Chapter 8

"GENOPEP", a Topical Cream in the Treatment of Burn Wounds

Jesse M. Jaynes*,[1] and V. Siva Rami Reddy[2]

[1]Tuskegee University, Integrative Biosciences, Tuskegee, AL 36811, United States
[2]Osmania Medical College, Plastic Surgery, Hyderabad 500 095, India
***E-mail: jjaynes@mytu.tuskegee.edu**

The loss of the skin's protective barrier as the result of burns fosters the susceptibility to bacterial infection, invasion, and sepsis. Infection remains the leading cause of death among patients who are hospitalized for burns. Current standards of treating the burned tissue have severe limitations and inherent risks of complications. Based upon the principles discovered in naturally occurring peptides, recent designs of synthetically engineered antimicrobial peptides have demonstrated increased potency and efficacy/tolerability, enhanced specificity, and reduced toxicity in comparison to the extant burn treatment modalities. These peptides termed as designed antimicrobial peptides (dAMP), are resistant to such effects of high solute levels and demonstrate even greater antibacterial activity than traditional antibiotics. One such peptide, GENOPEP, has shown significant antimicrobial activity and accelerated wound healing and is the first such peptide to be used to treat burns in humans. The impact of this treatment could improve patient survival or quality of life and reduce costs to the patient, their family, hospital and society.

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Introduction

The loss of the skin's protective barrier as the result of burns fosters the susceptibility of the wound to bacterial infection, invasion, and sepsis. Infection remains the leading cause of death among patients who are hospitalized for burns. The risk of burn wound infection is directly related to the extent of the burn (first degree burn; second degree burn; third degree burn) and is related to impaired resistance due to disruption of the skin's mechanical integrity and generalized immune suppression (*1–3*).

Current standards of treating the burned tissue include applying topical antibiotics such as silver sulfadiazine, mafenide acetate, or silver nitrate to the burn wounds to help prevent massive bacterial invasion and sepsis, and use of oral or intravenous antibiotics. Unfortunately, each of these agents has its limitations and inherent risk of complications (*4–7*).

The use of silver sulfadiazine, for example, has been demonstrated to increase wound epithelialization but can impair wound contraction (*8*). Mafenide acetate has been demonstrated to enhance angiogenesis, epithelialization, and dermal thickening in some studies, while in others it has been linked to decreases in keratinocyte growth rates and is a known source of metabolic acidosis through its inhibition of carbonic anhydrase (*9*, *10*). Both of these agents have a limited spectrum of antibacterial activity.

Other topical agents used to decrease the wound bacterial load have included Dakin's (sodium hypochlorite) solution, betadine, acetic acid, and hydrogen peroxide. Dakin's solution exhibits deleterious effects to fibroblasts and endothelial cells and can impair neutrophil migration and wound neovascularization (*11*). Studies on Betadine have shown slower rates of re-epithelialization compared to other topical antimicrobial agents and impairment of microcirculation at higher levels of concentration (*12*). Acetic acid alternatively does not demonstrate effective control to keep bacterial levels at less than 10^5 colonies per gram of tissue and is cytotoxic at its traditionally used concentration of 0.25% (*13*) . Hydrogen peroxide can also be toxic to fibroblasts (*14*).

Oral or intravenous antibiotics are often used in conjunction with topical antimicrobials to decrease the bacterial burden on tissue. As more focus is centered on the problem of multi-drug resistant bacteria, choices for effective selection of antimicrobial agents can become limited. Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin resistant Enterococcus faecium/faecalis (VRE) are two very resistant bacterial strains that are difficult to treat with current antibiotics (*15–17*).

Furthermore, these resistant bacteria have the potential of fostering cross-resistance through plasmid transfer (*18*). Transmission of multi-resistant organisms to other patients, particularly in contained burn units, not only increases morbidity, but also adds an enormous cost to the hospitals and society (*19*, *20*). One percent of all patient discharges from the hospital have ongoing Staphylococcus aureus infections (*21*). The hospital costs for people with Staphylococcus aureus infections were twice those of other patients (*22*). Clearly

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the need for effective antimicrobial agents is urgent as drug resistance continues to emerge.

It is clear that the topical agents are crucial in the ultimate eradication of the burn and infected wound pathogens since it is extremely difficult to administer the intravenous antibiotics to non-perfused tissue such as burned skin. The poorly vascularized, burned skin is, therefore, the portal of entry and the ongoing nidus of infection for burn victims. The ideal topical agent should be highly active against common and multi-resistant pathogens, such as methicillin resistant Staphylococcus aureus, vancomycin resistant Enterococcus faecium/faecalis, and extended spectrum β-lactamase producing Gram-negative organisms, while having a neutral or even beneficial effect on the wound healing process.

