EFFECT OF TOPICAL PHENYTOIN CREAM ON LINEAR INCISIONAL WOUND HEALING IN ALBINO RATS

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ABSTRACT

In this study, the effect of phenytoin cream on linear incisional wound healing was investigated. Thirty male Wistar rats were subjected to a linear 3cm incision made over the skin of the back. The animals were randomly divided into 3 experimental, control, cold cream and treatment groups. Control group did not receive any drug or cold cream. Cold cream group received topical cold cream once a day from the beginning of experiments until the day that wounds were closed. Treatment group were treated topically by 1% phenytoin cream at the same time. For computation of the percentage of wound healing, the area of the wound were measured at the beginning of experiments and the next 2,4,6,8,10,12,14 and 16 days. The percentage of the healing wounds were calculated by Walker formula after measurement of the wound area. Results showed that there weren't statistically significant differences between treatment and cold cream animals ($P>0.05$) in most of the days. It is concluded that phenytoin has possibly no significant effect on the rate of wound closing in acute wound model of incision in rat. Therefore further study is required for detection of the role of phenytoin on wound healing and the related parameters in various kinds of experimental wound models.

Keywords: Wound healing, Phenytoin, Skin, Rat

INTRODUCTION

Wound healing consist of several processes including cell migration, proliferation, differentiation and the formation of new extracellular matrix (1). Dehiscence of operative wounds and delayed wound healing express a significant clinical problem (2). Phenyton (PHT) was first used clinically in the treatment of epilepsy in 1938 and it has been used in the healing of a diversity of wounds since the 1950s (3-7). In a clinical study it was first showed that pretreatment with oral PHT enhanced healing of periodental wounds (8). In other investigations acceleration of the healing of war wounds as well as diabetic and leprotic ulcers have been reported (9-10).

The effect of topical PHT on large abscess cavities has also been reported. Patients treated with topical PHT showed earlier detachment of slough, decrease in edema and increase in wound healing. In spite of the above mentioned information, some contradictory results have been reported that invitro administration of PHT had no effect on human dermal fibroblasts or epidermal keratinocytes (11)and according to a recent study, subcutaneouse injection of PHT had a significant effect in an incisional wound model in rats (12). So the effect of topical administration of PHT for the promotion of wound healing seems promising but requires further trials (13,14). In this study the effect of topical PHT on the rate of wound healing in an incisional wound model in rats was investigated.

MATERIALS AND METHODS

Adult male Wistar rats (200-220g) were used in this study. They were housed individually per cage in a room with natural light cycle and constant temperature ($24 \pm 2\ ^0C$). Food and water were available ad libitum. The animals were anesthetised by intraperitoenal injection (75 mg/kg) of pentobarbital. After shaving the backs of the animals, a linear 3cm incision was made over skin of the back. Thirty animals were randomly divided into 3 experimental control, cold cream and treatment groups. Cold cream was prepared according to the USP (United State Pharmacopoeia) recommendations. For the prepatation of 1% Phenytoin cream, 1gr of phenytoin powder was added to 99gr. of cold cream. Control group did not receive any drug or cold cream. Animals of cold cream (basal vehicle of phenytoin cream) group received topical cold cream once a day from the beginning of experiments until complete wound closure. Animals of treatment group were treated topically by 1% phenytoin cream at the same time. For computation of the percentage of wound healing, a transparent paper was placed on the location of wound and its shape was drawn on the paper. Then

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the wound area was measured by matching the shape of wound with a graph paper. The percentage of wound healing calculated by walker formula after measuement of the wound area (15). Percentage of wound healing was computed at the beginning of experiments and the next 2, 4, 6, 8, 10, 12 ,14 and 16 days.

$$\% \text{ wound area} = \frac{\text{Wound area in the day of X}}{\text{Wound area in the first day}} \times 100$$

Percentage of wound healing = 100 – percentage of wound area

Statistical methods

The data had normal distribution by Kolmogorov-Smirnov test. Students t-test and ANOVA were used to test differences between the groups. Differences were considered significant when $P<0.05$. Results are given as means of values.

