Indian Journal of Science and Technology Vol. 4 No. 9 (Sep 2011) ISSN: 0974- 6846

Comparative study of collagenase and papain-urea based preparations in the management of chronic nonhealing limb ulcers

Hosamath Vijaykumar, Sreekar Agumbe Pai*, Vijay Pandey and Prasannakumar Kamble

Department of General Surgery, M.S.Ramaiah Medical College, Bangalore-560 054, Karnataka, India
drvkhosmath@gmail.com; sreekarpai76@yahoo.com*; drvijaypandey16@yahoo.co.in; prasan_kims@rediffmail.com

Abstract

The objectives of this study are to compare effectiveness of collagenase v/s papain- urea for debridement of chronic non-healing ulcers/wounds and to evaluate their role in promoting ulcer healing by granulation and reduction in ulcer/wound size. A comparative study of 100 patients was done at M.S. Ramaiah Hospitals in India from November 2007 to August 2009. Patients were selected, randomized, and divided into two groups consisting 50 patients each. Group- 1 treated with collagenase and Group 2 with papain- urea. Patients were evaluated at 0, 1, 2, 3, and 4 weeks for reduction in ulcer size, granulation, discharge and over all response to treatment. The mean age was 42 +/- 15yrs. The co-morbidities were diabetes 28.4 %, hypertension in 21.1%, others 14.0%. Culture and sensitivity test reveals that most frequently grown organism was *E. coli* (13%) *Staphyloccus aureus* (9%) and samples with no growth was 64%. In papain-urea group, ulcer was reduced from 24.8 sq. cms to 11.9 sq. cms and collagenase group 23.1 sq. cms to 9.7 sq. cms. There was significant reduction in slough and necrotic tissue *i.e*. in papain-urea group 22.54 sq. cms to 5.07 sq. cms and collagenase group 21.76 sq. cms to 6.12 sq. cms. Significant amount of increase in granulation tissue in papain-urea group *i.e*., 2.4 sq. cms to 6.82 sq. cms and collagenase group 1.4 sq. cms to 3.8 sq. cms was observed. But Papain-urea group showed better response in 2nd, 3rd, 4th weeks compared to collagenase (p value <0.05) and significant improvement in 28% in papain-urea, 12% in collagenase group. Mean follow-up period was 7.28- 8.14wks. Papain-urea and collagenase have shown proven efficacy in bringing out enzymatic wound debridement. Papain-urea is a better enzymatic debriding agent promotes faster granulation compared to collagenase.

Keywords: Collagenase, papain-urea, non-healing ulcer, enzymatic debridement, debridement.

Introduction

Management of chronic non-healing ulcers or wounds is a difficult clinical problem. Non healing ulcers represent a major health burden and drain on resources. Management of wounds though common in surgical wards, there is need for thorough knowledge about pathophysiology and various treatment options available which form corner stone in treating such patients. It helps the treating surgeon to achieve better outcome both in terms of patient compliance and reducing time and cost.

With continued research new information regarding processes involved in chronic non-healing ulcers is available we now know that a cellular burden, comprising phenotypically abnormal cells, exists in chronic wounds and needs to be removed or corrected. We have come to recognize the deleterious effects of excessive exudate, which breaks down extracellular matrix material and blocks the effectiveness of new forms of therapy, including growth factors and bioengineered skin. We are becoming more cognizant of the pathophysiologic abnormalities of chronic wounds and of ways to correct them. We have also come to recognize that chronic wounds may be in need of constant or more steady state debridement. Hence, the concept of maintenance debridement needs to be tested.

Many modalities of debridement are now available as surgical/sharp, mechanical, autolytic, enzymatic and biologic with major emphasis on enzymatic wound debridement. Enzymatic wound debridement has proven efficacy in management of ulcers. It uses topical enzymes to remove necrotic tissue by digesting and dissolving the devitalised tissue in the ulcer/wound bed. Efficacy of two such enzymatic debriding agents as collagenase and papain-urea has been compared in this study. There are not many studies available for chronic non healing limb ulcers in this group. Hence the present studyis made with the objectives of comparing the effectiveness of collagenase v/s papain - urea for debridement of chronic non-healing ulcers/wounds and to evaluate their role in promoting ulcer healing by granulation and reduction in ulcer/wound size.