Antimicrobial peptides represent a relatively new discovery in the immune system pathway. These small peptides are inducible elements of the immune system that serve as nonspecific effector molecules to eradicate infection caused by bacteria, yeast, and viruses, protecting host epithelial surfaces such as the tracheal mucous membrane and genitourinary tract (*23–26*). In mammals, several of these compounds are known to be present in high concentrations in neutrophilic granules and phagocyte vacuoles. These peptides differ significantly in their structure between species but, in common, appear to create amphipathic helical or beta-pleated structures. The mechanism of action is different from currently utilized antibiotics and appears to be based on their ability to insert into membranes, from channels or "pores", and destroy the cell by changing membrane conductance and altering intracellular function (*27*, *28*).

Based upon the principles discovered in the naturally occurring peptides, recent designs of synthetically engineered antimicrobial peptides have demonstrated increased potency and efficacy/tolerability, enhanced specificity, and reduced toxicity in comparison (*28–38*). These peptides termed as designed antimicrobial peptides (dAMP), are resistant to such effects of high solute levels and demonstrate even greater antibacterial activity (*39*). One such peptide, GENOPEP, has shown significant promise in in vitro studies against a large number of pathogens and is very solute resistant. GENOPEP is the trade name for a gel preparation containing the dAMP D2A21 that has been shown to improve survival and wound re-epithelization of full-thickness burns in rats compared to control treatments: vehicle, SSD and Sulfamylon (*40*, *41*).

This antimicrobial peptide shows sigificant promise in treating patients with chronic wounds or burn wound sepsis. The impact of this could improve patient survival or quality of life and reduce costs to the patient, their family, hospital and society (*40*, *41*).

Methodology

In Vitro Antimicrobial Studies Using GENOPEP

The proprietary test compound 'GENOPEP' showed high antibacterial activity on test organisms Staphylococcus aureus MTCC 96 and Pseudomonas aeroginosa MTCC 741. The test compound showed 100 % killing of Staphylococcus aureus on exposure to 1 μM (4.3 μg/ml) and 5 μM (21.5 μg/ml) concentrations for 1 hr at pH 7.2, and at pH 8.4 an exposure of 4 hrs was required to get 100% killing. Whereas, 100% killing of Pseudomonas aeruginosa was observed on exposure to the test compound for 1 hr at pH 8.4 and an exposure of 4 hrs was required for 100% killing at pH 7.2.

The microbiological studies with GENOPEP in vivo using a rat burn wound model were conducted. The observations on the bacterial growth in eschar and sub-eschar muscles on post burn day one, two or three in peptide treated and control treated groups were made. A substantial decrease in the microbial population level was observed in animals treated with peptide (unpublished pre-clinical studies).

Animal Studies Using GENOPEP

Sub-acute toxicity studies (conducted using well-established protocols) of GENOPEP in rats and rabbits demonstrated its safety when used topically. No abnormalities in physical, physiological, biochemical and histo-pathological parameters were observed by the topical application of the peptide. No mortalities in animals of any group were observed (unpublished pre-clinical studies).

There is evidence (dermal histopathology findings) to show that GENOPEP has stimulatory action on tissue growth (increased collagen content in granulation tissue and re-epithelialization) thus promoting improved wound healing (unpublished pre-clinical studies).

Phase-I Clinical Studies

The results of Phase-I clinical trial on healthy human patients revealed that GENOPEP cream administered topically twice a day was safe. GENOPEP was safe and adverse events were found to be minimal in the Phase-I Study. Treatment Groups were similar in efficacy/tolerability and safety parameters studied. With the conclusion of this study, GENOPEP cream was allowed to proceed to to Phase-II clinical trils as per Schedule Y (Amendment 2005) of Drugs and Cosmetics Rule 1940.

Phase II/III Study Design

The study was a double blind, randomized and placebo treatment controlled study in India. The study aimed to evaluate the efficacy of GENOPEP Cream in the treatment of burn wounds. Figure 1 describes the trial design in a schematic diagram.

Testing Procedures

Test Drug

GENOPEP 0.02% & GENOPEP 0.05%.

