

Effect of topical sodium fusidate, calcium mupirocin and papain—urea on wound healing in diabetic wistar rats

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ABSTRACT

Background: Poor wound healing is a complication of diabetes mellitus. Various agents have been used to promote wound healing in diabetic patients. Papain urea is used as a sloughing agent, whereas sodium fusidate and calcium mupirocin are used as topical antimicrobials for wounds in diabetics. **Aims and Objective:** To evaluate effect of topical sodium fusidate, calcium mupirocin, and papain—urea on wound healing in diabetic wistar rats. **Materials and Methods:** Incision and excision wound models were used. Each model had five groups of six rats each and one group was nondiabetic control. Diabetes was induced in the remaining four groups, one diabetic control, and three test groups in each model, using streptozotocin (30 mg/kg intraperitoneally). Wounds were made in diabetic rats with blood glucose levels of more than 250 mg/dL. Paraffin ointment base was applied to wounds of nondiabetic and diabetic control, whereas sodium fusidate, mupirocin, and papain—urea were applied topically to three test groups, respectively, in each model. **Result:** In sodium fusidate-treated group, there was a significant increase in breaking strength of incision wound, contraction rate, and hydroxyproline content of excision wound as compared to diabetic control group. There was significant decrease in period of epithelization in sodium fusidate-treated group compared to diabetic control group. There was no significant alteration in wound healing by mupirocin and papain—urea. **Conclusion:** Topical application of sodium fusidate promoted wound healing in diabetic rats.

KEY WORDS: Breaking strength; Epithelization; Wound contraction; Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is an important cause of poor wound healing.^[1] Hyperglycemia, neurological, and vascular complications play an important role in delayed healing of wounds in

diabetics.^[2] A decrease in growth factor production, function of macrophage, angiogenesis, and collagen production also contribute to impaired wound healing in diabetes.^[3–5] In diabetics, measures to promote healing of wounds include controlling blood glucose levels, dietary modifications, cleaning of wounds and debridement (wherever necessary), eradication of infection, and relieving pressure on foot ulcers.^[6,7] Despite treatment with antidiabetic agents, poor glycemic control remains a major problem and some patients with diabetes will have nonhealing wounds. About 15% patients with diabetes may develop foot ulcers due to poor wound healing.^[7]

A combination of papain—urea has been used in the debridement of diabetic foot.^[6,8] Papain is a proteolytic enzyme, whereas urea is a chemical agent which denatures

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nonviable protein. Though not recommended, various topical antimicrobials such as calcium mupirocin, sodium fusidate, framycetin, bacitracin, and neomycin are prescribed for prophylactic use for uncontaminated wounds.^[9] Sodium fusidate is used topically but may also be given systemically for wound infections.^[10,11] Mupirocin is indicated for the treatment of furuncles, impetigo, and traumatic skin lesions.^[12] But there has been conflicting evidence regarding their effectiveness in preventing wound infection. Studies have demonstrated that topical sodium fusidate and calcium mupirocin have a favorable effect on period of epithelization, wound contraction rate, and collagen content of burn wound in rats.^[13,14] Hence, this study was aimed to determine the effect of topical sodium fusidate, calcium mupirocin, and papain-urea on healing of incision and excision wounds in rats with diabetes.

MATERIALS AND METHODS

Animals

The study was carried out after obtaining approval from the Institutional Animal Ethics Committee (IAEC), Manipal. Male and female wistar, albino rats each weighing about 150–200 g were used in the study. They were housed individually in polypropylene cages and maintained at a temperature of $27^{\circ} \pm 3^{\circ}\text{C}$, relative humidity of $60 \pm 10\%$ and 12 h light/dark cycle in the Central Animal Research Facility, Manipal. Animal care and handling was carried out according to guidelines set by the committee for the purpose of control and supervision of experiments on animals (CPCSEA). All chemicals used in the study were purchased.

Induction of Diabetes Mellitus

Streptozotocin (STZ) (Sigma Chemicals, USA) solution was prepared by dissolving 600 mg in 0.1M of freshly prepared ice-cold isocitrate buffer (pH – 4.5) solution. All rats were fasted overnight and blood samples were collected from the tail vein of all the rats. Fasting blood glucose levels were estimated using glucose strips and Accu-Chek glucometer in rats. Then, STZ was administered intraperitoneally in a dose of 30 mg/kg body weight of the rat.^[15] On the third, fifth, and seventh day following STZ administration, fasting blood glucose level was estimated in rats. Rats with stable blood glucose levels above 250 mg/dL on seventh day were considered as diabetic and used in the experiment.^[16]

Experimental Design

Incision and excision wound models were used. A total of 90 rats were used. Five groups of six animals each (total 30 rats) were used in incision wound model. Five groups of 12 rats each were used in excision wound model. The groups in each model and drugs administered were as follows:

