THE EFFICACY OF COMBINED CREAM BROMELAIN - LIPOSOME AS ENZYMATIC DEBRIDING AGENT FOR DEEP BURN PATIENTS

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ABSTRACT

Burns remain a major source of severe injury involving all age groups, often resulting in high morbidity and mortality. Deep burns are characterized by necrotic tissue called eschar requiring early excision for patient's survival and recovery. Despite the advantages of early excision, there are often contraindications to surgical debridement. Enzymatic debridement is an alternative which effectively and selectively degrades necrotic tissue. Unfortunately, there is limited understanding and availability of this modality in Indonesia. The agent already in market requires a relatively long period to work, is very expensive and difficult to access. With a high incidence of burns, especially in Dr. Soetomo General Hospital Surabaya, efforts were made to develop the ideal enzymatic debriding agent, bromelain, which is extracted from pineapple, combined with liposome as a carrier drug. A clinical trial was performed using 'post-test only' design, 'simple randomized' and 'single blind' to compare the effectiveness of silversulfadiazine cream (SSD), collagenase ointment, bromelain 35% cream and combined cream bromelain 10%-liposome 6% for debridement in 24 deep burn patients (DBD). Photographic documentation of the burns was made and surface areas were measured daily with Visitrak digital. In one third of patients, histopathology preparations were made before treatment and on the fifth day of treatment, using hematoxylin eosin stain to compare lytic zone thicknesses. Statistic tests used were T-test, Anova, Post Hoc and Multivariate tests. Bromelain-liposome cream was found to be significantly more effective for enzymatic debridement of DBP compared with other the agents making it a comparable alternative for surgical debridement of DBP.

Keywords: *Burns, eschar, enzymatic debridement, bromelain-liposome cream*

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INTRODUCTION

Deep burns often cause high morbidity dan mortality. Eschar which consists of necrotic burned tissue is very toxic and immunosuppressive (Moenadjat 2001) and serves as a favorable media for bacteria. Therefore, early excision is mandatory to allow patient's recovery and wound healing. The number of burn patients admitted to the emergency unit at Dr. Soetomo Hospital from years 2001 to 2004 were 1192 comprising 20.1 ‰ of all surgical cases admitted to the hospital in 2002 (Josh 2003).

From the total, 46.77% were moderate and severe burns requiring hospitalization in the burn unit. The proportional mortality rate was 31% (Perdanakusuma 2005) and remains high today. One of the major reasons for this has been delayed surgical debridement due to the patients' very poor general condition impeding general anesthesia besides the limited donor skin for immediate coverage. On the other hand, there is very limited knowledge of non surgical debridement.

There are 5 debriding methods i.e autolytic, mechanical, enzymatic, biological and surgical debridements. Enzymatic debridement is superior in selective degradation with minimal pain. However, the available debriding enzyme, collagenase, is costly and necessitates lengthy time to work, the average of which is not less than 5 to 12 days (Rosenberg et al. 2004). Efforts were made to acquire the ideal debriding agent, bromelain, from pineapple (Ananas comosus) which grows abundantly in Indonesia, combined with liposome as a carrier drug expediting the penetration and localization of the agent through eschar.

In a previous study in Surabaya, bromelain 35% was proven to be efficacious for debridement of deep burns in rats (Maluegha & Marzoeki 2005) whereas in Israel, it has already been demonstrated to be remarkably effective for DBD (Rosenberg et al. 2004). The objective of this study was to prove the efficacy of combined cream bromelain 10%-liposome 6% for debridement in DBP.

MATERIALS AND METHODS

This clinical trial was conducted at the Departments of Plastic Surgery and Pathology Anatomy at Dr. Soetomo Hospital Surabaya. Twenty four DBP, comprising deep second and third degree burns, aged 14 years and above, were allocated into 4 groups by random and single blind mode. Patients with multi trauma and other life-threatening conditions, systemic illnesses, allergy to sulphur and pineapple, burns already treated before admission, present steroid use and, alcohol intoxication, mental retardation, or who were pregnant and lactating were excluded.

An isolated wound with ≤1% surface area was selected on each patient. Every day for 10 days, group I was treated with silversulfadiazine cream, group II with collagenase ointment, and after an allergy test, group III was treated with bromelain 35% cream and group IV with combined cream bromelain 10% liposome 6%.

Clinical evaluation was made on days I to X, documented with Nikon E2200 2 mega pixel and surface areas were measured with an instrument called Visitrak Digital. In one third of patients, 2 patients from each group, histopathology examination was done before treatment and on the fifth day after treatment, using hematoxylin eosin stain. Lytic zone thicknesses were measured on a scale of a graticule under 100 X magnification. Statistical tests were performed using normal distribution testing, ANOVA test, T-Test, Post Hoc Test and multivariate testing.

