

## Effect of topical phenytoin on burn wound healing in rats

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To evaluate the effect of phenytoin on burn wounds and to compare the effect of the combination of topical phenytoin preparation in dexamethasone treated burn wounds in rats, partial thickness thermal burn wounds were inflicted upon five groups of six rats each. Group I was assigned as control, Group II received the standard silver sulphadiazine, Group III was given topical phenytoin and Group IV received injection dexamethasone, Group V received the combination of the phenytoin and the dexamethasone. The parameters observed were epithelialization period, percentage of wound contraction and histopathological analysis as indicative of the process of healing. Phenytoin group showed significant improvement in burn wound contraction in comparison to standard silver sulphadiazine group, the combination group of topical phenytoin and dexamethasone also showed significant contraction compared to dexamethasone group. The period of epithelialization also decreased significantly in groups II, III and V. In conclusion, phenytoin promotes burn wound healing as evidenced by decrease in period of epithelialization and faster wound contraction.

**Keywords:** Epithelialization, Phenytoin, Silver sulphadiazine, Wound contraction

Burns remain a major public health issue all over the world, especially in developing countries. Superficial burns comprise a spectrum of injury severity depending on the depth of the wound and proportion of the body affected<sup>1</sup>. While current approach to burn injury management has improved patient prognosis, however increased morbidity and mortality remain a major challenge for clinicians. Infection due to new strains of drug resistant bacteria is the major impediment topical factor in healing process and also prolongs treatment period<sup>2</sup>. Improving the methods of wound healing and tissue repair offers tremendous opportunities to enhance the quality of life for burn patients<sup>3</sup>. Phenytoin is an antiepileptic that has been clinically used since 1938<sup>4</sup>. The common clinical finding in patients taking oral phenytoin is gingival hyperplasia, which led Shapiro<sup>5</sup> to carry out first clinical study to evaluate the effect of phenytoin on gingival wounds. Collagen is the main component of fibrous and cartilage tissue which provides structural support and the synthesis is stimulated by various growth factors which in turn

hasten wound healing process<sup>6</sup>. Phenytoin causes a dose dependent adverse effect of gingival hyperplasia due to collagen proliferation. This property could be useful to hasten superficial burn wound healing; it also appears to enhance the healing of full-thickness skin graft in normal individuals and in diabetic ulcer patients as shown in the previous studies<sup>7,8</sup>. Effect of topical phenytoin in the healing of decubitus ulcers, traumatic ulcers and a variety of chronic non-healing ulcers in clinical and experimental studies has been reported<sup>9</sup>. This study has been designed to evaluate its healing efficacy in superficial burn wounds in rats.

### Materials and Methods

**Drugs and chemicals**—Phenytoin base (Orgamine Chemicals, Mumbai), silver sulphadiazine cream 1% w/w (12 g) (Rexcin Pharmaceuticals), dexamethasone injection 2 mg/1 ml (Zyodus Allidac), liquid paraffin, hard paraffin (Rankem), alcohol, xylene, eosin and hematoxylin, (Sigma Chemical Co. ,St Louis, USA) were used . All other chemicals were of analytical grade.

**Animals**—A total of 120 healthy (150-200 g) Wistar rats, of either sex aged 12 weeks bred locally in the animal house of Kasturba Medical College Manipal, were selected for the study. They were

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housed under controlled conditions of temperature  $23^{\circ}\pm 2^{\circ}\text{C}$ ,  $50\pm 5\%$  RH and 10-14 h of light and dark cycles and were maintained as per the guidelines of Indian National Science Academy New Delhi, India. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food and water *ad libitum*. Animals were kept under fasting for overnight and weighed before the experiment. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee (IAEC approval letter No. IAEC/KMC/ 07/ 2008-2009, dated June 16, 2008).

**Study design and drug administration**—Rats (30) were divided randomly into 5 groups of 6 rats each. Group I served as control, Group II received silver sulfadiazine topically, Group III received phenytoin cream topically, Group IV received dexamethasone (0.17mg/kg, im) daily and Group V received dexamethasone (0.17mg/kg, im) daily plus topical phenytoin cream. All the drugs were given daily for 21 days or till complete epithelialization whichever was earlier.

**Preparation of phenytoin cream**—Prewedged phenytoin base (2.5 g) was added to liquid paraffin (12.5 ml) in a mortar while triturating. To this mixture added molten hard paraffin (10 g) and triturated until a cream (10 %) was formed.

**Wound model**—A partial thickness burn wound model was employed as per Bairy *et al*<sup>10</sup>. Briefly, the rats were anaesthetized with ketamine (100 mg/kg, im) and the hair on the dorsum was clipped. Then burn wounds were created by pouring hot ( $80^{\circ}\text{C}$ ) molten wax in the metal cylinder, placed on the shaven back of the animal at the nape of the neck. After animals recovered completely from anesthesia, they were kept in individual cages and followed all norms of good laboratory practice in caring the animals.