Placebo Treatment

GENOPEP Base

Dosing

Group 1: GENOPEP cream 0.02%, half a gram/cm2 applied every alternate day for 21 days or Healing of wound which ever was earlier.

Group 2: GENOPEP cream 0.05%, half a gram/cm2 applied every alternate day for 21 days or Healing of wound which ever was earlier.

Group 3: Placebo, half a gram/cm2 applied every alternate day for 21 days or Healing of wound whichever was earlier.

Method of Administration and Instructions for Use

Selected Patients instructed to report to the investigator every alternate day in the morning

Site of Application

Apply the prescribed treatment to the patient on wound area

Measurements (Area of Application)

Complete Wound Area

Procedure

After thorough cleaning of the site of application, the given formulation was applied uniformly in complete burn wound area. The site was covered with sterile pad and bandage. The patient was instructed to report any adverse event either to the investigator or the study personnel.

Duration of Treatment

21days (11 Visits) or Healing of the Burn Wound which ever was earlier.

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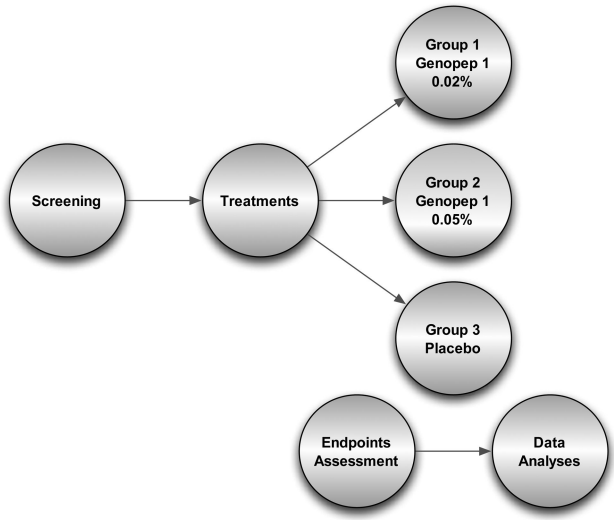

Figure 1. Trial design flow diagram.

Evaluation Criteria

Efficacy/Tolerability Variables

Primary End Point: The primary endpoint of the treatment was taken as complete closure / healing of the wound. At each visit form visit 2 the functional assessment of the wound was determined using the following scale below.

100 % wound closure: with complete epithelialization and no drainage or scab present

Less than 100% closure with drainage present.

The primary efficacy criteria are defined as the percentage of patients achieving a complete wound closure (functional assessment of score of 0) within the three-week treatment period. If a score of 0 is achieved for any patient then the medication will be stopped and recorded in the CRF as having reached the primary endpoint.

In addition to complete closure of the wound the endpoint of the treatment also considered the following:

Extent of non-viable tissue by clinical evaluation % of wound covered with non-viable tissue

- 76-100%
- 51-75%
- 26-50%
- 1-25%
- No Necrotic Tissue

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Degree of granulation by visual Score % of wound filled with granulation tissue

- No Granulation
- Scanty Granulation
- Healthy Granulation

Besides the above parameters for an assessment for the primary efficacy, the secondary efficacy is assessed based on average wound evaluation score.

Wound Evaluation Score

Wound Evaluation Done on Four Parameters:

- Erythema (redness of the skin caused by dilatation and congestion of the capillaries, often a sign of inflammation or infection)
- Edema (excessive accumulation of serous fluid in tissue spaces)
- Purulence (the state or condition of containing or secreting pus)
- Necrotic Tissue (dead, devitalized tissue)

Each of these parameters is measured on a scale of 0-3 as follows: 0 = Absent; 1 = Mild; 2 = Moderate; 3 = Severe.

A Wound Evaluation Score (WES) of 0 is considered as a secondary efficacy criterion. The closer this score is to 0 the more significant the healing and revitalization of the wound.

Statistical Methods

The study aims to evaluate the safety and efficacy of treatment in three groups. The primary end point parameters of patients with epithelialization/healing of wound in different groups was assessed and analyzed by $\chi 2$ test to hypothesis testing between groups to measure the efficacy of the test groups and for the complete healing of patients. The Secondary efficacy variable being a categorical variable, the difference was analyzed by $\chi 2$ test. Safety analysis with $\chi 2$ test for categorical variables and GLM (ANOVA) for continuous variables were conducted. Statistical significance was considered when P value is < 0.05.