Approval for this study was given by the Ethical Committee of Dr. Soetomo Hospital Surabaya. Patients and families received oral and written information and were informed about the possible risk of allergy which was minimized by allergy testing, and when indicated they could be withdrawn from the study, that biopsy or treatment could be associated with discomfort and that all records were confidential. Informed consent was obtained.

RESULTS

Table 1. Characteristics of subjects

Variables	Group				Total
	SSD	Collagenase	Bromelain	Brom+L	
- Sex					
Male	5 (83.3)	5 (83.3)	4 (66.7)	5 (83.3)	19 (79.2)
Female	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)	5 (20.8)
- Age	28.8±11.6	28.3±7.4	40.5±8.9	32.8±19.7	32.6±12.9
- Burn Surface Area	17.7±12.8	35.0±25.4	9.3±10.7	17.5±21.3	19.9±19.8
- Burn Depth					
IIAB	3 (50)	4 (66.7)	2 (33.3)	1 (16.7)	10 (41.7)
IIB	2 (33.3)	1 (16.7)	2 (33.3)	3 (50)	8 (33.3)
III	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	6 (25)

All patients underwent treatment without complication.

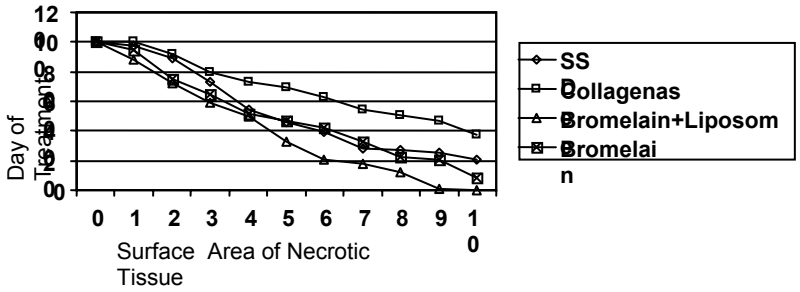

Figure 1. Mean surface area of necrotic tissue in groups of treatment

In group treated with SSD, the reduction of eschar surface area to 75% initial area was obtained on day III, on day V reduction of eschar surface area to 50% initial area was obtained and 25% initial area reduction of eschar surface area was obtained on day X. In group treated with collagenase, reduction of eschar surface area to 75% initial area was obtained on day IV , and 50% initial area was obtained on day IX. In group

treated with Bromelain, reduction of eschar surface area to 75% initial area was obtained on day II, 50% initial area was obtained on day V and 25% initial area was obtained on day VIII. In group treated with Bromelain + Liposome, reduction of eschar surface area to 75% initial area was obtained on day II, day IV obtained to 50% initial area of reduction and day VI obtained 25% initial area.

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Table 2.Change in surface area of necrotic tissue in groups of treatment

Days	Group							
	SSD		Collagenase		Bromelain		Brom+L	
	x±SD	P	x±SD	P	x±SD	P	X±SD	P
0/pre therapy	100	-	100	-	100	-	100	-
1	97.2±4.4	0.175	100±0	1.000	95.1±7.9	0.184	88.5±9.7	0.033
2	89.4±8.8	0.032	91.4±15.8	0.241	74.8±33.4	0.124	72.0±14.8	0.006
3	73.1±24.1	0.041	79.5±31.6	0.173	64.9±30.1	0.035	59.4±19.0	0.003
4	54.8±29.3	0.013	73.3±37.1	0.138	52.0±38.9	0.029	49.3±19.7	0.001
5	46.3±35.4	0.014	68.9±34.9	0.081	47.1±33.0	0.011	32.5±31.0	0.003
6	39.5±30.2	0.004	63.2±31.6	0.036	42.3±26.5	0.003	21.1±24.5	0.001
7	28.1±33.6	0.003	54.6±31.0	0.016	33.1±17.9	0.000	17.7±21.0	0.000
8	27.4±32.5	0.003	51.0±32.3	0.014	22.7±14.9	0.000	12.6±15.7	0.000
9	25.3±30.8	0.002	47.0±31.8	0.010	20.7±16.2	0.000	0.8±2.0	0.000
10	20.2±31.5	0.002	37.8±26.9	0.002	8.2±7.3	0.000	0.5±1.1	0.000

