

A STUDY OF THE EFFECT OF MAGNESIUM HYDROXIDE ON THE WOUND HEALING PROCESS IN RATS

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SUMMARY: Wound healing is the restoration of physical integrity to internal and external structures and involves intricate interactions between the cells and numerous other factors. Appropriate treatment and care are essential for acceleration of the healing process, prevention of infection and chronicity of the wound and different means and approaches have thus far been used to this end. The aim of this study was to evaluate the effect of magnesium hydroxide that was used for prevention of bed sores erstwhile on the wound healing process.

The effect of magnesium hydroxide on the healing process in two models of skin wound; length wounds of 15 mm full-thickness and round wounds of 5 mm in diameter full thickness incision were created in the paravertebral area, 1.5 mm from midline on the back of rats was evaluated through measuring the length and area of the healed region and process pathological on different days, and conducting tensiometry experiments after complete wound healing.

The percentage of wound healing on days 3, 6, 9, 12 and 15 in control group of length wounds changed in the group treated with magnesium hydroxide from 10.13%, 31.88%, 52.46%, 78.75% and 100% to 11.63%, 49.75% ($p<0.05$), 94% ($p<0.01$), 100% and 100% respectively; also the percentage of wound healing on days 3, 6, 9, 12, 15 and 18 in control group of round wounds changed in the group treated with magnesium hydroxide from 9.88%, 21.25%, 52.13%, 69.63%, 88.21% and 100% to 12.25%, 37.25% ($p<0.05$), 60.5%, 76%, 93.4% and 100% respectively.

Stress (maximum tensile force causing skin rupture) changed from 13.19 Newton (N) in the control group of length wound to 20.87 N, also from 11.78 N ($p<0.05$) in the control group of round wound to 16.9 N ($p<0.05$) in group treated with magnesium hydroxide.

Strain (tissue length under maximum strain) changed from 9.98 mm in the control group of length wound to 15.43 mm ($p<0.05$), also from 10.53 mm in the control group of round wound to 17 mm ($p<0.05$) in group treated with magnesium hydroxide.

The result of pathological samples of view histological (wound healing and cell aggregative) accelerated in magnesium groups on length and round wounds partition to control groups.

Our findings suggest that magnesium hydroxide may have accelerated the skin wound healing process in rat and increased tissue strength through stimulating collagen formation.

Key Words: Wound healing, magnesium hydroxide, strain, stress.

INTRODUCTION

Wound healing is the restoration of physical integrity to internal and external structures and involves complex

interactions between the cells and various factors (1). Wound healing is a homeostatic mechanism for restoration of physiological balance and is triggered by the interruption of the connection between adjacent cells or cell death. The healing process consists of a sequence of

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Table 1: The means percentage of wound healing in the control and magnesium hydroxide groups on length wounds with computation changes length relation to 0 day.

Day \ Group	0	3	6	9	12	15
LC	0	10.13 ± 2.64	31.88 ± 2.79	42 ± 5.5	78.75 ± 4.6	100
LM	0	11.63 ± 2.3	49.75 ± 2.79 ^a	94 ± 5.5 ^b	100	100

LC: Length wound control group, LM: Length wound magnesium hydroxide group. Values are Means ± SD, Sample size is eight rats in each group. Tests: t-test and Tuky.

a: $p < 0.05$, compared to control, b: $p < 0.01$, compared to control.

overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodeling (2,3). Appropriate treatment and wound care accelerate the healing process and prevent infection and chronicity of the wound. Thus far, different methods and approaches have been used to achieve shorter healing times. Despite extensive efforts to improve wound healing, the outcomes of existing methods are far from optimal.

Patino studied the effect of electromagnetic fields on the healing of rectangular wounds on the back of rats and monitored changes in wound length compared to day 0 (4). In a different experiment, Patino used wound area and circumference measurements to evaluate the wound healing process. Another highly valuable measure for evaluating the wound healing process is the tissue tensile strength. There is evidence of increased collagen synthesis in the hours immediately following injury. Multiple bonds and special arrangement of collagen fibers are responsible for tissue strength, hence tensiometry can be used to assess the tensile strength of the healed wound (5-7).

