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EFFICACY OF MUPIROCIN WITH CHITOSAN AND COLLAGEN AS COMPARED TO MUPIROCIN ONLY ON EXPERIMENTALLY INDUCED INCISION WOUND HEALING IN WISTAR RATS

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ABSTRACT

Introduction: Wound healing is a process involving hemostasis, inflammation, proliferation and remodeling. Any insult in the process delays the wound healing. The antibiotics have a role in preventing infections and thereby facilitate process of wound healing. Mupirocin is used for *Staphylococcus aureus*, *Streptococcus pyogenes* or methicillin-resistant *Staphylococcus aureus* (MRSA) skin infections. On the other hand, chitosan and collagen are shown to facilitate wound healing. Thus, this study intends to compare mupirocin application versus mupirocin with chitosan and collagen application on incised wounds in Wistar rats. **Methods**: Fifteen male Wistar rats were divided into three groups (5 rats per group) namely control, mupirocin and mupirocin+chitosan+collagen groups. After overnight fasting, the incision wounds with interrupted sutures were created on dorsum of animals. The animals in group 2 and 3 received topically mupirocin and mupirocin plus chitosan and collagen respectively daily for ten days. The control group received no topical ointment. The sutures were removed on day 7 and wound breaking strength was measured on day 10. **Results**: The breaking strength of both mupirocin and mupirocin+chitosan+collagen groups were statistically higher than control group. Although the chitosan and collagen addition had increased the breaking strength, there was no statistical difference between mupirocin and mupirocin+chitosan+collagen groups. **Conclusion**: Mupirocin and mupirocin+chitosan+collagen groups show better healing in rat incision wound model when compared to control group.

KEYWORDS: Wound healing, mupirocin, chitosan, collagen.

1. INTRODUCTION

Wound healing is a physiological process of repair in tissue injury and it comprises of multiple biochemical stages such as hemostasis, inflammation, proliferation and remodeling.[1] The initial phase, involves vasoconstriction and thrombus formation which stops blood loss. The platelets then release several cytokines such as platelet-derived growth factor (PDGF) that attract inflammatory cells like neutrophils, polymorphonuclear lymphocytes (PMN) and macrophages. The platelets and local injured tissues release vasoactive amines. These amines including histamine and serotonin, increases vascular permeability. Macrophages remove tissue debris and microorganisms, and regulate the fibroblast activity in the proliferative phase of healing. Fibrinogen produces fibrin, which provides initial structural support for cells. The proliferative phase consists mainly of fibroblast activity including collagen and ground substance (glycosaminoglycans and proteoglycans) production, angioneogenesis and re-epithelialisation of the wound surface.[2]

Fibroblasts require vitamin C to produce collagen.[3] As collagen increases, tensile strength of the wound increases, consisting of type 3 collagen. Collagen maturation occurs in the remodeling phase, where type 3 collagen fibres are replaced by type 1 collagen fibres and there is a realignment of collagen fibres, and collagen fibres rearrange themselves along the lines of tension.

There is also a reduction in vascularity and wound contracts due to fibroblast and myofibroblast activity. Whenever there is a diversion in the healing process from the usual, it is not rare to have non-healing, limited healing or exaggerated healing.

Studies have shown beneficial effects of chitosan and collagen composite in medicated dressing on wound healing.[4,5] However, whether chitosan and collagen retains its activity when combined with mupirocin ointment is yet to be evaluated. Hence, this study is planned to evaluate and compare mupirocin + chitosan + collagen ointment with mupirocin ointment on experimentally induced incision wound healing.

2. METHOD AND MATERIALS

The study, an in vivo experiment, was done at the Central Animals House Facility, Ras Al Khaimah Medical and Health Sciences University (RAKMHSU), United Arab Emirates. The experiment was performed using 15 male Wistar rats that weighed 150-200g and aged 3 months old, housed under controlled condition of temperature 23±2˚C, humidity of 50±5% and 10-14hr of light and dark cycle respectively. The animals were housed individually in polypropylene cages containing sterile wood dust bedding throughout the study and were given free access to food (animal chow) and water ad libitum. The study was conducted after obtaining the approval of the RAKMHSU Research Ethics Committee (RAKMHSU-REC-174-2019-F-M). The mupirocin + chitosan + collagen ointment was locally prepared and mupirocin, ointment (T-bact) of GlaxoSmithKline (GSK) was procured from local pharmaceuticals for research purpose.

The animals were divided into 3 groups of 5 each, as follows.
Group 1 was the control group and there was no drug application.
Group 2 animals were treated with T-bact ointment (mupirocin 2%) (GSK).
Group 3 animals were treated with mupirocin + chitosan + collagen ointment (mupirocin 2% w/w along with chitosan and collagen)

2.1 Incision wound model

After overnight fasting, the animals were weighed and anesthetized using light ether. The dorsal fur of the animals was shaved. Asepsis was ensured by wiping the area with 70% alcohol. A single incision wound of 6 cm length was created paravertebrally as previously described.[6,7,8] The wound was sutured by simple interrupted sutures with nylon thread placed at 1 cm apart. The drugs were applied for animals in group 2 and 3, daily for 10 days. Group-1 being control group received no drug. The sutures were removed on day 7 and wound breaking strength was measured on day 10.