Assessment Schedule

After screening, the patients were allotted to Treatment Groups as per the randomization schedule. The assessment schedule for all three groups was the day of reporting burn wound i.e. on 0th day, 12th day and 20th day. The maximum number of visits was expected were 11 during the study period of 21 days. The assessment schedule, major study milestones and drug description are given in Figure 2 and Tables I & II, respectively.

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Table I

Step	*Milestone*	*Dates*
1	Filing of Clinical Trial Protocol	August 2007
2	Clinical Trial Protocol Approval	August 2007
3	Investigators Meeting	October 2007
4	IRB/EC Approval	November 2007
5	Site Initiation	November 2007
6	Patient Screening and Recruitment	November 2007
7	Last Patient In	September 2008
8	Last Patient Out	September 2008
9	Trial Report	March 2010
10	Report Submission	May 2010

Table II

Item	*Description*
Study Drugs	GENOPEP Cream 0.02%, 0.05% and Base
Manufacturer	ISSAR Pharmaceuticals
Purity	97.8%
How Supplied	5 gm tubes
Precautions	Test at Room Temperature
Shelf Life	24 Months at Room Temperature
Route of Administration	Topical Cream
Dosing	Sufficient for Burn Wound
Contraindications	Nil
Drug Interactions	None
Use During Pregnancy	Can be Used
Drug Supplies & Labels	Yes as per Stipulated Guidelines
Drug Accountability	Yes
Intercurrent Illness	Yes

Assessment Schedule

The study was conducted at Osmania General Hospital, Hyderabad on 60 patients. Twenty patients each on 0.02% & 0.05% peptide containing cream and Placebo Treatment Groups formed the study samples.

Criteria for Inclusion or Exclusion

Inclusion Criteria:

- Adult male or female patients aged above 18 years of age.
- Patients with partial thickness burn wounds.
- Total surface area of the burn less than 20%
- Willing to give written informed consent.

Exclusion Criteria

- Patients with more than 20% of burns.
- Patients with full thickness burns
- Patients who need skin grafting.
- Patients with diabetes.
- Immune compromised patients.
- Patients with infectious diseases.

Disposition of Subjects

The efficacy data was analyzed for evaluable patients. Table III shows the number of subjects and the reasons for excluding the subjects from the data set for evaluable subjects. Thus, a total of 60 subjects were included and completed this study.

Table III. Disposition of Subjects

	0.02% GENOPEP	*0.05% GENOPEP*	*Placebo*
Number Treated	20	20	20
Non Compliance	0	0	0
Efficacy/Tolerability	20	20	20
Number Completed	20	20	20
Number Withdrawn	0	0	0
Absence to Treatment	4	3	4

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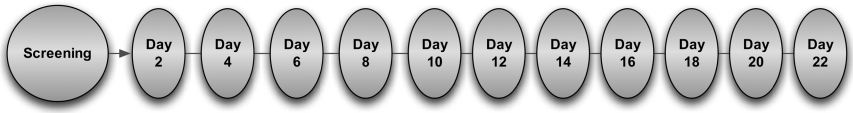

Figure 2. Days of assessment.

Demography

The data was analyzed at visit 1 (baseline) with respect to demographic characteristics. There was no significant statistical difference observed between Drug groups in the parameters such as Age, Weight and Height. The Age group ranged from 18 to 72 years, the majority belonged to age group 18-48 years, the weight ranged from 40 kg to 92 kg and height ranged from 142 cm to 176 cm (Table IV).

Table IV. Demography of Subjects

Group	*N*	*Variable*	*Mean*	*SD*	*Minimum*	*Maximum*
GENOPEP-0.02%	20	Age	29.35	9.40	18.00	48.00
		Weight	60.15	11.44	44.00	84.00
		Height	159.25	7.85	142.00	176.00
GENOPEP-0.05%	20	Age	28.35	7.32	18.00	45.00
		Weight	61.80	13.37	46.00	89.00
		Height	159.65	6.92	148.00	174.00
Placebo	20	Age	31.90	11.41	18.00	55.00
		Weight	64.15	12.51	48.00	92.00
		Height	160.60	6.49	146.00	175.00

Burn Characteristics

The characteristics of the burns at baseline (visit-1) are presented in Table V below. Nearly 90% had multiple burns. As per inclusion criteria only patients with ≤20% burn were selected into the study and were assigned at random to the treatment groups. The percent of the burns ranged from 3 to 20%. The average burn size was 17.25% in the GENOPEP 0.02% group, 15.55% in the GENOPEP 0.05 % group and 18.75% in the Placebo Group of the total body surface area for each patient. By Analysis of Variance (ANOVA) the group means were found to be statistically non-significant. Thus indicating the groups were similar in burn characteristics at visit-1.