Control (nondiabetic) group – Topical paraffin ointment base

Control (diabetic) group – Topical paraffin ointment base

Test group – Topical calcium mupirocin 2% (GlaxoSmithKline Pharmaceuticals, Mumbai)

Test group – Topical sodium fusidate 2% (Ranbaxy Pharmaceuticals, Goa)

Test group – Topical papain-urea (Lupin Pharmaceuticals, Mumbai)

Incision wound

Incision wound model was utilized to study the breaking strength of the wound in rats. An incision wound was made on the seventh day after induction of diabetes. Ketamine (Sun Pharmaceuticals, Mumbai) was the anesthetic agent (80 mg/kg intraperitoneally) used. The dorsum of rats was shaved cleanly. Two straight incisions, each 6 cm, were made on the back of the rats, 1 cm on each side of the vertebral column under aseptic conditions.^[17] They were closed with intermittent sutures. The drugs were applied topically once a day from day 0 (day of wounding) upto day 9 (postwounding day). Sutures were removed on day 7 (postwounding day). The rats were sacrificed on day 10 using excess ketamine. The breaking strength of the wound was measured on both sides on day 10 (postwounding) by continuous constant waterflow technique of Lee.^[18] Three readings were taken for incision wound on each side thus obtaining six readings for each rat. The mean breaking strength (g) of each rat was calculated.

Excision wound

Excision wound model was used to study the effect of drugs on the rate of wound contraction and time required for full epithelization of the wound. An excision wound was made on the seventh day after induction of diabetes. Animals were anesthetized using ketamine (80 mg/kg) intraperitoneally. After shaving the area, a round seal of 2.5 cm diameter was used to make an impression 5 cm away from the ears, in the dorsal interscapular region. Then full thickness skin from the area was excised to get a wound measuring 400 mm² and 2 mm depth.^[19] The drugs were applied topically from day 0 (day of wounding) till day 21 or till the wound was completely healed, whichever was earlier. The rate of wound contraction was studied by tracing the raw wound area on a butter paper on every alternate day starting from day of wounding till 21st postoperative day or till the wound was completely healed, whichever occurred earlier. The measured wound area was transferred to 1 mm² graph sheets. The wound contraction was calculated as percentage of original wound size (400 mm²) taken as 100% for each animal.^[19,20] The mean of the group was calculated for 4th, 8th, 12th, and 16th day for final analysis of results.

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Formation of scar along with absence of raw wound area indicated complete epithelization. The number of days required for eschar to fall away leaving behind no raw wound was taken as the period of epithelization.^[19] Using a punch biopsy needle

Table 2: Effect of topical sodium fusidate, mupirocin, and papain-urea on wound contraction rate and period of epithelization of an excision wound in diabetic rats

Group	Drugs	Wound contraction rate (%)				Period of epithelization (days)
		Day 4	Day 8	Day 12	Day 16	
1	Paraffin (nondiabetic control)	5.75 ± 0.94	34.33 ± 5.77	80.12 ± 2.01	97.16 ± 1.11	18.16 ± 0.47
2	Paraffin (diabetic control)	9.91 ± 2.05	31.04 ± 1.91	74.2 ± 1.22	80.35 ± 1.15 ^a	22.35 ± 0.44
3	Calcium mupirocin	20.50 ± 2.74 ^{a,b}	71.2 ± 2.96 ^{a,b}	80.62 ± 2.79	90.08 ± 2.02	19.0 ± 0.77
4	Sodium fusidate	19.75 ± 1.16 ^{a,b}	74.37 ± 1.66 ^{a,b}	85.50 ± 1.43 ^b	98.95 ± 0.67 ^b	17.0 ± 0.44 ^a
5	Papain-urea	14.0 ± 1.17 ^a	60.16 ± 1.67 ^{a,b}	75.58 ± 2.61	87.08 ± 2.68	20.50 ± 1.31

Values are expressed as Mean ± SEM.

^a*p* < 0.05 versus nondiabetic control; ^b*p* < 0.05 versus diabetic control.

(5 mm), granulation tissue was obtained from six rats in each group under ketamine anesthesia (80 mg/kg intraperitoneally) on 10th postwounding day. Histological examination was conducted using hematoxylin and eosin stain. Estimation of hydroxyproline was done in the granulation tissue obtained. [21,22]

Statistical Analysis

Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's posthoc test. A *p*-value < 0.05 was considered as statistically significant.

RESULT

Incision Wound Model

The mean breaking strength of a 10 day old wound was measured. In rats treated with sodium fusidate, it was significantly (*p* < 0.05) increased to 301.66 ± 7.65 g as compared to diabetic control group (235.0 ± 8.23 g). It was not significantly altered in groups treated with calcium mupirocin or papain-urea (Table 1).