Tabel 3.Change in surface area of necrotic tissue between types of treatment

Days	Group				p Value
	SSD	Collagenase	Bromelain	Brom+L	
1	-2.9±4.4[a]	0.0±0.0 [a]	-5.0±7.9 [ab]	-11.6±9.7 [b]	0.042
2	-10.6±	-8.6±15.8	-25.2±33.4	-28.1±14.8	0.265
3	-27.0±	-20.5±31.6	-35.1±30.1	-40.6±19.0	0.585
4	-45.2±	-26.7±37.1	-48.0±38.9	-50.7±19.7	0.572
5	-53.8±	-31.1±34.9	-52.9±33.0	-67.6±31.0	0.332
6	-60.8±	-36.8±31.6	-57.7±26.5	-79.0±24.5	0.116
7	-72.0±	-45.4±31.0	-66.8±17.9	-82.3±21.0	0.141
8	-72.7±	-49.0±32.3	-77.3±14.9	-87.4±15.7	0.091
9	-74.7±30.8[ab]	-53.0±31.8[a]	-79.4±16.2[ab]	-99.2±2.0[ab]	0.025
10	-79.8±31.5[ab]	-62.2±26.9[a]	-91.8±7.3[ab]	-99.5±1.1[ab]	0.032

Note: Difference in superscript alphabets indicates significant difference at α =0.05

There was a significant difference in the surface areas of burn necrotic tissue between types of treatment on day I, IX and X (p value < 0.05). Results of multivariat test which controls degree of burn depth revealed that burn depth did not affect the speed of reduction of eschar surface area in which p value in Wilk's Lamba was 0.774.

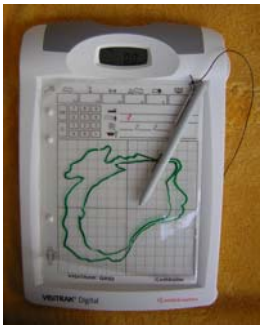

Figure 2. The instrument Visitrak Digital used to measure eschar surface area

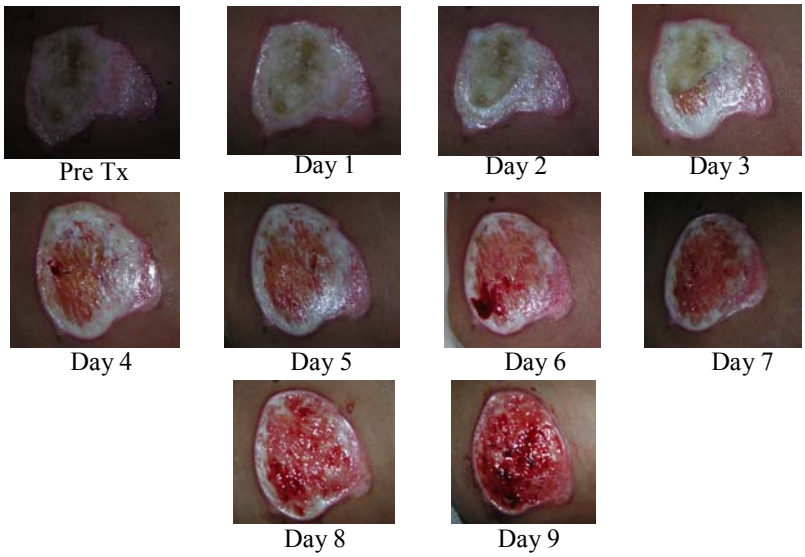

Figure 3.Sample treated with combined cream bromelain-liposome. On day VI, eschar surface area was significantly reduced to 1/3 initial area. On day IX, eschar was totally reduced.

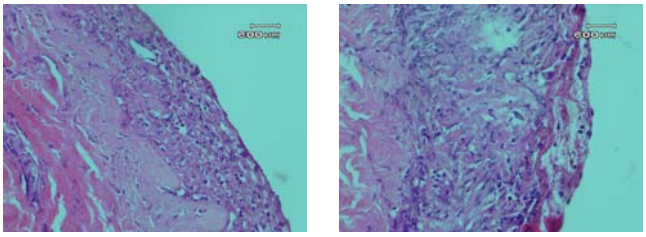

Figure 4.Histopathology preparation with HE staining and 100 X magnification before treatment and on day V post treatment day with bromelain-liposome. Lytic zones increased 2 times in width, characterized with necrotic collagen tissue and inflammatory cells.