Materials and methods

This is a comparative study of 100 patients made at M.S. Ramaiah Hospitals, Bangalore, India between November 2007 to August 2009. Patients were selected, randomised, and divided into two groups 50 patients in each group.

Group - 1 was treated with collagenase; Group 2 was treated with papain - urea.

Method of collection of data

Clinical assessment done at time of inclusion in the study

a) Detailed history and examination done, b) Ulcer and devitalised tissue assessed and measurements taken using sterile burns mesh or gauze and graph paper used to calculate the area, c) Swab for gram stain and culture/sensitivity taken prior to inclusion in the study and

Indian Journal of Science and Technology Vol. 4 No. 9 (Sep 2011) ISSN: 0974- 6846

d) Ulcer cleaned with normal saline and daily dressed for a week.

Treatment phase:

a) Patients were randomised using random table when ulcer with were stable (< 20% change in size) or improving (decreasing in size), b) Patients were evaluated at 0 (randomisation) , 1, 2, 3, and 4 weeks, c) Debridement of slough/nonviable tissue, reduction in ulcer size, granulation noted, d) Discharge, odour, induration noted for over all response to treatment and e) Dressings were done using same technique - cleaning with saline and application of ointment (collagenase/papain - urea) and putting a dressing.

Application of ointment was done once daily in the following manner: Step 1: Prior to application, the lesion was cleaned of debris and digested material by gently rubbing with gauze pad by normal saline. Step 2: Whenever infection is present, an appropriate topical antibiotic powder should be applied to the lesion prior to the application of Ointment. Step 3: Ointment was applied directly to deep lesions with a wooden tongue depressor or spatula. For shallow lesions, Ointment was applied on sterile gauze pad, which was then applied to the wound and properly secured.Step 4: All excess ointment was removed each time dressing was changed. Use of ointment should be terminated when debridement of necrotic tissue is complete and granulation tissue is well established.

Specimen collection and handling for culture and sensitivity; a) The pus swab from the infected wound is taken before applying antiseptic dressing. Use aseptic precautions while collecting the specimen and use sterile swab while to collect the sample. b) Microscopy - Gram stain is performed after making a smear and c) The pus swab is inoculated on blood agar, Mac Conkey agar and thioglycollate broth. The media is incubated aerobically for 72 hours and examined for growth of bacteria. Bacteria that are isolated in culture are identified using standard methods and antibiotic sensitivity tests are put according to CLSI guidelines.

Patients included in the study are those who have chronic limb ulcers with slough and for which debridement is required for healing. Chronic non healing ulcers are those that do not show healing for a period of 8 weeks. Patients who have severe infection, cellulites or uncontrolled diabetes, peripheral vascular disease, neuropathy and patients on steroids, immunosuppressive agents, radiation or chemotherapy are excluded from the study. Patients are evaluated with Complete Blood Count, Renal Function Test, Liver Function Test, Culture and sensitivity (table of c/s follows), X- ray of involved part if necessary. Statistical analysis was done by Chi-square test and student T test.

Results

Mean age in papain-urea and collagenase group was 42.43 +/- 15.03 yrs. No significant difference between groups with respect to age and gender noted. 30% of ulcers were in Upper limb and 70.0% in Lower limb.

Table 1. Distribution of site of ulcer involved in study group

Site	Group		Total	Chi Square Value	'p' value
	Papain/ Urea	Collage-nase			
Upper Limb	16	14	30	2.198	0.333
	32.0%	28.0%	30.0%		
Lower Limb	34	36	70		
	68.0%	72.0%	70.0%		
Total	50	50	100		
	100.0%	100.0%	100.0%		

(Table 1).Some of the patients had diabetes 28.4 %, hypertension 21.1 %, and bronchial asthma, coronary artery disease, etc. and amounted to 14.0 %. (Table 2). Culture and sensitivity patterns in both groups were similar and most frequently grown organism was *E. coli* (13%), *Staphyloccus aureus* (9%), *Streptococcus* (6%) and no growth in (64%). (Table 3)Baseline ulcer sizes were similar in both groups 23-24 sq. cms (4 - 94 sq. cms) before starting the treatment and there is similar gradual reduction in size over a period of 4 weeks. In

