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Research Article

Evaluation of wound healing activity of topical phenytoin in an excision wound model in rats

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ABSTRACT

Background: Wound healing is a significant healthcare problem in today's medical practice. Despite extensive treatment modalities that are supposed to hasten the wound healing process, the outcomes of existing methods are far from optimal. One such agent that has been tried previously and found controversial in wound healing is phenytoin. Therefore, this study was planned to evaluate and compare wound healing effect of topical phenytoin with povidone iodine ointment in rats.
Methods: This study was conducted after approval from Institutional Animal Ethics Committee (IAEC). Wound healing activity of topical phenytoin (1 g% and 2 g%) was assessed in excision wound model in Sprague Dawley rats (n=8), which was compared with topical petroleum jelly and povidone iodine ointment. Parameters studied included wound area on day 0, 4, 8, 12, 16, 20, percentage wound contraction, percentage wound healing from day 0 to day 20 and period of re-epithelisation.
Results: Wound surface area decreased in all treatment groups from day 0 through day 20 and the percentage wound closure was better in both the preparations (1% and 2%) of phenytoin when compared wih control and povidone iodine, but this was not statistically significant. Furthermore, the days required for complete re-epithelisation were less with phenytoin treated groups. There was no statistical difference between both the preparations of phenytoin.
Conclusion: In this study, it was found that topical phenytoin accelerates wound healing process in an excision wound model.

Keywords: Epithelization, Phenytoin, Wound contraction

INTRODUCTION

Wound is defined as a disruption of cellular and anatomical or functional continuity of living tissue.[1] Wound healing is the process of restoration of physical integrity of internal or external body structures and involves a complex interaction between the cells and various other factors. The healing process consists of a sequence of overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodelling.[2]

Wound healing is a significant healthcare problem in today's medical practice. Appropriate treatment and wound care accelerate the healing process and prevent infection and chronicity of the wound.[2]

Oral phenytoin was introduced into therapy in 1938 and widely used for management of convulsions. On long-term administration of phenytoin, it produces gingival hypertrophy and by virtue of this property, studies on its effect on wound healing were undertaken.[3] According to few studies, phenytoin possesses wound healing activity, but there are few contradictory studies. In view of these conflicting results, the present study was planned to evaluate the effect of topical phenytoin on the rate of wound healing in an excision wound model and compare with petroleum jelly and povidone iodine.

METHODS

The present study was carried out in Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, Maharashtra, after approval from Institutional Animal Ethics Committee (IAEC).

Animals

A total of 32, healthy (150-200 g) Sprague-Dawley rats of either sex, aged 12 weeks bred locally in the animal house of our college were used. The animals were kept in

the laboratory for 3-4 days for acclimatization, with free access to food and water. Animals were housed in standard polypropylene cages with wire mesh top and husk bedding.

Prior to the day of the experimental procedure, they were starved over night, and the area of skin, where wound was to be made, was depilated.

Drugs used in the study

- Petroleum jelly
- 1% Phenytoin
- 2% Phenytoin
- 10% Povidone iodine.

Pure powder form of phenytoin obtained from JPN Pharma Pvt. Ltd. Petroleum jelly and 10% povidone iodine were obtained from commercial resources.

Preparation of 1% phenytoin cream: 1 g of phenytoin powder was added to 99 g of petroleum jelly.[4]

Preparation of 2% phenytoin cream: 2 g of phenytoin powder was added to 98 g of petroleum jelly.

Excision Model

Procedure

Sprague Dawley rats (150-250 g) were used for the study. They were individually housed and maintained on normal standard diet and water ad libitum. Prior to creating excisional wounds the rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg). Then the animals were shaved on the back, and the skin were disinfected using cotton and alcohol wipes. Using sterile surgical instruments, round full thickness skin wound measuring 500 mm^2 was created in the paravertebral area, 1.5 mm from the midline on the back of rats, and then thoroughly disinfected using povidone iodine. Haemostasis was obtained by blotting the wound with cotton swabs soaked in normal saline solution. Dressing of the wound was done daily, and the study formulations were applied daily on the wound surface till complete wound healing was observed. Physical attribute of healing *viz*, wound contraction which mainly contributes for wound closure in first 2 weeks was studied by tracing wound area on transparent plastic on wounding day 0, 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th} and subsequently on alternate day till complete epithelisation has occurred. The criteria for complete epithelisation were the fall of scab without any raw area. Wound area was measured by retracting wound on millimetre graph paper.[4,5]

Grouping of the animals

32 Sprague Dawley rats were randomly divided into 4 groups, 8 rats in each group.

Group I: Topical petroleum jelly
Group II: Topical 1% phenytoin
Group III: Topical 2% phenytoin
Group IV: Topical 10% povidone iodine.