The role of trace elements in the wound-healing process is still controversial (8). Magnesium hydroxide is current antacid that can be used for prevention bed sores erstwhile on the wound healing process. Pollack showed the importance to wound healing of trace elements and minerals (such as Mg) (9). Pastoride showed that the dual topical therapy with zinc chloride spray and magnesium hydroxide ointment proved to be safe and effective in accelerating wound healing in obstetric and gynecologic patients (10). Lange showed that Mg^{2+} and Ca^{2+} differentially regulate beta 1 integrin-mediated adhesion of dermal fibroblasts and keratinocytes to various extracellu-

lar matrix proteins. These components may be able to accelerate the healing process (11). Hence this study was conducted to evaluate the effect of magnesium hydroxide on the wound healing process on laboratory rats.

MATERIAL AND METHODS

The experiments were conducted on wistar rats weighing 200-300 grams (supplied from Razi Vaccine and Serology Research Center). The animals were caged individually in a controlled environment at 23-25°C and 50% humidity with a 12 hours artificial light cycle. Two groups of rats were studied:

a) *Length wound group*; This group was divided into two subgroups:

1. Control group (12) that no material used on this wound group after incision until the end of the study.
2. Experimental group (12) that magnesium hydroxide was used on this wound group after incision two times daily until the end of the study.

b) *Round wound group*; This group was divided into two subgroups:

1. Control group (12) that no material used on this wound group after incision until the end of the study.
2. Experimental group (12) that magnesium hydroxide was used on this wound group after incision two times daily until the end of the study.

Prior to incision, the rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and Xylazine 2% (5 mg/kg). Then the animals were shaved on the back and the skin was disinfected using cotton and alcohol wipes. Using sterile surgical scalpels, in two models skin wound; length wounds of a 15 mm full-thickness and round wounds of a 5 mm diameter full thickness incision were created in the paravertebral area, 1.5 mm from midline on the back of rats. After incision, the wound was thoroughly disinfected using povidone iodine and injected gentamicin (5mg/kg, single dose) as antiseptic and antibiotics respectively. In control group, no material used on wound and case group magnesium hydroxide used two times daily on the wound after incision until complete healing of the wound.

Table 2: The means percentage of wound healing in the control and magnesium hydroxide groups on round wounds with computation changes diameter relation to 0 day.

Day \ Group	0	3	6	9	12	15	18
RC	0	9.88 ± 2.63	21.25 ± 3.4	52.13 ± 6.4	60.5 ± 6.4	87.41 ± 3.6	100
RM	0	12.25 ± 2.63	37.25 ± 3.2 ^a	60.5 ± 6.4	76 ± 4.98	93.4 ± 3.6	100

RC: Round wound control group, RM: Round wound magnesium hydroxide group. Values are Means ± SD, Sample size is eight rats in each group. Tests: t-test and Tukey. a: p<0.05, compared to control.

Evaluation of the healing process

Two methods were used to evaluate the wound healing process.

a) Measurement of wound length and area: Every third day as of the first day of creating the incisions (day 0) until complete wound healing, the animals were anesthetized with ether and the shape of the wound was drawn on a piece of transparent plastic using a special marker. To measure the wound length or area, using a negatoscope and the software Video Image Analyzer. The wound area was accurately measured and the percentage of healing was calculated according to the following formula on different days.

b) Measurement of tissue tensile strength (tensiometry): To conduct this measurement, the animals were killed by chloroform inhalation after complete healing of the wound. The skin of the back was excised at the deep fascia region and submersed in normal saline to prevent drying.

$$\text{The wound size percentage} = \frac{\text{Difference wound length or area on Xday relation to 0 day}}{\text{Wound length or area on 0 day}} \times 100$$

$$\text{Healing percentage on Xday} = 100 - \text{Wound size percentage on Xday}$$

Tissue tensile strength was then measured using a tensiometer. In this method, a narrow strip of skin, 4 cm in length and 3 cm in width, is attached to tensiometer holders. The healed wound lies at the center and at right angle to the length of the skin. The movement of holders is controlled by computer. The tension exerted on the skin is increased and is stopped as soon as the skin ruptures. The following parameters are calculated by tensiometer and the results are displayed by computer:

Stress: Maximum tensile force causing skin rupture.