Table 2. Distribution of co-morbidities seen in study group

Co morbidity	Group		Total	Chi Square Value	'p' value
	Papain-Urea	Collage-nase			
Diabetes Mellitus	14	13	27	0.001	0.973
	28.6%	28.3%	28.4%		
Hypertension	12	8	20	0.719	0.396
	24.5%	17.4%	21.1%		
Other:					
BA	3	2	5	3.333	0.649
	6.0%	4.0%	5.0%		
CAD	3	2	5		
	6.0%	4.0%	5.0%		
OA	1	2	3		
	2.0%	4.0%	3.0%		
COPD	0	1	1		
	.0%	2.0%	1.0%		

Table 3. Culture and sensitivity patterns in the study

Bacteria	Group		Total	Chi Square Value	'p' value
	Papain-Urea	Collagenase			
E.coli	4	5	9	3.427	0.634
	8.0%	10.0%	9.0%		
Pseudomonas aeroginosa	4	3	7		
	8.0%	6.0%	7.0%		
Staphylococcus aureus	9	4	13		
	18.0%	8.0%	13.0%		
Streptococcus pyogenes	3	3	6		
	6.0%	6.0%	6.0%		
Actinobacter beaumani	0	1	1		
	.0%	2.0%	1.0%		
No Growth	30	34	64		
	60.0%	68.0%	64.0%		
Total	50	50	100		
	100.0%	100.0%	100.0%		

Indian Journal of Science and Technology Vol. 4 No. 9 (Sep 2011) ISSN: 0974- 6846

Table 4. Percent reduction in slough/necrotic tissue from baseline

Visit	Group	N	Mean	Std. Deviation	Minimum	Maximum	't' value	'p' value
Week 1	Papain/Urea	50	33.14	15.328	10	70	1.395	.240
	Collagenase	50	29.78	12.950	12	58		
Week 2	Papain/Urea	50	58.52	17.958	22	91	5.237	.024
	Collagenase	50	50.65	16.434	20	87		
Week 3	Papain/Urea	50	77.47	19.647	33	100	6.426	.013
	Collagenase	50	67.97	17.758	29	100		
Week 4	Papain/Urea	50	89.22	15.162	41	100	4.208	.043
	Collagenase	50	82.51	17.450	36	100		

papain-urea group, ulcer reduced from 24.8 sq. cms to 11.9 sq. cms and collagenase group 23.1 sq. cms to 9.7 sq. cms and there is similar week wise comparison of

reduction in size of ulcer. The mean amount of slough/necrotic tissue at baseline i.e. 0 weeks (21-22 sq. cms) in both groups and subsequent weeks showed significant reduction in slough and necrotic tissue *i.e*., papain-urea group slough area reduced from 22.54 sq. cms to 5.07 sq. cms and collagenase group 21.76 sq. cms to 6.12 sq. cms. But papain-urea group showed better response in 2nd, 3rd, 4th weeks compared to collagenase (p value < 0.05). (Table 4)There was significant amount of increase in granulation tissue over the four week period. In papain-urea group 2.4 sq. cms to 6.82 sq. cms and collagenase group 1.4 sq. cms to 3.8 sq. cms. But there was significant difference in percentage increase in granulation tissue in papain-urea group compared to collagenase group. Week wise comparison between the two groups showed that papain-urea showed better granulation after 1st, 2nd, 3rd, & 4th

Table 5. Comparison of amount of granulation tissue between the groups

Visit	Group	N	Mean	Std. Deviation	Minimum	Maximum	't' value	'p' value
Baseline	Papain/Urea	50	2.400	2.8631	.0	8.0	4.415	.038
	Collagenase	50	1.400	1.7687	.0	7.0		
Week 1	Papain/Urea	50	4.148	2.8005	1.0	10.0	5.394	.022
	Collagenase	50	3.080	1.6522	.0	6.0		
Week 2	Papain/Urea	50	5.464	4.5083	1.0	18.4	8.147	.005
	Collagenase	50	3.500	1.8296	.8	9.0		
Week 3	Papain/Urea	50	6.628	7.0073	.0	29.2	8.270	.005
	Collagenase	50	3.600	2.5166	.2	14.0		
Week 4	Papain/Urea	50	6.828	8.1590	.0	30.0	6.927	.010
	Collagenase	50	3.580	3.0942	.0	16.0		

weeks compared to collagenase group. (Table 5) Overall response to treatment by clinical assessment and it was found that there was significant improvement was noted in 28% patients of papain-urea group, as it was 12% in collagenase group. The difference was statistically significant (p value < 0.05). Mean follow-up in weeks was less for papain-urea group (7.28wks) and collagenase group (8.14wks). Difference in mean follow up was not statistically significant.

