demonstrated. The working hypothesis was that such continuous streaming of fresh supply of proteolytic enzyme solution, providing environmental conditions optimal for maximal expression of the proteolytic activity, and effective distribution within the treated wound will provide high efficacy, allowing the completion of wound debridement within an additional 2–5 runs, providing substantial reduction in currently used treatment times.

The selection of papain as the proteolytic enzyme for this study was based on its long history of safe application in debridement (ointments, creams, and sprays). Papain debriding solutions were formulated for this study and fortified by the incorporation of hypertonic agents. A hyperosmolar environment is commonly used for washing chronic wounds, providing reduced microbial load and effective exudate removal.

Comparison of debridement affected by the 2 mg/ml papain solution to the effect of the corre-
spending running buffered solutions clearly indicated that the debridement observed should be attributed to papain’s proteolytic activity—debridement was not observed in any of the experiments with running buffered solutions. Continuous streaming of 2 mg/mL papain solution resulted in removal of about half of the necrotic material developed in the pig model wounds within a single run of 6 h.

Furthermore, in accord with previously published results from the authors’ lab,16 no adverse effects or noticeable morphological changes of the wound surface or its immediate surroundings were observed in the histological sections taken from either treated necrotic wounds or freshly cut acute wounds. This observation demonstrated that under the working conditions employed, this enzymatic debridement method is selective with clear preference to digest and remove necrotic nonvitalized tissue and blood clots without impairing adjacent vitalized healthy tissues.

This observation also correlates with results observed following a short 3-hour papain solution streaming indicating that the mechanism involved in this mode of debridement starts with swelling of the necrotic tissue, papain penetration, equilibration, softening, dissolution, and washing. Extension of running time from 6 h to 11 h combined with strong hypertonic medium resulted in a preliminary observation of hyperemia.

Conclusion

The results demonstrate the feasibility of debridement by continuous streaming of a papain solution and indicate that this system carries potential for wound debridement in humans through a few streaming sessions. This system is capable of paving the way for an extension of its use in clinical trials and applications.

References

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