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Comparison of the Royal Jelly and Povidone Iodine on Wound Healing in Rabbits

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Abstract: The effects of Royal Jelly (RJ) on wound healing were investigated and compared to commonly used 10% povidone iodine and 0.9% sodium chloride treatment. Three full-thickness skin defects, 3,14 cm in diameter, were created on dorsal aspects of 12 rabbits. Wound surfaces were macroscopically examinated from the points of exudation, beginning of the contraction, granulation tissue and epithelization during the postoperative period. Biopsy specimens which were collected on the 4th, 8th, 12th and 16th Postoperative Days (POD) were evaluated for histopathological changes. Specimens were evaluated according to several histopathologic parameters, such as the thickness of scar tissue, the density of vascular proliferation and the degree of inflammatory cell infiltration. SPSS 11.0 for windows was used for statistical analyses. Thickness of scar tissue was significantly changed between the treatments of RJ and 0.9% sodium chloride ($p<0.05$), while the wound treatment with 10% povidone iodine and 0.9 % sodium chloride created similar results ($p>0.05$). The density of vascular proliferation between study groups was significantly different only on POD 4 and 16 ($p<0.05$). The degree of inflammatory cell infiltration was similar in all groups ($p> 0.05$), but a significant difference between the 10% povidone iodine and 0.9 % sodium chloride treatments on PODs 12 as an exception ($p<0.05$). In conclusion, daily topical royal jelly application to full-thickness skin defects in rabbit accelerated wound healing. Other adventages of RJ are ease of application and the low cost.

Key words: Royal jelly, skin wound, rabbit, specimens, inflammatory,histopathological

INTRODUCTION

Royal Jelly (RJ) has been used worldwide for many years as medical products, health foods and cosmetics (Hidaka *et al*., 2006). A number of biological and immuno-regulatory actions attributed to RJ have been reported. These include vasodilative and hypotensive activities induction of decrease in serum cholesterol levels, antitumor activities, antioxidative activities and protective activity aganist hematopoietic dysfunction in X-irradiated mice (Inove *et al*., 2003).

Ten % povidone-iodine aqueous solution is a microbicidal, antiseptic agent. It is only the unbound free iodine that is active against a range of microbes, including gram-positive and gram-negative bacteria, viruses, fungi, protozoa and some spores. Widespread clinical application of povidone-iodine includes preoperative skin preparation and a wound dressing soaked in gauze (Burks, 1998). Systemic toxicity can occur from topical absorption of iodine in patients with extensive burns (>20%), large open wounds and renal impairment (Kramer, 1999).

In this study, we have investigated efficacy of RJ on healing wound standing on the clinical and histopathologic comparing with 10% povidone iodine and 0,9% sodium chloride.

MATERIALS AND METHODS

Six male and six female rabbits weighing about 2500±200 g. were taken for the study. The dorsal aspects (backs) of rabbits were clipped and prepared for aseptic surgery. All rabbits were anesthetized with i.m. administration of 10 mg kg^{-1} xylazine hydrochloride (Rompun, Bayer) and 50 mg kg^{-1} ketamine hydrochloride (Ketanes, Albe). On dorsal aspect of each animal, two

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cranially and one caudally located full-thickness skin wounds in 3,14 cm diameter were created using a template prepared from an X-ray film.

Following incision different wounds of each animal were treated with RJ (83 mg mL^{-1} Royal Jelly-Arýjel Co., Ltd.), 10% povidon iodine and 0,9% sodium chloride as the control, respectively. Then the wounds were closed with sterile gauze and fixed with circular adhesive bands. The animals had free access to water and standart laboratory pellet and were housed individually to prevent them from tampering with the others' wounds. The dressing were changed every day until complete wound healing. During the postoperative period, all rabbits were checked daily for general health condition, bandage slippage and other unspecified abnormalities. Macroscopically wounds were examinated and exudation, the beginning of the wound contraction to mark the beginning of healing, granulation tissue and the first day of epithelization were noted regularly.

Skin samples from all three incision sites for histologic examination were collected from each animal on Postoperative Days (PODs) 4,8,12 and 16. The biopsy specimens were fixated in 10% formalin solution and were placed into ototechnicon for routine tissue processing. They were embedded in parafin blocks, cut into 4 μm thickness slices and stained with Hemetoxylin-Eosin for routine light microscopic evaluation. The processed specimens were evaluated according to several histopathologic parameters, such as the thickness of scar tissue, the density of vascular proliferation and the degree of inflammatory cell infiltration. Thickness of scar tissue was expressed as μm, the density of vascular proliferation and the degree of inflammatory cell infiltration was scored as follows: 0 = normal, 1 = light increase 2 = mild increase, 3 = marked increase. According to number of inflammatory cells in the wounded tissue per 40x magnification field, the increase was considered as light (3-10 cells), mild (11-30 cells) or marked (>30 cells).