Discussion

Wound healing involves a well-orchestrated, complex process leading to repair of injured tissues. However, chronic wounds do not follow the normal pattern of repair. This is due to underlying physiological problems associated with their development, which unless corrected would continue to cause wound deterioration. The key to effective wound care lies in a combination of three approaches: treatment of underlying medical problems, assessment and treatment of local wound bed and effective management of any patient-centered concerns. An essential component of this recommended approach is restoration of healthy granulation tissue in the wound bed. Wound bed preparation brings a number of existing procedures, including debridement, treatment of infection, and management of exudate levels, together into a systematic approach to help restore the chronic wound bed environment. The aim of wound bed preparation is to remove the barriers to healing and initiate the repair process (Falanga, 2002).

By defining what is that prevents chronic wounds from progressing to wound closure, wound bed preparation provides a clinical strategy that will ultimately lead to the removal of all local barriers to the healing process, so that wound repair can progress normally (Stuart Enoch, 2003). The use of debridement as a standard procedure for proper wound management is based largely on expert consensus as opposed to randomized clinical trials. However, some clinical trial evidence for debridement does exist. One landmark trial supporting its use in chronic wounds was reported by Steed *et al*. (1996). In this study, which was part of the data that led to the approval of rhPDGF for diabetic neuropathic foot ulcers, higher healing rates were observed in those treatment centers that performed more frequent surgical debridement of diabetic foot ulcers compared to other centers that did not debride as often. Other data from clinical series exist. For example, in one study, 26 out of 30 refractory ulcer patients showed successful healing following two-stage surgical debridement (Hobson *et al*., 1998b). For bio surgical debridement, a study reported 68% decrease in the mean area of slough and necrotic tissue in leg ulcers following maggot therapy, while the area of granulation tissue increased by 26% and there was also a reduction in the amount of exudate, odour, and bacteria present (Levenson *et al*.,1981).

Enzymatic debridement

Evidences exist that enzymatic ulcer debridement is effective in debridement of chronic nonhealing ulcers and also decrease exudates, bacterial burden and promote ulcer healing. Enzymatic debridement is a highly selective method of wound debridement that uses naturally occurring proteolytic enzymes that are manufactured by the pharmaceutical and healthcare industry specifically

Indian Journal of Science and Technology Vol. 4 No. 9 (Sep 2011) ISSN: 0974- 6846

for wound debridement. These exogenously applied enzymes work alongside the endogenous enzymes in the wound. Several enzyme debriding agents have been developed including bacterial collagenase, papain/urea, fibrinolysin/DNAse, trypsin, streptokinase-streptodornase combination, and subtilisin. Only the first three products are widely available commercially in those markets where they are registered, although availability varies geographically

Papain-urea-based combinations

A well-known and widely used enzymatic system is the papain-urea combination (Berger, 1993; Westerhof, 1994). In this system, papain is used to attack and break down any protein containing cysteine residues. This property of papain renders the combination quite nonselective because most proteins, including growth factors, contain cysteine residues. Collagen contains no cysteine residues and is thus unaffected by papain. The urea component of the most widely used of these combinations will also attack a wide variety of proteins. However, urea's role in this enzymatic combination is to facilitate the proteolytic action of papain by altering the three-dimensional structure of proteins and disrupting their hydrogen bonds, as well as exposing by solvent action the activators of papain. Urea also plays a role in the reduction of disulfide bridges; as the disulfide bridges are reduced, cysteine residues become exposed and are, therefore, more susceptible to the action of papain (Miller *et al*.,1958).The combination of papain and urea is probably twice as effective in protein digestion as papain alone (Miller *et al*.,1958). An advantage of the papain-urea combination may be nonspecific bulk debridement within a broad pH range (3.0-12.0). The papain-urea preparations have been used clinically for decades, especially in pressure ulcers. The available literature indicates that these debriding systems are effective when properly used, especially if one keeps in mind that they cannot substitute for surgical debridement when that is required (Rao, 1975). The addition of the chlorophyllin may have improved the product by reducing pain. There have been concerns that these papain-based enzyme preparations can destroy locally active growth factors, such as PDGF (Gosiewska, 1998). In experimental wounds in animals, the papain-urea combination has been shown to be quite effective for debridement (Rao, 1975). However, in both experimental and human burns, these preparations may behave too aggressively, both in terms of affecting viable tissue and in causing pain.