**Assessment of burn healing**—Animals were inspected every alternate day and the healing was assessed based on physical parameters namely wound contraction and epithelialization as well as histologically.

**Wound contraction**—Wound contraction was monitored by measuring the progressive changes in raw wound area, planimetrically on a transparent paper, every two days, excluding the day of wounding. The tracing was then transferred to

1 mm<sup>2</sup> graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm<sup>2</sup>, as 100% by using the following equation<sup>11</sup>:

$$\text{Wound contraction(\%)} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

**Epithelialization**—Time taken for full epithelialization was measured by recording the days required for fall of eschar leaving no raw wound area behind.

**Histopathology examination**—After the fall of eschar, rats were sacrificed by administering intravenous thiopentone sodium. The wound bed was dissected and preserved in 10% formalin for seven days and histopathological examination was done by H & E staining. Stained slides were observed for collagen content, inflammatory changes, polymorphonuclear cells, vacuolization and fibroblast number, these observations were compared with control groups.

**Statistical analysis**—The results were expressed as mean  $\pm$  SE. The results were analysed for statistical significance using one way ANOVA, followed by Scheffe's test. *P* value  $< 0.05$  was considered significant. Computer statistical package SPSS (version 16) was used for analysis.

## Results

The percentage of wound contraction was significantly increased in the topical phenytoin group compared to silver sulphadiazine and control group, and in phenytoin+dexamethasone group in comparison to dexamethasone group (Table 1).

The mean period of epithelialization was significantly decreased in phenytoin group ( $13.66 \pm 0.12$  days) in comparison to the standard silver sulphadiazine ( $19.00 \pm 0.68$  days) and control ( $22.66 \pm 0.66$  days). However the mean period of epithelialization was increased in the combination group of phenytoin and dexamethasone ( $20.00 \pm 1.63$  days) in comparison to dexamethasone group ( $30.83 \pm 1.75$  days).

**Histopathology**—Inflammatory changes were seen in all the five groups. There was mild to moderate infiltration of polymorphonuclear cells and appearance of fibroblasts and myofibroblasts in all the

groups. More collagenation was seen in phenytoin and silver sulfadiazine group in contrast to the control and combination group of phenytoin + dexamethasone. Remodelling of epidermis and dermis was more mature in the phenytoin and silver

sulfadiazine group compared with the combination group of phenytoin and dexamethasone which showed ulcers in epidermis. Increased neovascularization and lymphocytes were also observed in phenytoin and silver sulfadiazine group (Figs 1-4).

Table 1—Effect of topical phenytoin on burn wound contraction in rats  
[Values are mean  $\pm$  SE ]

Groups	Wound contraction (%)				
	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	20 <sup>th</sup> day
Control	5.53 $\pm$ 0.39	18.33 $\pm$ 1.39	31.53 $\pm$ 3.64	53.87 $\pm$ 6.45	71.68 $\pm$ 5.78
Silver sulphadiazine	13.32 $\pm$ 0.53 <sup>ac</sup>	30.82 $\pm$ 2.89 <sup>ac</sup>	45.12 $\pm$ 2.62 <sup>a</sup>	61.30 $\pm$ 2.42	77.81 $\pm$ 2.27
Phenytoin	22.84 $\pm$ 0.64 <sup>abcd</sup>	40.59 $\pm$ 1.54 <sup>acd</sup>	67.76 $\pm$ 2.37 <sup>abc</sup>	81.10 $\pm$ 1.97 <sup>abc</sup>	91.98 $\pm$ 0.73 <sup>abc</sup>
Dexa*	5.96 $\pm$ 1.14 <sup>bd</sup>	16.50 $\pm$ 1.28 <sup>bd</sup>	37.51 $\pm$ 2.23 <sup>d</sup>	55.37 $\pm$ 2.30	69.12 $\pm$ 1.24 <sup>d</sup>
Phenytoin+ Dexa	13.15 $\pm$ 2.12 <sup>ac</sup>	28.49 $\pm$ 3.12 <sup>cd</sup>	52.89 $\pm$ 1.42 <sup>ac</sup>	69.67 $\pm$ 1.72	83.53 $\pm$ 2.12 <sup>c</sup>

\* Dexa = Dexamethasone. *P* value : <0.05; compared to <sup>a</sup>control; <sup>b</sup>silver sulphadiazine; <sup>c</sup>dexamethasone; <sup>d</sup>phenytoin+dexamethasone