RESULTS

There was statistically significant difference ($P<0.05$) between phenytoin cream and cold cream groups of animals only in the day of 8 of experiments (Fig1). These data suggest that the net effect of topical PHT on wound healing in an incised model in rats dose not have any clinical value.

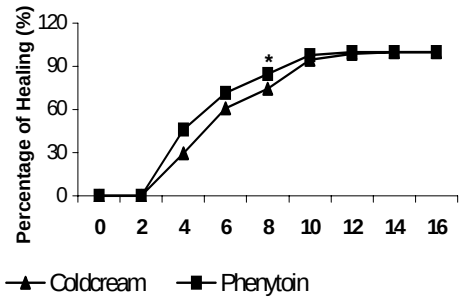

Fig 1. Effect of PHT in comparison with control group on the percentage of wound healing in days after beginning of experiments. *P<0.05

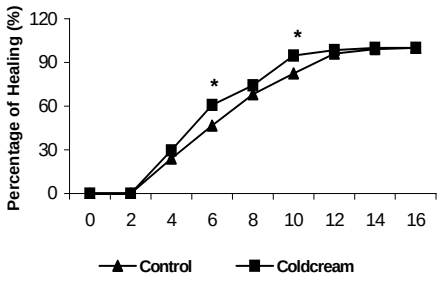

Fig 2. Effect of cold cream with comparison of control group on the percentage of wound healing in days after beginning of experiments. *P<0.05

There were statistically significant difference ($P<0.05$) between cold cream and control animals only in the days of 6 and 10 of experiments (Fig2).A comparison between control and treatment groups (Cold cream + Phenytoin) showed that there were significant differences in days of 4, 6, 8 and 10 of experiments. It is clear that the addition of cold cream to PHT in three days of expriment as mentioned above may be the cause of acceleration of wound healing processes in this experiment [(Additive synergism)(Fig 3)].

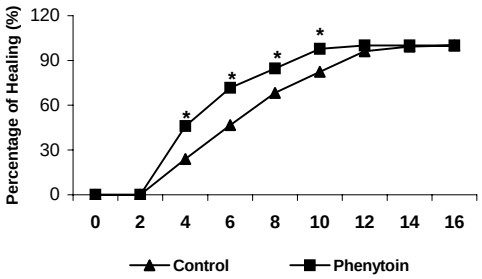

Fig 3. Effect of PHT+Cold cream in comparison with control group on the percentage of wound healing in days after beginning of experiments. *P<0.05

DISCUSSION

While the mechanism of action of PHT in wound healing is not clear yet, there are some contradictions about its effect on various kinds of wounds especially in the experimental models (12, 13, 14). There are various conditions for the application of this drug to increase wound healing, especially in clinical conditions where age or underlying diseases such as diabetes and sepsis cause significant morbidity or mortality resulting from weak healing or wound dehiscence. Considering the physicochemical propertis of PHT and its weak circulation in chronic wounds, it is possible that PHT is stayed there for longer periods and could be metabolized by surronding tissue and cells (16). It seems that the high beneficial effect of PHT on wound healing will be mostly via injections(12).Topical application of PHT, results in direct access of the drug to the target site without undergoing classical metabolic pathways, and there isn't a higher risk of causing dose-related side-effects which is seen with systemic therapy for wound healing (10). The effect of topical use of PHT for promotion of wound healing seems promising but requires further trials (13,14). Our data suggest that topical administration of PHT does not have the potential to change the natural history of the wound healing in incised wound model in rats (Fig 1). According to our results cold cream had a positive effect in days 6 and 10 on wound healing (Fig 2). This effect may be related to control of

microbial growth by preservatives in cold cream and its moisturizing effect. A comparison between cold cream and treatment (PHT) groups showed that the net effect of PHT on the rate of wound closing was not significant in most days of experiment (Fig 1). Apparently on the basis of results of this investigation PHT has little effect on the rate of Re-epithelialisation in incised models of wound healing in rat, but it must be considered that the enhancement of wound closing which was observed in our experiments depend on either Re-epithelialisation or wound contraction. According to some reports PHT reduces wound contraction (17,18). Therefore it seems that in this study the net effect of PHT must be enhanced in re-epithelialisation.