Available as Debridace Ointment (Virchow Pharmaceuticals) - each gram of ointment contains: Papain USP 521700 Units of activity Urea USP 100mg. It contains papain and urea in emulsified wax ointment base. Papain is proteolytic enzyme derived from the fruit of Carica papaya, is a potent digestant of non viable protein matter, but is harmless to viable tissue. Papain is active over a wide range of pH (3 - 12). Papain is relatively ineffective when used alone as debriding agent and requires the presence of activators to stimulate its digestive potency. Papain is combined with urea, a denaturant of proteins to provide two supplemental chemical actions:1) to expose the sulfhydryl groups (activators of papains) by solvent action and 2) to denature the non viable tissue in the lesions and render it more susceptible to enzymatic digestions. Pharmacological studies have shown that the combinations of papain and urea result in twice as much digestive activity as papain alone.

Collagenase preparations

Collagenase is another well known and established enzyme preparation used for debridement. Its development as a debriding agent as well as for other applications came to a peak in the early 1970s. The commercially available preparation of collagenase is derived from bacteria (*Clostridium histolyticum*). Collagenase is a water soluble proteinase that specifically attacks and breaks down collagen in the necrotic tissue (Rao, 1975; Herman, 1996). Collagenase is reported to be most effective in a pH range of 6 to 8 at the physiological pH and temperature makes it particularly effective in the removal of debris from the wound. It has been shown that collagenase can hydrolyze native collagen and thereby facilitate rapid debridement and healing of chronic wounds. The mechanism of action of collagenase is to degrade collagen and convert it to gelatin, upon which less specific enzymes can then act. However, until collagenase cleaves collagen, no other enzyme is capable of breaking it down. An interesting observation is that the collagenase preparation may be selective for nonviable collagen. This effect needs to be studied further, but it is thought that viable collagen is surrounded and protected by mucopolysaccharide sheaths (Altman *et al*., 1978; Falanga, 2002). Available as 20 gm tube of SALUTYL Ointment (Elder Pharmaceuticals) contains: Collagenase - 250 units per gram of White Soft Paraffin I.P.

Collagenase and papain/urea formulations have been demonstrated to have degrading effects on wound components, such as collagen, fibrin, and elastin both in vitro and clinically. A recent study showed that collagenase *in vitro* was capable of degrading both collagen and elastin, while papain/urea was effective for fibrin and collagen degradation (Hobson *et al*., 1998a). In another study by Alvarez *et al*. (2000), papain-urea proved to be significantly more effective than collagenase for pressure ulcer debridement. Papain-urea also appeared to be more effective in promoting granulation tissue than collagenase. In two separate studies using different *in vitro* models for debridement (Levenson *et al*.,1981; Hobson *et al*.,1998) it was shown that the combination of an enzyme (papain) with a mucolytic nonenzymatic agent (urea) was significantly more effective than enzymatic agents alone (collagenase or

Indian Journal of Science and Technology Vol. 4 No. 9 (Sep 2011) ISSN: 0974- 6846

DNase/fibrinolysin).The only RCT on papain reported more visible NTR and granulation tissue formation during weeks 2, 3, or 4 of debridement on pressure ulcers using papain combined with urea in a hydrophilic ointment vehicle compared to collagenase in petrolatum ointment in long-term care. There was no significant difference in healing rates (n = 26) (Ramundo, 2008). Papain-urea is in a white hydrophilic ointment, whereas collagenase debriding ointment has a petrolatum vehicle and is considerably more hydrophobic. Differences in the hydrophilic nature of the ointment vehicles between these two formulations may be of importance, since hydrophilic formulations have been shown to be more effective in releasing enzymes than hydrophobic formulations (Alvarez *et al*., 2000). In this study efficacy of collagenase and papain-urea was compared for ulcer debridement. It was found that there was no difference in reduction in ulcer size between the two groups. Papain-urea showed significant reduction in slough/necrotic tissue compared to collagenase .Granulation was better with papain-urea compared to collagenase. On clinical assessment of wound/ulcer significant improvement was noted in papain-urea group compared to collagenase.