2.2 Tensile strength assessment

The breaking strength of the wound was measured on 10th day of wounding by performing a tensile strength test using method of Lee *et al.*[9,10] Rats were anesthetized using light ether and secured to the operation board with the help of clippers. Two forceps were firmly held, at each side of the incised wound area. One of the forceps was fixed while the other forcep was connected to a freely suspended lightweight polypropylene container with the aid of a 100-cm string and pulley.[10] Water was allowed to flow from another reservoir slowly and steadily into the polypropylene container until the wound just opened up. After completion of the study, the animals were sacrificed by decapitation. The data were compared using SPSS version 24.0 and results statistically analyzed by one-way ANOVA followed by Bonferroni post hoc test. The p value of 0.05 was considered as statistically significant.

3. RESULTS

The breaking strength in control group was 188.66 ± 23.40 g (mean ± SE) whereas it was 274.66 ± 23.52g, 284.33 ± 29.67g respectively in mupirocin and mupirocin+chitosan+collagen groups (Figure 1). Though the addition of chitosan and collagen has increased the breaking strength, there was no statistical significance among the groups with P value 0.72. The unpaired t test between control and test groups showed statistical significance. The unpaired t test between mupirocin and mupirocin + chitosan + collagen groups showed no statistical significance.

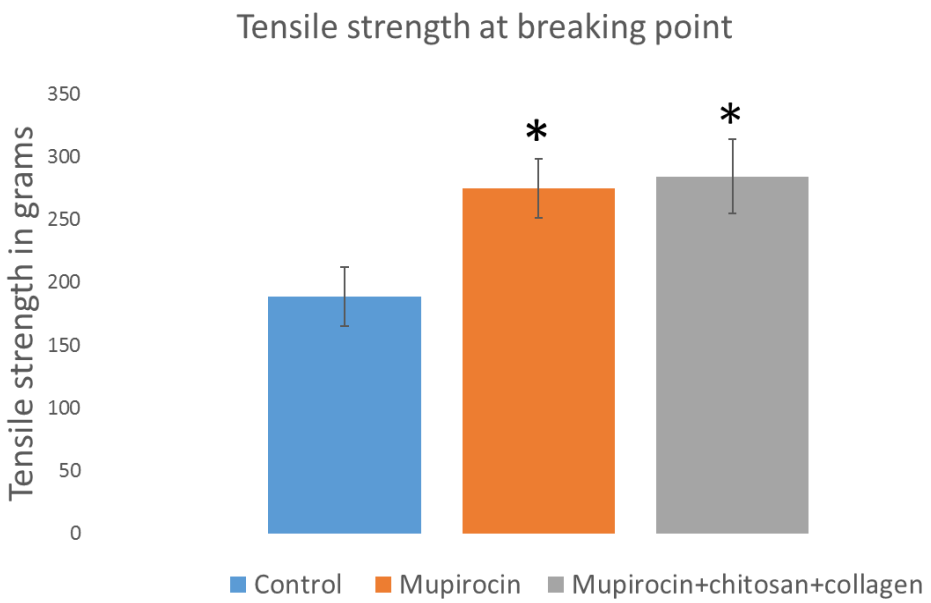

Figure 1. The bar chart of tensile strength (g) among the three groups, shown as mean ± SE. The comparison among the groups was done by one-way ANOVA. The groups were not statistically significant. The comparison between two groups was done by un-paired t test. * P<0.05 vs control. The p value <0.05 considered as statistically significant.

4. DISCUSSION

Role of antibiotics by its antimicrobial action in impaired wound healing as seen in chronic wounds has been studied previously.[11] In an in-vivo study mupirocin was shown to benefit mouse wound infection models involving *S pyogenes* and *S aureus*. Mupirocin had shown statistical significant results as compared to no-treatment group. Our study showed beneficial effect of mupirocin in wound healing as compared to no-treatment group. This may be attributed to antimicrobial property of mupirocin, though incision wounds induced were all clean in physical appearance.

Mupirocin embedded in chitosan hydrogel was demonstrated to exhibit better bio adhesiveness and longer drug release in burn infections such as *Staphylococcus aureus* and *Bacillus subtilis*.[12] Moreover, chitosan coated liposomes and hydrogels were independently shown to have antimicrobial properties when used as excipients against *Staphylococcus epidermidis* and *Staphylococcus aureus*.[13] Collagen and collagen mimetic peptides are used as various forms for promoting wound healing.[14] Collagen matrix dressings are also shown to improve cytological migration and epithelization during wound healing.[15] The outcome measure of our study tensile strength, measures organization of the collagen fibers in the wound base. Tensile strength has been used widely to measure the strength of wounds.[10,16] The tensile strength of the incision is proportional to amount of collagen in the healing tissue as reported previously.[16] Collagen is a prime extracellular protein in the granulation tissue of healing wound attributed for in wound strength and integrity.[17] The synthesis and deposition of new collagen plays crucial role in wound healing.[18] Thus, combination of mupirocin+ chitosan+collagen, theoretically all have beneficial effect on wound healing. However, our results did not show any significant effect when mupirocin is combined with chitosan and collagen. Our results also showed the trend for benefit even as compared to only mupirocin group. The lack of statistical significance may be due to marginal benefit or small sample size. Since animal model has been used in our study, results obtained cannot be directly extrapolated on to humans, for which there is a need for clinical assessment in humans to confirm the observations made. We were not able to study the bacterial counts from the wounds, which would have given clear picture about healing effect seen with mupirocin. This is one of the limitations of our study. The results of our study would pave way for future wound healing studies with microscopical evaluation of tissues.

5. CONCLUSION

Mupirocin and mupirocin+chitosan+collagen groups showed better healing effect in incision wound model of rats when compared with no-treatment group, as demonstrated by better tensile strength. Although there is an increase in breaking strength in the groups that received collagen and chitosan, there was no statistical difference between mupirocin and mupirocin+chitosan+collagen.

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