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REFERENCES

1. Clark RAF, Basis of cuetaneous wound repair. J Dermatol Surge Oncol 1993; 19: 693-706.
2. Angelopoulos AP, Goaz PW. Incidence of diphenylhydration gingival hyperplasia. Oral Surg Oral Med Oral Pathol 1972; 31: 808-906.
3. Bogaert H, Saleta B, Sanchez E, Garcia B. Trophic leprosy ulcer: treatment with topical and systemic phenytoin. Int J Dermatol 1990; 29: 156-7.
4. Mendiola GJ, Espego PI, Rodrigues NE. Sodium diphenylhydnation in burns: Effect on pain and healing. Invest Med Int 1983; 10: 443-7.
5. Simpson GM, Kunz E, Slafta J. Use of sodium diphenylhydnation in the treatment of leg ulcers. NY State J Med 1965; 65: 886-8.
6. Rodrigues NE, Esparza AS, Andrade PJS, Espejo PI, Chapa ARJ. Treatment of soft tissue ulceration with topical diphenylhydnation. Invest Med Int 1983; 10: 18-16.
7. Bansal NK. Comparison of topical phenytoin with normal saline in the treatment of chronic trophic ulcers in leprosy. Int J Dermatol 1993; 32: 210-3.
8. Muthukumarasamy MG, Sivakumar G, Manoharan G. Topical phenytoin in diabetic foot ulcers. Diabetes Care 1991; 14: 909-11.
9. Modaghegh S. Use of phenytoin in the healing of war and non-war wounds. Int J Dermatol 1989; 28: 347-50.
10. Talas G, Brown RA, McGrouther DA. Role of Phenytoin in Wound Healing, A Wound Pharmacology Perspective. Biochem Pharmacol 1999; 57: 1058-1094.
11. Vijayasingham SM, Dykes PJ, Markes K. Phenytoin has little effect on in vitro models of wound healing. Br J Dermatol 1991; 125: 136-9.
12. Habibipour S, Oswald TM, Zhang F, Joshi P, Zhou XC, Dorsett-Martin W, Lineaweaver WC. Effect of sodium diphenylhydantoin on skin wound healing in rats. Plast Reconstr Surg. 2003; 112: 1620-7.
13. Carneiro PM, Rwanyuma LR, Mkony CA. A comparison of topical phenytoin with Silverex in the treatment of superficial dermal burn wounds. Cent Afr J Med 2002; 48: 105-8.
14. Scheinfeld N. Phenytoin in cutaneous medicine: its uses, mechanisms and side effects. Dermatol Online J. 2003; 9: 6.
15. Walker HL, Mason AD. A standard animal burn. J Trauma 1969; 8: 1049-1051.
16. Hobson MI, Brown RA, Green CJ, Terenghi G. Interrelationships between angiogenesis and nerve regeneration: A histochemical study. Br J Plast Surg. 1997; 50: 125–131.
17. Talas G, Adams TST, Eastwood M, Rubio G, Brown RA. Phenytoin reduces the contraction of recessive dystrophic epidermolysis bullosa fibroblast populated collagen gels. Int J Biochem Cell Biol 1997; 29: 261–270.
18. Talas G, Adams TST, Porter RA, Eastwood M, Brown RA. Phenytoin alters dermal fibroblast contraction in vitro and wound contraction in vivo. Wound Rep Reg 1994; 4: A177.