Conclusions

Papain-urea and collagenase have proven efficacy in bringing out enzymatic wound debridement. Papain-urea (89.2%) is a better enzymatic debriding agent than collagenase (82.2%). Papain urea (from 2.4 sq. cms to 6.8 sq. cms) promotes faster granulation compared to collagenase (1.4 sq. cms to 3.5 sq. cms). Papain-urea and collagenase have proven efficacy in bringing out enzymatic wound debridement. Papain-urea is a better enzymatic debriding agent promotes faster granulation compared to collagenase.

Acknowledgement

Competing interest - The authors declare that they have no competing interests.

References

1. Altman MI, Goldstein L and Horowitz S (1978) An adjunct to healing trophic ulcerations in the diabetic patient. *J. Am. Pod. Assoc*. 68, 11-15.
2. Alvarez OM, Fernandez-Obregon A, Rogers RS, Bergamo L, Masso J and Black M (2000) Chemical debridement of pressure ulcers: A prospective, randomized comparative trial of collagenase and papain/urea formulations. *Wounds.* 12 (2), 15-25.
3. Berger MM (1993) Enzyme debriding preparations. *Ost/Wound Manag*. 39, 61-66.
4. Falanga V (2002) Wound bed preparation and the role of enzymes: A case for multiple actions of therapeutic agents. *Wounds.* 14(2), 47-57.
5. Gosiewska A, Yi C-F, Brown L and Geesin JC (1998) The effect of enzyme debriders on the biological activity of recombinant human platelet-derived growth factor-BB (rhPDGF-BB), the active agentofRegranex gel. *Wound Rep. Regen*. 6, A501.
6. Herman I (1996) Stimulation of human keratinocyte migration and proliferation *in vitro*: Insights into the cellular responses to injury and wound healing. *Wounds.* 8, 33-41.
7. Hobson D, White E, Anderson L and Lira L (1998a) Development and use of a quantitative method to evaluate the action of enzymatic wound debriding agents *in vitro*. *Wounds*. 10(4), 105-110.
8. Hobson D, Lira L and Nijeha F (1998b) Evaluation of the effects of formulation factors on the action of wound debriding agents *in vitro*. Abstract presented at the 30th Ann. Conf. of the Wound Ostomy and Continence Nursing Soc. in Salt Lake City, Utah, June. 13-17.
9. Levenson SM, Gruber DK and Gruber C (1981) Chemical debridement of burns: Mercaptans. *J. Trauma*. 21, 632-644.
10. Miller JM and Howard F (1958) The interaction of papain, urea, and water-soluble chlorophyll in a proteolytic ointment for infected wounds. *Surgery*. 43, 939-948.
11. Ramundo J and Gray M (2008) Enzymatic wound debridement. *J. Wound Ostomy Continence Nurs*. 35(3), 273-280.
12. Rao DB, Sane PG and Georgiev EL (1975) Collagenase in the treatment of dermal and decubitus ulcers. *J. Am. Geriatr. Soc*. XXIII, 22-30.
13. Steed DL, Donohoe D, Webster MW and Lindsey L (1996) Effect of extensive debridement & treatment of healing Diabetic foot ulcers. *J. Am. Coll. Surg*.183, 61-64.
14. Stuart Enoch (2003) Wound bed preparation: The science behind the removal of barriers to healing. *Wounds*. 15 (7), 213-229.
15. Westerhof W (1994) Futureprospects of proteolytic enzymes and wound healing. In: Proteolytic enzymes and wound healing. Westerhof W & Vanscheidt W (eds). Springer-Verlag & Co., NY. pp: 99-102.