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Table V. Mean Percentage of Burns by Group

Group	*N*	*Mean*	*SD*	*Minimum*	*Maximum*
GENOPEP-0.02%	20	17.25%	4.70	3.00	20.00
GENOPEP-0.05%	20	15.55%	6.26	3.00	20.00
Placebo	20	18.75%	3.02	9.00	20.00

Results and Conclusions

Primary Efficacy/Tolerability Conclusions

Primary efficacy assessment was carried out on the patients with epithelialization/healing of the wound. Statistical significance was considered at $P<0.05$ assuming a null hypothesis that the efficacy parameter was significantly different among Treatment Groups. To determine the effective dose and regimen, the above analysis was performed between placebo, 0.02% Peptide and 0.05% Peptide Treatment Groups.

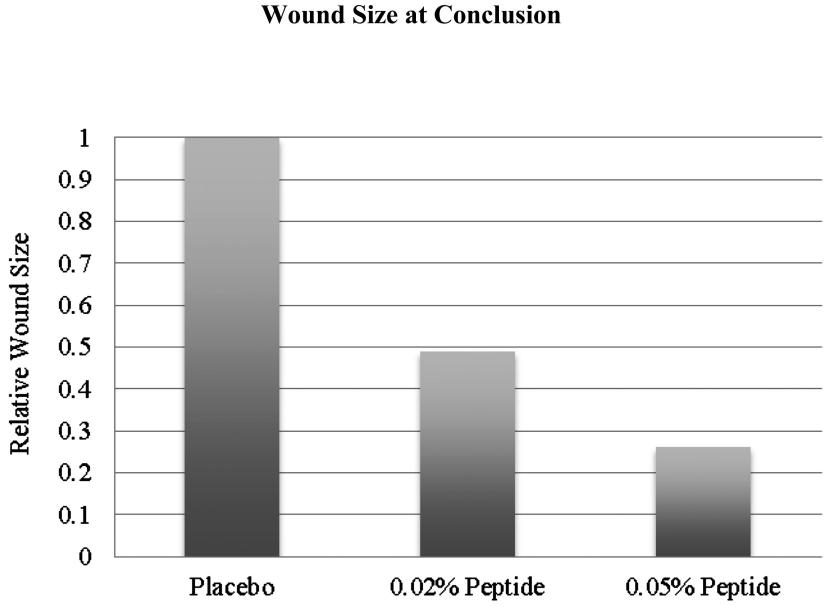

Figure 3. Both peptide treated groups achieved accelerated wound healing from that of the placebo with a greater level of significance than $P<0.05$ (0.02% peptide group $P<0.011$ and 0.05% peptide group $P<0.0044$).

Table VI. Wound Evaluation Score

Group	*Ery-thema*		*Edema*		*Purulence*		*Necrosis*		*WES*		*Ave WES*	
Visit	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last
0.02% Pep	44	1	21	0	0	3	60	7	125	11	6.25	0.55
0.05% Pep	50	0	17	0	0	2	60	5	128	7	6.40	0.35
Placebo	41	1	12	2	0	9	60	14	113	26	5.65	1.30