Excision Wound Model

Sodium fusidate significantly (*p* < 0.05) increased the rate of wound contraction on 4th, 8th, 12th, and 16th day as compared

to the diabetic control group (Table 2). There was a significant (*p* < 0.05) increase in wound contraction rate on the 8th day in rats treated with calcium mupirocin and papain-urea as compared to diabetic control, but no significant changes were seen on 12th and 16th days in these groups (Table 2).

The mean period of epithelization in diabetic control group was 22.35 ± 0.44 days. It was significantly (*p* < 0.05) decreased in sodium fusidate treated group but not significantly altered in groups treated with mupirocin and papain-urea (Table 2).

Hydroxyproline Content in Granulation Tissue of Excision Wound

The hydroxyproline content in diabetic control group was 101.15 ± 1.35 mg/g of tissue which was significantly (*p* < 0.05) less as compared to nondiabetic control group. The hydroxyproline content in granulation tissue of rats treated with sodium fusidate was significantly (*p* < 0.05) high, 154.5 ± 0.80 mg/g, as compared to diabetic control group (Table 3).

Histopathology Studies

Granulation tissue of nondiabetic control group (Figure 1) showed less macrophages, moderate collagen fibers, and fibroblasts, whereas that of diabetic control group (Figure 2) had more

Table 1: Effect of topical sodium fusidate, mupirocin, and papain-urea on breaking strength of incision wound in diabetic rats

Group	Drug	Breaking strength (g) Mean ± SEM
1	Paraffin (nondiabetic control)	280.33 ± 13.40
2	Paraffin (diabetic control)	235.0 ± 8.23 ^a
3	Calcium mupirocin	268.5 ± 7.53
4	Sodium fusidate	301.66 ± 7.65 ^b
5	Papain-urea	252.33 ± 8.32

^a*p* < 0.05 versus nondiabetic control; ^b*p* < 0.05 versus diabetic control.

Table 3: Effect of topical sodium fusidate, mupirocin, and papain-urea on hydroxyproline content of granulation tissue in excision wound

Group	Drugs	Hydroxyproline (mg/g) Mean ± SEM
1	Paraffin (nondiabetic control)	145.7 ± 1.10
2	Paraffin (diabetic control)	101.15 ± 1.35 ^a
3	Calcium mupirocin	127.6 ± 0.8
4	Sodium fusidate	154.5 ± 0.8 ^b
5	Papain-urea	120.6 ± 2.4

^a*p* < 0.05 versus nondiabetic control ; ^b*p* < 0.05 versus diabetic control.

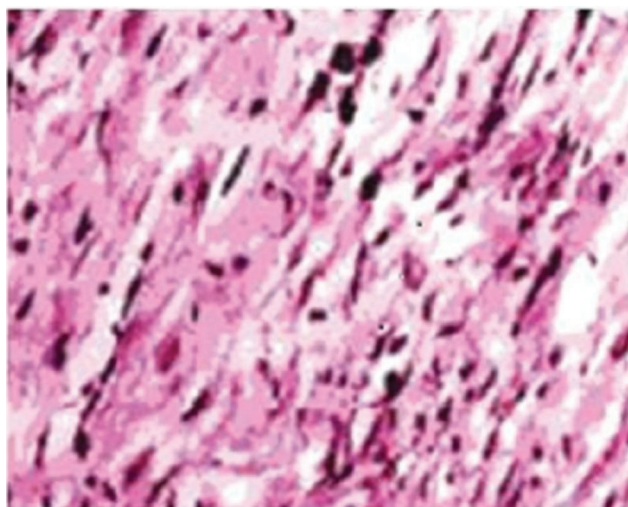


Figure 1: Histopathology of granulation tissue of nondiabetic control group.

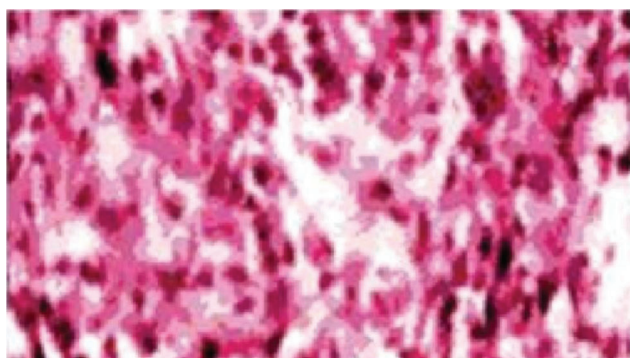


Figure 2: Histopathology of granulation tissue of diabetic control group.

macrophages and less collagen fibers. There was moderate collagen deposition, few scattered fibroblasts and some macrophages in the calcium mupirocin treated group (Figure 3). Tissue obtained from sodium fusidate-treated group showed significant increase in collagen content and more fibroblasts (Figure 4). Granulation tissue in the papain-urea treated group had more macrophages, with less collagenation and fibroblasts (Figure 5).