SPSS 11.0 for windows was used for all statistical analysis (SPSS, 2001). Comparison of the changes in the thicknesses of scar tissue analysed via variance analysis and Tukey test. Nonparametric values (vascular proliferation, the degree of inflammatory cell infiltration) were analysed by Kruskal ssWallis and Mann-Whitney U test.

RESULTS

All rabbits survived until the end of study with no sign of pain or discomfort. During healing, all wound boundarise became progressively shapeless. All control wound surface were covered by a thin gelatinous exudate (POD 4). After this exudate was removed, an ongoing healthy granulation and epithelization tissues were determineted underneath. Macroscopically, the wounds treated with 10% povidon iodine and 0.9% sodium chloride appeared to have marked fibrinous exudate and crust formation which caused adherence to the gauze. RJ gauze-applied wounds showed strong adherence and a lesser degree exudate Therefore, they required greater tearing force for removal of the dressing. According to other groups, the acceleration of epithelization in the RJ treated group appeared to occur between 7 and 9 days clinically as well as histologically. However, adhered strongly and the frequent dressing may delay the healing. The epithelization was completed on POD 16 on RJ and 10% povidone iodine gauze-applied wounds, whereas it wasn't completed on 0.9 % sodium chloride gauze-applied wounds and ulceration in central wounds was seen. The granulation tissues on all wounds were noted on PODs 3-5, while epithelization was seen on PODs 6-8. The expansion process (to POD 4) was followed by the contraction process (to PODs 6-8). The contraction in RJ gauze-applied wounds (to PODs 16) became more than other wounds.

The thickness of scar tissue was significantly different PODs 4 and 12 between RJ groups ($p<0.05$). It was similar in all groups on PODs 16 ($p>0.05$). Such a relation was significantly different on PODs 8 between 10% Povidone iodine and 0.9% sodium chloride ($p<0.05$). But, it was similar in 10 % Povidone iodine and 0.9 % sodium chloride on PODs 4 and 12 ($p>0.05$). The graphical expression of the change of scar thickness in study groups according time is presented in Table 1.

The density of vascular proliferation progression was significantly different PODs 4, 8 and 16 between RJ groups. Such a relation was not found on PODs 12 ($p>0.05$).

The degree of inflammatory cell infiltration was significantly different PODs 8, 12 and 16 between RJ groups. Such a relation was not found on PODs 4 ($p>0.05$).

Table1: According to days, the thickness of scar tissue as average between groups (μM) are shown

Groups		4. Days	8. Days	12. Days	16. Days
RJ	Mean	540.5000	1149.1667	2048.3333	1689.1667
	Std. deviation	67.4500	77.0984	187.1274	278.2160
10% Povidon Iodine	Mean	1048.3333	1248.3333	1185.8333	1565.8333
	Std. deviation	116.0029	129.0607	179.5109	192.5206
0.9% Sodium Chloride	Mean	996.6667	1105.8333	1394.3333	1701.6667
	Std. deviation	137.7921	32.9267	49.6817	135.3021

DISCUSSION

The wound healing process involves considerable complex factors. Consequently, a detailed evaluation of curative nature of healing matearial in an inflicted skin wound may require a wide range of observations including groos and microscopic examinations and biochemical and pharmacological analyses (Kilic *et al*., 2002). However, we adopted an experimental design including macroscopically (the exudation, the day wound healed with contraction started, granulation tissue and the day epithelization), histological (the thickness of scar tissue, the density of vascular proliferation and the degree of inflammatory cell infiltration) and quantitative evaluations of wound healing process. Some authors (Norman, 2003) conflicted to the use of antiseptics in open wounds, moreover some others (Yavuzer *et al*., 1997) claimed that antiseptics might disorder epithelization layer and disturb progressive vascular proliferation. In our study we did not observe any adverse effects of antiseptics on the thickness of scar tissue, the density of vascular proliferation and the degree of inflammatory cell infiltration in full thickness skin wounds. Such a conclusion was reported before by Niedher and Schoph (1986) that antiseptics had not resulted any undesirable effects on granulation tissue formation in open wounds.

According to current study, RJ gauze-applied wounds was showed strong adherence, the dressing every day and required an extra force to separate it from them. This force could cause epithelial damage and thus may increase the thickness of scar tissue. The thickness of scar tissue in the RJ treated group was noted lesser than other groups on PODs 4. But its showed thicker than other groups on PODs 12. Meantime, the density of vascular proliferation was significantly increase wounds treated with RJ dreesing than other groups.

Honey and related products, such as RJ and propolis has appeared anti-inflamator activity. Topical application of honeys to burn wounds and other wound has benen found to be effective in controlling infection and producing a clean granulating bed (Sabrahmany, 1998). Kohno *et al*. (2004), mentioned RJ efficiently inhibits the production of proinflammatory cytokines by activated macrophages. We have shown that the wounds treated with RJ the degree of inflammatory cell infiltration was decreased on PODs 8, 12 and 16.

CONCLUSION

RJ application to full-thickness skin defects in rabbits accelerated wound healing. Thus, we conclude from this study that, RJ as an antiseptic not only improves the outcome of wounds but it's also, non-toxic, easily available and cheap.

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