Treatment

The study formulations were applied daily to the wound surface till complete healing of the wound was observed.

Parameters evaluated were:

A. Measurement of wound contracture:
   To measure the contracture of wound, a transparent plastic paper was placed on the location of wound and its shape was drawn on the same paper with a marker and then were matched with the graph paper for finding the area of the wound (expressed in mm^2).[2]
B. Percentage of wound closure by using Walkar formula:[2]
   Percentage of wound area = wound area on day X/ wound area on day 1 × 100.
   Percentage of wound healing = 100 - Percentage of wound area.
C. Days required for complete epithelisation:
   The criteria for complete epithelization were the fall of scab without any raw area.[2]

Statistical analysis

The data of surface area of wound and percentage wound closure was analyzed using two-way ANOVA. Bonferroni's test was applied for post hoc analysis. Data for a period of complete re-epithelization was analyzed using one-way ANOVA, followed by *post hoc* tukey's test. Data analysis was performed using GraphPad Prism 5.0 software. $p<0.05$ was considered as statistically significant.

RESULTS

Table 1 and Figure 1 depict the wound surface area (mm^2) measured on day 0, day 4, day 8, day 12, day 16 and day 20 in excision wound model.

The wound surface area decreased in all study groups, but this was not statistically significant.

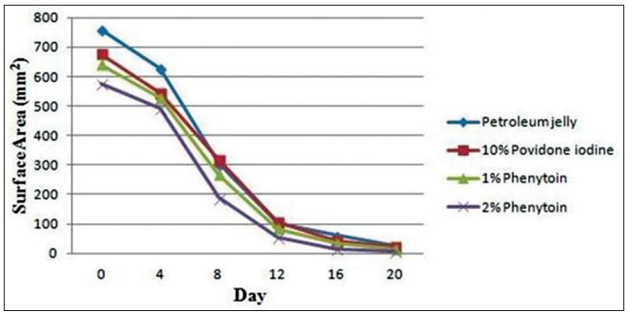

Figure 1: Effect of treatments on wound surface area.

Table 1: Measurement of wound surface area (mm^2) in all studied groups (Mean±SEM).

Groups	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Petroleum jelly	759±40.93	628.50±32.88	303.38±26.53	103.13±15.63	62.13±12.63	25.50±8.90
Povidone iodine	676.75±42.76	544.38±42.38	316.63±36.37	106.63±10.74	42.88±10.13	22.38±5.11
1% phenytoin	640±25.02	528.37±16.88	266.87±30.40	84.25±10.78	33.75±8.27	14.5±7.44
2% phenytoin	575.25±33.05	492.75±31.61	188±15.76	54.38±3.92	16.38±1.96	6.38±1.35

SEM: Standard error of mean

Table 2 and Figure 2 depict the percentage wound closure measured on day 4, day 8, day 12, day 16 and day 20.

Percentage wound closure was more with 2% phenytoin as compared to other groups, but on comparison, there was no significant difference between the groups. Furthermore, there was no significant difference between the two preparations of phenytoin.

Table 3 and Figure 3 depict the number of days required for complete re-epithelisation of the wound.

Days required for complete re-epithelisation was significantly less in 10% povidone iodine group when compared with petroleum jelly and 2% phenytoin. There was no significant difference between the two formulations of phenytoin.

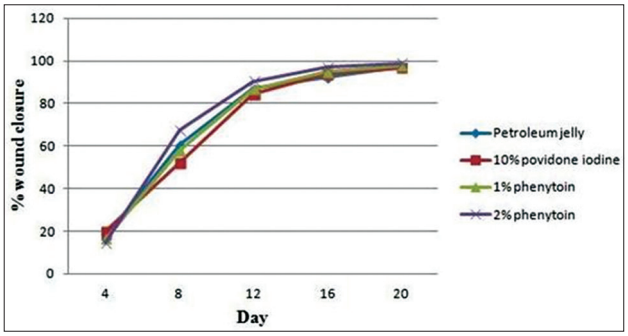

Figure 2: Effect of treatments on percentage wound closure.

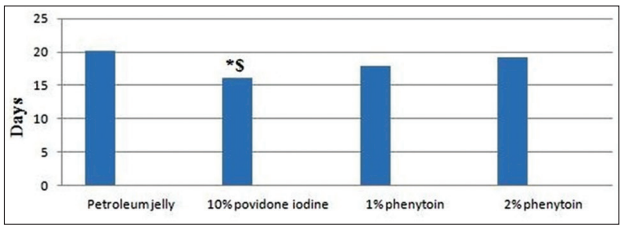

Figure 3: Effect of treatments on complete re-epithelization. (*p<0.001 as compared to petroleum jelly, $p<0.001 as compared to 2% phenytoin).