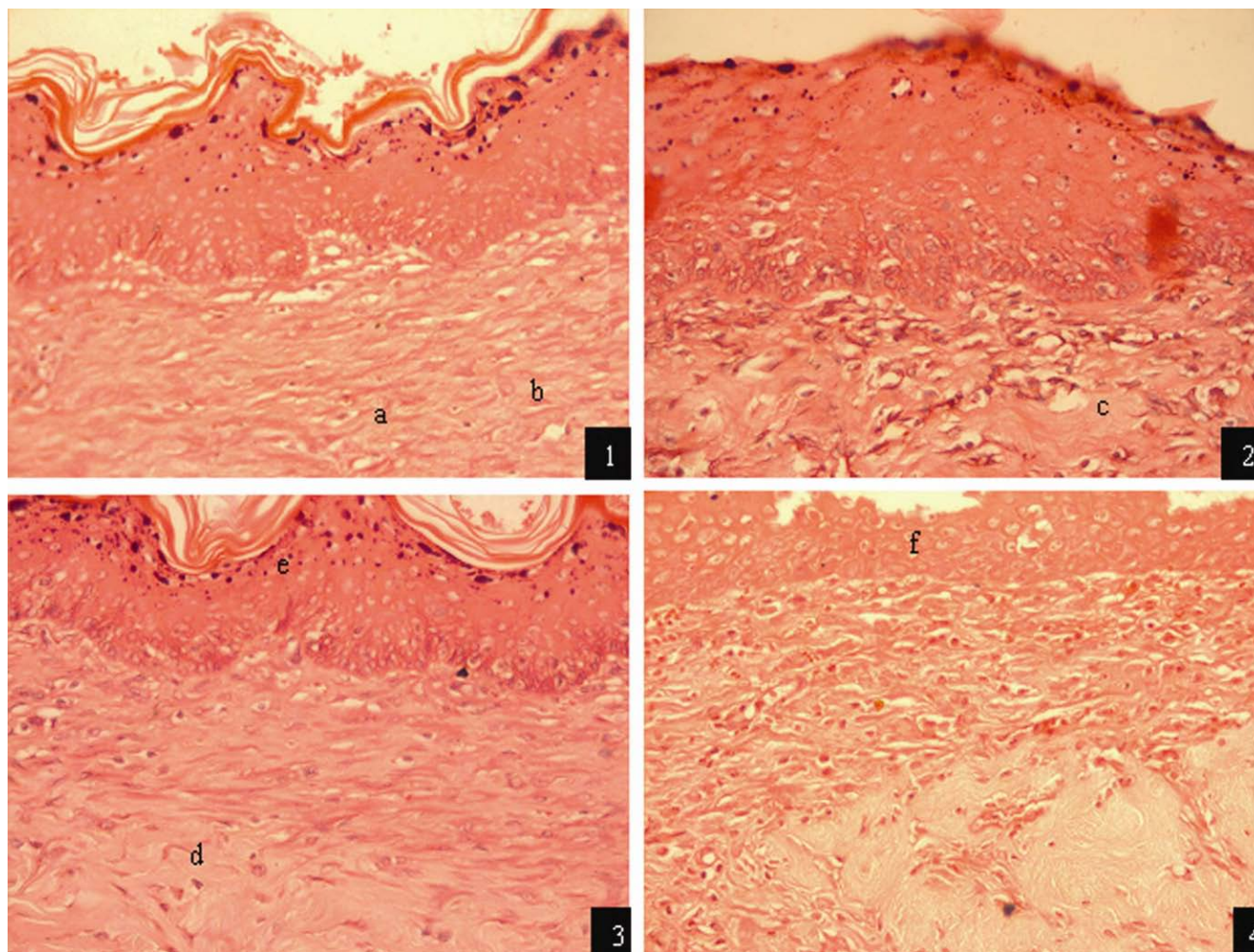


Fig. 1—Histopathology (1) control group- there are inflammatory changes, infiltration of polymorphnuclear cells, fibroblasts (a) as well as collagenation (b), (2) silver sulphadiazine- there are fibroblasts, collagenization, neovascularization (c), (3) phenytoin treated group- there are fibroblasts, myofibroblasts (d), lymphocytes (e), collagenization and neovascularization, (4) the phenytoin and dexamethasone treated group- the epidermis is not completely healed (f), areas of ulceration are present. H & E  $\times$  40.

## Discussion

The results show that phenytoin topically enhanced wound contraction and period of epithelialization; this is in agreement with histopathological findings which showed a large amount of fibroblasts, myofibroblasts, lymphocytes, collagenization and neovascularization<sup>6,13</sup>. Topical application of phenytoin significantly accelerated wound healing and improved the quality and vascularity of granulation tissue, possibly by increasing fibroblast proliferation, maturation of collagen content on one hand and decreasing collagenase activity on the other<sup>12,13,15</sup>.