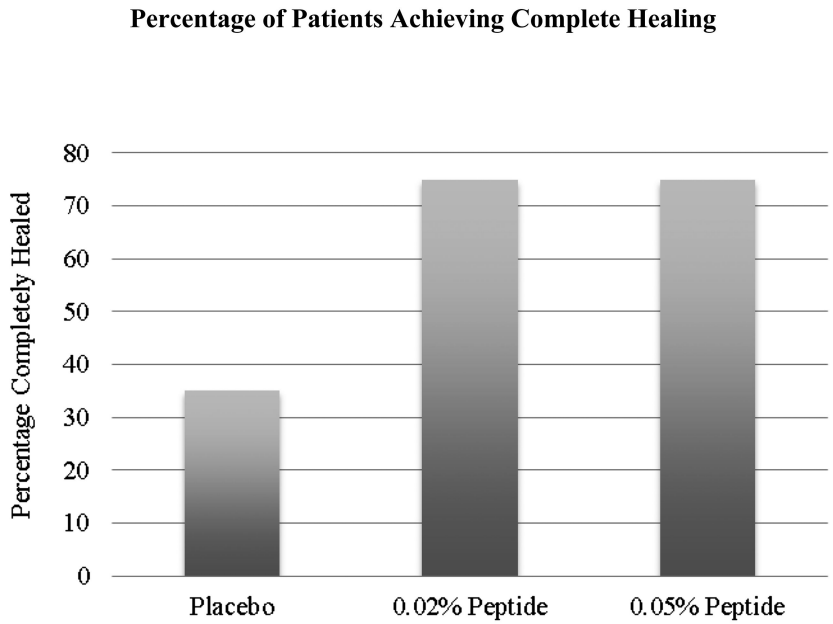

Figure 4. Both peptide treated groups achieved a greater number of patients that were completely healed than the placebo with a lower than P<0.05 level of significance (both 0.02% and 0.05% at P<0.011).

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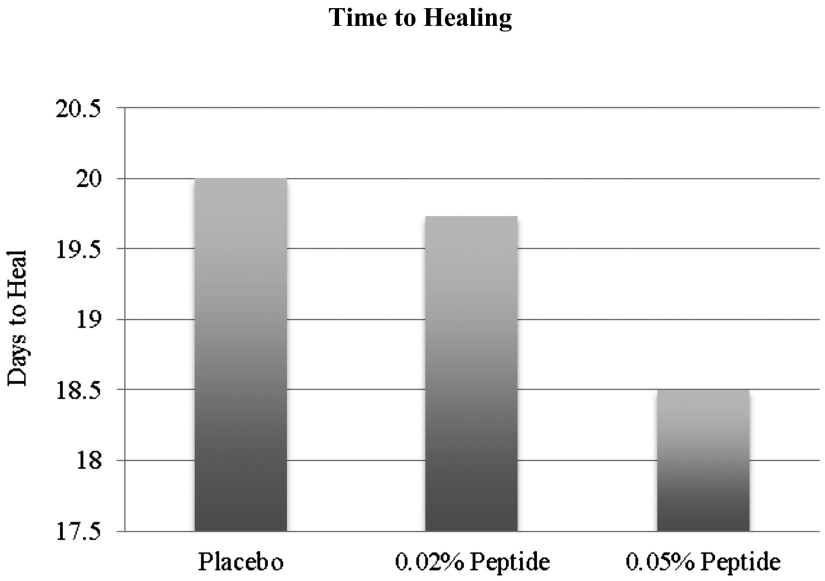

Figure 5. Both peptide treated groups achieved a lower average time of healing. A significant difference was seen between the 0.05% peptide group and Placebo treatment with a P<0.017.

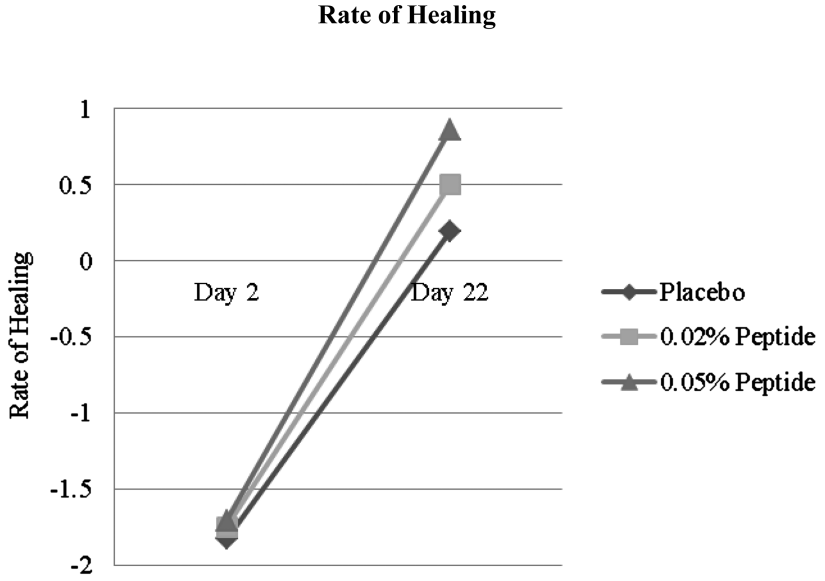

Figure 6. Both peptide treated groups achieved a lower average time of healing and an increase in the rate of wound closure than that of the Placebo. A significant difference was seen between the 0.05% peptide and Placebo Treatments P<0.05.

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Secondary Efficacy/Tolerability Conclusions

Descriptive statistics were calculated for all the secondary efficacy variables and compared between groups at endpoint using Analysis of Variance for significance between groups at $P < 0.05$.