DISCUSSION

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity and involves a complex process, which includes vascular, inflammatory phase, re-epithelisation, granulation tissue formation, matrix, and collagen remodelling. Any agent that promotes any of the above processes is a promoter of wound healing.

Phenytoin was introduced into therapy in 1938 for the effective control of convulsive disorders. In 1939, Kimball first observed that gingival hyperplasia occurred in some patients treated with phenytoin[6] and Shapiro carried out the first clinical trial in 1958 and found out that periodontal patients with surgical wounds who were pre-treated with oral phenytoin had less inflammation, less pain and accelerated healing when compared to controls.[7] This stimulated the study regarding the potential use of phenytoin in wound healing. Subsequently, it was seen that phenytoin promoted the healing of dental extraction sockets and also increased the tensile strength of skin wounds.[8,9] In spite of all these reports, there are few studies with contradictory reports stating that topical application of phenytoin has no effect on dermal or epidermal growth suggesting that it does not possess wound healing property.[3]

The mechanism by which phenytoin accelerates wound healing is unknown. Clinical, animal and in vitro studies suggest that phenytoin may be involved in the healing process at several levels including stimulation of fibroblast proliferation, enhancing the formation of granulation tissue, decreasing collagenase activity (by reducing its production or secretion or both), promoting deposition of collagen and other connective tissue components, decreasing bacterial contamination and decreasing wound exudates. Biopsies of phenytoin treated open wounds show neovascularisation, collagenisation, decreased polymorphonuclear infiltrate cells and eosinophils.[10]

A number of clinical studies indicate that phenytoin decreases the bacterial load of wounds. Topical phenytoin was reported to eliminate *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas* spp. from wounds within 7-9 days. In a guinea pig model of wound healing, it was found that phenytoin cleared Gram-negative organisms from the wounds more readily than Gram-positive bacteria. It is unknown if phenytoin has intrinsic antibacterial activity, or if the effect of phenytoin on the bacterial load of wounds may be mediated indirectly by effects on inflammatory cells and neovascularisation.[11-15]

In a study reported by Hasamnis et al.[2] in excision wound model, topical phenytoin had been compared with control (no

Table 2: Percentage wound closure (Mean±SEM).

Groups	Day 4	Day 8	Day 12	Day 16	Day 20
Petroleum jelly	17.03±2.14	60.42±1.84	86.89±1.44	92.23±1.17	96.89±0.84
Povidone iodine	19.84±3.21	52.32±5.38	84.26±1.25	93.88±1.17	96.79±0.59
1% phenytoin	16.40±3.07	58.06±4.65	86.74±1.69	94.76±1.27	97.75±1.13
2% phenytoin	14.43±2.09	67.28±2.19	90.50±0.57	97.22±0.26	98.92±0.24

SEM: Standard error of mean

Table 3: Days required for complete re-epithelisation (Mean±SEM).

Study groups	Days required for complete re-epithelisation
Petroleum jelly	20.12±7.11
Povidone iodine	16.12±0.41*$
1% phenytoin	17.87±0.80
2% phenytoin	19.13±0.60

SEM: Standard error of mean

treatment). The results of this study showed that phenytoin accelerated the healing of excision wounds.

Qunaibi et al.[16] studied the effect of phenytoin ointment on complete wound closure, as well as its biochemical and histological effects in an excision wound model. They observed that phenytoin hastened the healing, increased protein and hydroxyproline content as well as histological collagenisation of excision wounds. Therefore, the study concluded that phenytoin not only shortens the time for wound healing but also improves the quality of the healing tissues.

The results of the present study are in accordance with the above studies suggesting that phenytoin promotes wound healing process in excision wound model.

CONCLUSION

The results of all the parameters included in our study showed comparable results in phenytoin treated group when compared to the povidone iodine treated group. This signifies not only an experimental use, but also a possibility of use of topical phenytoin clinically in treatment of wounds.

In a therapeutic area of wound healing the current scenario for the treatment of majority of wounds include more of supportive measures like maintenance of hygiene, proper wound dressing and prophylactic use of antibiotics rather than agents which inherently improve the healing process. Ideally the drugs for wound healing should be designed in a way that they should be effective and promise fast results to reduce the morbidity and sufferings of the patients and most important of all to be cost-effective. Formulation of phenytoin shows great promise by promoting wound healing and is cost-effective. Even though, there are studies since history, which describes the role of phenytoin in wound healing activity, these studies are limited and comparative studies using different formulations, route of administration and dosing intervals are lacking. Reflecting these findings, a large number of animal studies should be conducted, and extensive clinical trials should be carried out to support the animal study based evidence.

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Ethical approval: Obtained from Institutional Animal Ethics Committee

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