Lipid peroxidation is an important process in several types of injuries like burns, inflicted wound and skin ulcers, and a drug which inhibits lipid peroxidation is believed to increase the viability of collagen fibrils<sup>16</sup>. The exact mechanism by which phenytoin increased granuloma strength cannot be explained with present data. In the present study the test drug phenytoin increased collagenation, this could be one of the possible assumption.

As a consequence of post burns injury it may jeopardise return to normal accustomed work due to cosmetic disfigurement<sup>17</sup>. Topical application of phenytoin in such patients may enhance healing by its faster epithelialization property and patients will be able to resume their daily work at an early date.

Topical phenytoin effectively reduced the antihealing effect of dexamethasone<sup>14</sup>. This property of phenytoin can be used to promote burn wound healing in patients who received steroids for the management of burns or for some other comorbid conditions.

In conclusion, phenytoin cream is a less costly and efficacious treatment to enhance the wound healing in superficial burns or to reverse antihealing effects of known wound healing suppressants such as non steroidal anti-inflammatory agents, steroids, anticancer agents etc. A systematic clinical evaluation of topical phenytoin in burn patients is warranted before advocating topical phenytoin into clinical practice.

## References

- 1 Moore O A, Smith L A, Campbell F, Seers K, Mc Quay & Moore R A, Systematic review of the use of honey as a wound dressing, *BMC Complement Altern Med*, 1 (2001) 2.
- 2 Jaladi F S, Saifzadeh S, Tazik H & Farshid A A, Experimental evaluation of repair process of burn wounds treated with natural honey, *J Anim Vet Adv*, 6 (2007) 179.
- 3 Blanks T, Brown S, Cosgrave B, Woody J, Bentley V, O' Sullivan N, et al. *The body shop book of wellbeing mind, body, and soul*, (Ebury Press London) 1998, 173.
- 4 Merritt H H & Putnam T J, Sodium diphenylhydantoinate in the treatment of convulsive disorders, *JAMA*, 251 (1984) 1062.
- 5 Shapiro M, Acceleration of gingival wound healing in non-epileptic patients receiving diphenylhydantoin, *Exp Med Surg*, 16 (1958) 41.
- 6 Kiran K & Asad M, Wound healing activity of *Sesamum indicum* Linn seed and oil in rats, *Indian J Exp Biol*, 46 (2008) 777.
- 7 Albsoul-Younes A, Younes NA & Badran DH, Topical phenytoin ointment increases autograft acceptance in rats, *Saudi Med J*, 27 (2006) 962.
- 8 Younes N, Albsoul A, Badran D & Obedi S, Wound bed preparation with 10% phenytoin ointment increases the take of split-thickness skin graft in large diabetic ulcers, *Dermatol Online J*, 12 (2006) 5.
- 9 Pendse AK, Sharma A, Sodani A & Hada S. Topical Phenytoin in wound healing, *Int J Dermatol*, 32 (1993 ) 214.
- 10 Bairy K L, Somayaji S W & Rao C M, Experimental model to produce partial thickness burn wound, *Indian J Exp Biol*, 35 (1997) 70.
- 11 Srivastava P & Durgaprasad S, Burn wound healing property of *Cocos nucifera*: An appraisal, *Indian J Pharmacol*, 40 (2008) 144.
- 12 Houck J C, Jacob R A, Maengwyn-Davies G D, The effect of sodium dilantin administration upon chemistry of the skin, *J Clin Invest*, 39 (1960) 1758.
- 13 Bauer E A, Cooper T W, Tucker D R & Esterly N B, Phenytoin therapy of recessive dystrophic epidermolysis bullosa: clinical trial and proposed mechanism of action on collagenase, *N Eng J Med*, 303 (1980) 776.
- 14 Ganesh B, Sanjeeva & Bairy K L, Effect of *Tridax procumbens* on burn wound healing, *Indian Drugs*, 40 (2003) 488.
- 15 Habibipour S, Oswald T M, Zhang F, Joshi P, Zhou X C, Dorsett-Martin W & Lineaweaver W C, Effect of sodium diphenylhydantoin on skin wound healing in rats, *Plast Reconstr Surg*, 112 (2003) 1620.
- 16 Senel O, Cetinkale Ozbay G, Ahcioglu F & Bulan R, Oxygen free radicals impair wound healing in ischemic rat skin, *Ann Plast Surg*, 39 (1997) 516.
- 17 Hwang YF, Chen-Sea MJ & Chen CL, Factors related to return to work and job modification after a hand burn, *J Burn Care Res*, 30 (2009) 661.