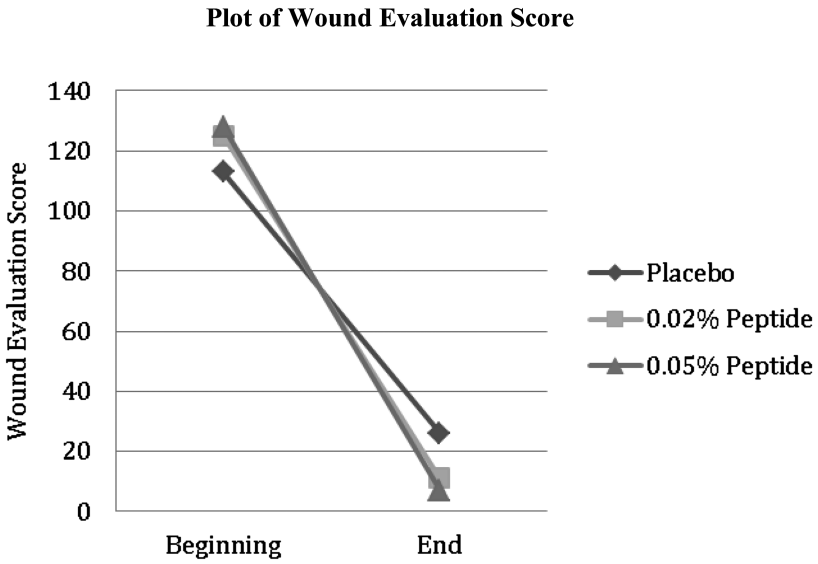

Figure 7. Wound Evaluation Scores of all groups were calculated and showed that there was statistical significance (for both groups P<0.011) between the peptide treated groups and the placebo group on the last day of evaluation (Table VI and The rate at which the WES changed over time) is shown.

The closer to 0 indicates more positive characteristics of wound healing with 0 being completely healed.

Figure 8 A and B demonstrate visually the difference between groups with A = Placebo, B = 0.02% Peptide and C = 0.05% Peptide.