Strain: Tissue length under maximum tension.

Statistical methods

Data pertaining to the duration of healing and tissue strength were evaluated using t-test. Comparison of data pertaining to duration and percentage of healing was performed using t-test and Tukey test. Significance level was set at 0.05. All results were reported as Mean ± SD.

RESULTS

The percentage of wound healing on days 3, 6, 9, 12 and 15 in control group of length wounds changed in the group treated with magnesium hydroxide from 10.13%, 31.88%, 52.46%, 78.75% and 100% to 11.63%, 49.75% (p<0.05), 94% (p<0.01), 100% and 100% respectively; also the percentage of wound healing on days 3, 6, 9, 12, 15 and 18 in control group of round wounds changed in the group treated with magnesium hydroxide from 9.88%, 21.25%, 52.13%, 69.63%, 88.21% and 100% to 12.25%, 37.25% (p<0.05), 60.5%, 76%, 93.4% and 100% respectively (Tables 1, 2).

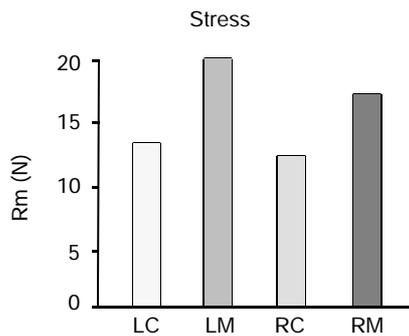
Stress (maximum tensile force causing skin rupture) changed from 13.19 Newton (N) in the control group of length wound to 20.87 N, also from 11.78 N (p<0.05) in the control group of round wound to 16.9 N (p<0.05) in group treated with magnesium hydroxide (Figure 1).

Strain (tissue length under maximum strain) changed from 9.98 mm in the control group of length wound to 15.43 mm (p<0.05), also from 10.53 mm in the control group of round wound to 17 mm (p<0.05) in group treated with magnesium hydroxide (Figure 2).

The result of pathological samples of view histological (wound healing and cell aggregative) accelerated in magnesium groups on length and round wounds partition to control groups.

Figure 1: Stress (Maximum tensile force causing skin rupture) in the control and magnesium hydroxide groups.

LC: Length wound control group, **LM:** Length wound magnesium hydroxide group, **RC:** Round wound control group, **RM:** Round wound magnesium hydroxide group, Sample size is eight rats in each group. Tests: t-test, Tukey. Wound healing in the drug group has occurred at a faster pace than in the control group and a significant difference is seen between the two groups in the percentage of healing on different days.



DISCUSSION

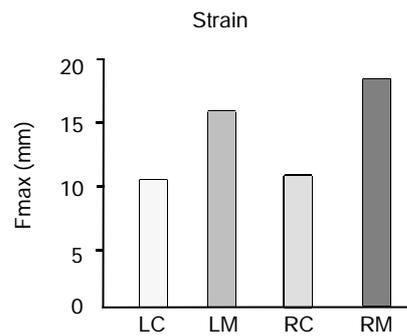
Tables 1 and 2 show the effects of magnesium hydroxide on wound healing in rats treated with the drug compared to control rats. The table shows the percentage of healing based on changes in wound area on different days compared to the first day (day 0) of the experiment.

Statistical analyses, including t-test and Tukey show a significant difference in the percentage of healing on days 6 ($p < 0.05$) and 9 ($p < 0.01$) on length wound groups; 6 ($p < 0.05$) on round wound groups of the study.