Table 4.Lytic zone thickness in histopathologic examination of eschar

No	Patient	Treatment	Pre Treatment	Day V
1.	Ys	SSD	30 µ	90 µ
2.	At	SSD	20 µ	90 µ
3.	Ss	Collagenase	10 µ	10 µ
4.	Sw	Collagenase	-	5 µ
5.	ES	Bromelain	-	100 µ
6.	Bdy	Bromelain	-	-
7.	AS	Bromelain + Liposome	40 µ	80 µ
8.	Amd	Bromelain + Liposom	-	40 µ

DISCUSSION

The effectiveness of the agents used was evaluated clinically by measurement of eschar surface area reduction. Eschar removal was performed by enzymatic debridement in treatment using collagenase, bromelain and bromelain-liposome. Furthermore, debridement also occured by involving autolysis endogenic proteases. In moist condition, wound healing occurs 50% faster than in dry condition (Perdanakusuma 2005). In this research, enzymatic debridement with combined cream bromelain 10%-liposome 6% was fastest in reducing eschar surface area compared to bromelain cream 35%, collagenase ointment and SSD cream.

Collagenase was slowest in reducing eschar. Accordingly to literature, enzymatic debridement required approximately 10-12 days except with collagenase. Bromelain- liposome reduced eschar surface area to 25% initial area on day VI while with bromelain 35%, 25% reduction was attained on day VIII. Topical bromelain 35% removes eschar by the action of its non proteolytic or hydrolytic component, called escharase without degrading normal protein substrate. In deep burns, heat source exceeding 50°C causes necrosis of skin tissue or eschar due to protein coagulation. Eschar consists mainly of interstitial or fibrillar collagens type I and III, elastin fibers and proteoglycan.

Endogenically, collagenase endopeptidase enzyme is produced in the forms of MMP-1 by fibroblasts, MMP-8 by neutrophils and MMP-13. Collagenase of human skin is synthesized and secreted by skin fibroblasts in the form of zymogen which is a proenzyme with a molecular weight of 52,000 Dalton. This zymogen can not catalyze its substrate collagen and this procollagenase is activated into active collagenase by limited trypsin activated proteolysis (Jeffrey 1992). Bromelain with its non proteolytic component plays a role here to activate procollagenase into active collagenase.

This activated collagenase can then degrade necrotic collagen fibers between the eschar and viable tissue underneath, producing products which become degradable by other proteases in the tissue which are less specific. SSD and bromelain 35% yielded similar results which could be explained by the moist condition provided by SSD which was applied every day and by the application of occlusive dressing promoting autolytic debridement.

Moreover SSD has a very good spectrum of bactericidal activity. Silver in SSD also reduces excessive inflammation allowing faster autolysis, increases reepithelization and regulates the activity of metalloproteinase matrix.

In the majority of samples which underwent effective debridement, spontaneous epithelization followed. Variation in wound healing process was considered as the result of the various endogens in each individual. There was no complication from use of enzymatic debridement except minimal pain and bleeding. Liposome, a synthetic lipid bilayer molecule, as a carrier drug proved to be effective in enabling passive diffusion of bromelain through non vital stratum corneum and its localization to its target site at the layer of necrotic collagen. Thus, bromelain in only 10% concentration significantly yielded superior results compared to bromelain 35%.

The result of multivariat analysis with Wilks' Lambda p value of 0.774 showed that burn depths and total surface areas did not effect speed of eschar surface area reduction. Histopathology examination in 8 cases revealed significant changes in the groups treated with bromelain-liposome and SSD, demonstrated by a wide lytic zone consisting of collagen fibers and necrotic inflammatory cells. The finding of lytic zones in the SSD group before treatment could be explained by the process of autolysis which increased on post therapy day V.

CONCLUSION

Combined cream bromelain 10%-liposome 6% is effective was a debriding agent for deep burn patients enabling wound bed preparation so that wound could be covered by spontaneous epithelization or other modality. This agent was significantly more effective compared with bromelain 35% and collagenase ointment for enzymatic debridement of DBP. Combined cream bromelain 10% liposome 6% can be used as an alternative therapy for surgery in debridement of deep burns. This cream can also replace the widely known collagenase ointment yet very expensive and difficult to access. Considering that it is extracted from pineapple, hopefully it can be produced widely. We suggest further research to compare the efficacies of SSD cream, collagenase ointment, bromelain 35% and combined cream bromelain 10% liposome 6% can be performed using more homogeneous samples in terms of burn depths.

ACKNOWLEDGMENT

We are grateful to Prof. DR. Indro Handojo, dr., SpPK (K.lm) who has developed the debriding agent

combined cream bromelain 10%-liposome 6%. And also to the Research Project of Medical Research Unit, Airlangga University, Faculty of Medicine, Surabaya, Indonesia, 2006

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