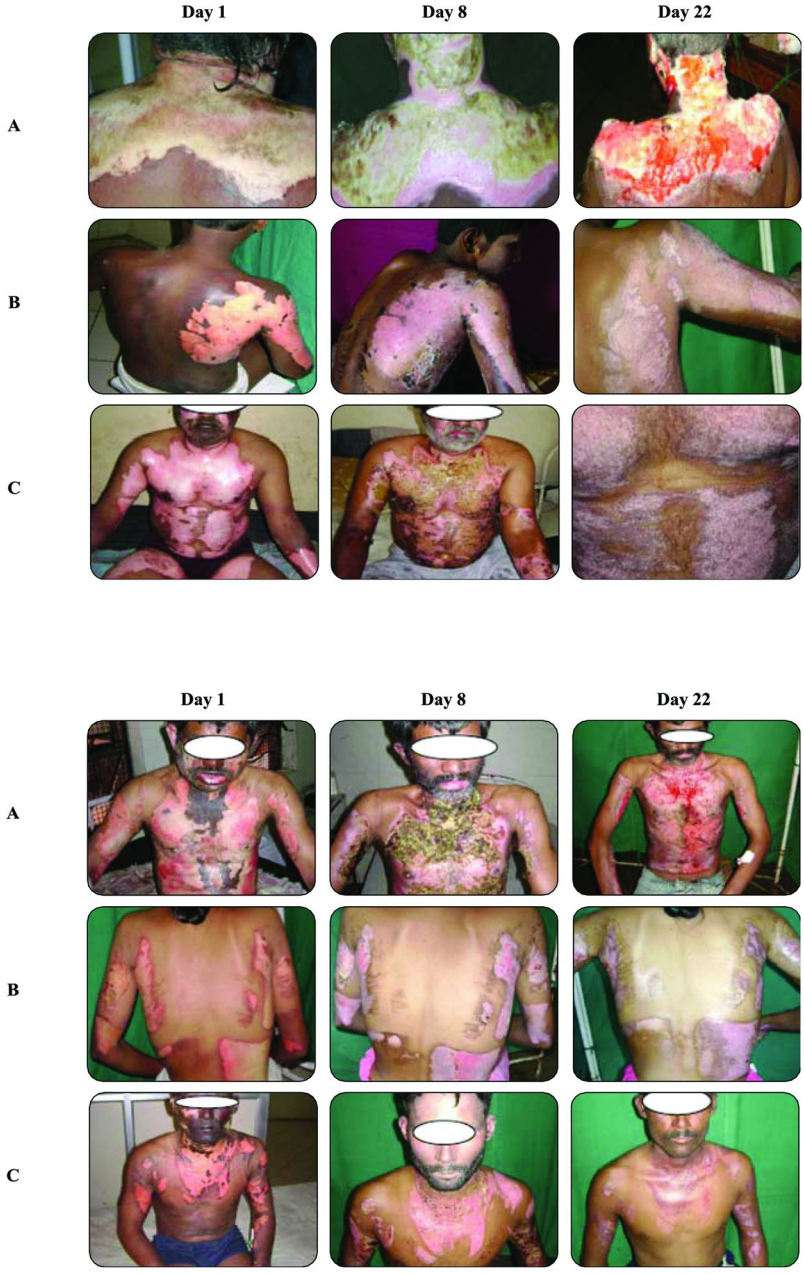

Figure 8. In A and B, the increased healing can be clearly seen in the 0.02% and 0.05% peptide groups while the Placebo Group is clearly lagging in healing rate. (see color insert)

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Safety Conclusions

The lab investigations included standard hematology and biochemical parameters. These investigations were used to assess the safety of the product. These conclusions were reached at both hospitals

General Linear Model (GLM) analysis was done to test the hypothesis that the lab-investigations were similar between baseline and study termination and there was no statistically significant difference found. However, a significant change between baseline and study termination in the leukocytes was observed.

It was observed that except the one variable (Total Protein) others were statistically non significant among the Treatment Groups (GENOPEP 0.02 %, GENOPEP 0.05% & Placebo).

Hence the overall results indicate that the safety variables are similar between time points and between groups.

The Vital signs includes Blood Pleasure, Pulse Rate, Heart Rate, Respiratory Rate and Temperature. These vital signs were used to assess the safety of the product and two sets of vital sign measurements were taken, one at the time of the baseline (visti-1) and another at the time of the termination visit. The General Linear Model (GLM) analysis was done to test the hypothesis that the vital sign measures are similar between base line and study termination. In about 11 units in 0.02% GENOPEP and 8 units in GENOPEP 0.05% as well as Placebo Group's subjects of pulse and heart rate reduction were observed form the baseline to termination day, however, they were all within the normal range.

It was observed that none of the variables were statistically significant between the Treatment Groups. Hence the overall results indicate the safety variables were similar between time points and between groups.

The Pharmacokinetic evaluation showed that the drug was not absorbed into the system as it was not detected in the serum samples of patients.

Summary and Final Conclusions

These double blind studies were conducted on 60 patients who were above 18 years of age with less than or equal to 20% partial thickness burns. They were randomly divided into three study groups of 20 patients each. The primary end point taken was complete wound closure or complete healing of burns of study subjects and the secondary end point was added to assist in the complete wound healing of the patient.

Twenty five percent of the patients in the Placebo Group completely healed while, remarkably, 15 (75%) patients in the GENOPEP 0.02% Peptide Treatment Group and 15 (75%) patients in the GENOPEP 0.05% Peptide Treatment Group completely healed in the stipulated study time period.

It was also found that in the GENOPEP Peptide Treatment Groups, there was significantly decreased time to healing from that of the Placebo Groups.

In the GENOPEP Peptide Treatment Groups, the incidence rate of wound healing was better as the scar formation was significantly lower compared to that in the Placebo Group, indicating that treatment with GENOPEP enables better healing with less morbidity.

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Treatment compliance was good and there were no side effects or adverse reactions or toxic effects noted in hematological or in biochemical tests with both study groups as compared with the Placebo Groups.

The pharmacokinetic samples, at 0 hr, 30 mins and study termination day, showed that there was no drug present in the sera found in patients at both study sites.

It is clear that the GENOPEP medication can be used as a long-term medication for burn patients without any side effects. It is concluded that the GENOPEP cream is safe and is highly effective in promoting burn wound healing (compared to vehicle control) for patients with partial thickness burns that are less than or equal to 20% without any side effects even if the drug is used as a longer term medication.

Therefore, it is worthwhile studying the efficacy and safety of the GENOPEP cream in both 0.02% & 0.05% forms in treating larger groups of burn patients with more than 20% partial thickness burns and comparing it to the standard treatment with SSD or Sulfamylon. These proposed studies are underway.

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