The role of trace elements in the wound-healing process is still controversial (8). Pollack showed the importance to wound healing of trace elements and minerals such as Mg^{2+} (9). Spectrophotometric studies have demonstrated the role of trace elements in healing of burn wounds (12). Pastorfiide showed that the dual topical therapy with zinc chloride spray and magnesium hydroxide ointment proved to be safe and effective in accelerating wound healing in obstetric and gynecologic patients (10). Njau and colleagues investigated fluctuations of trace elements Mg, Ca and Zn concentrations with time on skin-induced injuries. The results were correlated with time of injury. Suggestive alterations in trace elements mean con-

Figure 2: Strain (tissue length under maximum tension) in the control and magnesium hydroxide groups.

LC: Length wound control group, **LM:** Length wound magnesium hydroxide group, **RC:** Round wound control group, **RM:** Round wound magnesium hydroxide group, Sample size is eight rats in each group. Tests: t-test, Tukey. Wound healing in the drug group has occurred at a faster pace than in the control group and a significant difference is seen between the two groups in the percentage of healing on different days.



centrations with time were confirmed. The ratios of the mean in pairs (Ca/Zn, Ca/Mg, Mg/Zn) versus time were graphed. Effectively, the curves achieved by analysing postmortem tissues, serve to estimate the time of an injury induced in vivo (13). Also, Domres and colleagues showed that serum levels of magnesium and zinc increased after thermal trauma of the rat because of the anabolic process of wound healing (14). Spasov showed local treatment with polykatan, a magnesium-containing drug based on bischofite mineral, promoted healing of infected skin wounds. Wound cleansing from bacteria was due to a direct antibacterial effect of the drug (15). A number of studies conducted by Grzesiak showed that elements such as magnesium cause migration of keratinocytes and mediators of the healing process such as E-cadherin and integrin to the wounded area (16,17). In the present study, magnesium hydroxide apparently led to a notable decrease in length and area of the wound, as demonstrated by the significant difference in the percentage of healing on different days of the study as compared to the control group. In view of the results (Tables 1 and 2), it can be concluded that magnesium hydroxide significantly accelerated the wound healing process.

As shown in Figures 1 and 2, the tensile strength of the skin tissue from rats receiving magnesium hydroxide increased compared to the control groups. It has been demonstrated that the tensile strength of the skin is related to the number of collagen fibers and how they are connected to magnesium hydroxide probably increases collagen synthesis by increasing fibroblasts, as well as increasing DNA and protein production. It may also have positive effects on the maturation, deposition, and correct orientation of collagen fibers. Lange showed that Mg^{2+} and Ca^{2+} differentially regulate beta 1 integrin-mediated adhesion of dermal fibroblasts and keratinocytes to various extracellular matrix proteins (11). Also, Lange suggests that extracellular divalent cations differentially influence the integrin-mediated cell migration. A concentration gradient of Mg^{2+}/Ca^{2+} , as reported in tissue injury, thus may play a regulatory role in cell migration required for tissue remodeling (18). Experiments conducted by Grzesiak and colleagues (16), showed that the amount of magnesium and calcium increases in wound exudates. Analysis of wound exudates showed that elements such as magnesium promote tissue adhesion, migration of macrophages, keratinocytes, fibroblasts and production of type I collagen, all of which contribute to the wound healing process (19-21).

Neovascularization is an important factor in wound healing. Banai showed influence of extracellular magnesium on capillary endothelial cell proliferation and migration, and suggested that magnesium deficiency might adversely influence the healing and reendothelialization of vascular injuries and the healing of myocardial infarction and might also result in delayed or inadequate angiogenesis, effects potentially leading to infarct expansion and inadequate collateral development (22).

The result of pathological samples of view histological (wound healing and cell aggregative) accelerated in magnesium groups on length and round wounds partition to control groups.

It can be concluded from the obtained evidence that magnesium hydroxide has probably had an accelerating effect on the healing process.

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