DISCUSSION

DM is a public health problem worldwide. An important complication of DM is impaired wound healing which is distressing for the patient. This study was carried out to assess the effect of topical sodium fusidate, mupirocin, and papain-urea on wound healing in diabetic wistar rats. Various cytokines and growth factors such as transforming growth factor- β , platelet derived growth factor, epidermal growth factor together with

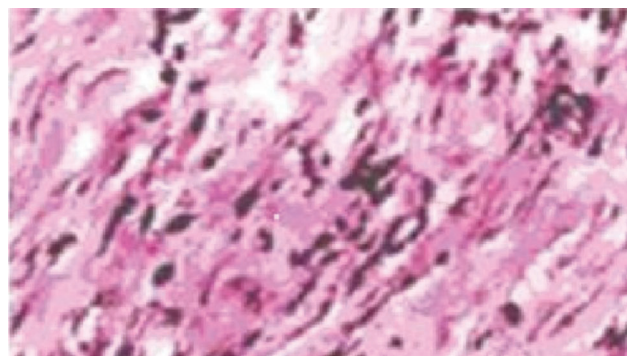


Figure 3: Histopathology of granulation tissue of calcium mupirocin-treated group.

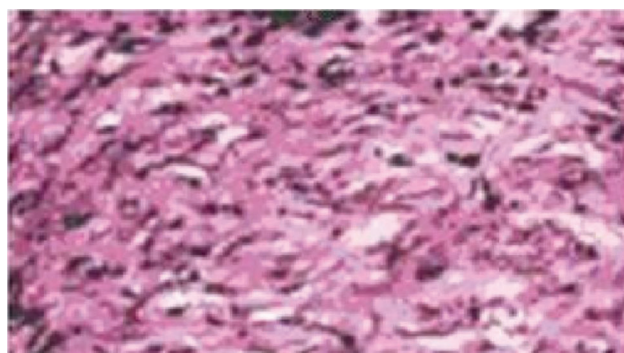


Figure 4: Histopathology of granulation tissue of sodium fusidate-treated group.



Figure 5: Histopathology of granulation tissue of papain-urea treated group.

macrophages, neutrophils, fibroblasts, and keratinocytes play an important role in wound healing.^[23] In this study, wound healing rate was delayed in diabetic rats as compared to nondiabetic control. All stages of healing are known to be impaired in diabetes. A decrease in chemotaxis, phagocytosis, growth factors, and antioxidant levels occurs in the initial stages of wound healing in diabetes.^[24–26] Increased free radicals, apoptosis, and reduction in cell proliferation impair granulation tissue

formation.^[24,27,28] Studies have shown decreased secretion of collagen in tissues in diabetic animals.^[29] Histology of granulation tissue of this group in our study showed few fibroblasts with less collagen content. Hydroxyproline content was also low. Alteration in these components could have impaired wound healing in the diabetic rats.

In this study, the breaking strength of incision wound and wound contraction rate was significantly increased whereas the period of epithelization of excision wound was decreased in sodium fusidate-treated rats. In the initial phase of wound healing, the strength of a wound depends on collagen content and its cross-linking.^[30] Increase in breaking strength of incision wound indicates improved collagen formation. This was confirmed histopathologically where there was abundant collagenation in the fusidate-treated group. Since hydroxyproline is relatively abundant in collagen, it is used as a marker of collagen content.^[31] There was an increase in hydroxyproline which reflects an increase in collagen. Excision wound, an open wound, heals by secondary intention. Wound contraction is important along with epithelization and connective tissue formation for healing.^[32] Fibroblasts play an important role in wound contraction.^[33] There was an increased number of fibroblasts in granulation tissue in rats treated with sodium fusidate which may have contributed favorably to the rate of wound contraction. Earlier studies with sodium fusidate hydrogel for infected and noninfected wounds has shown increased keratinocyte migration and accelerated epithelization.^[34] Topical application of sodium fusidate to burn wound has shown to increase collagen content of wound and rate of wound contraction and decrease period of epithelization.^[13] Though topical mupirocin and papain—urea have shown to improve wound healing in some studies, they did not significantly alter wound healing in rats with diabetes in this study.^[12,17]

CONCLUSION

The results of this study indicate that antimicrobials, such as sodium fusidate, have a positive impact on various parameters of wound healing and can be useful both prophylactically for infection control as well as promoting wound healing in diabetes.

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