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ORIGINAL ARTICLE

The Effect of Topical Phenytoin Application on Traumatic Tympanic Membrane Perforation

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Objective: To determine the effect of topical phenytoin on healing of tympanic membrane in rabbits.

Materials and Methods: Under otomicroscopy, a 4 mm perforation was created in the posterosuperior quadrants in both ears of eighteen healthy adult New Zealand white rabbits. Daily applications of topical phenytoin solution (0.5ml) to the right and isotonic saline (0.5 ml) to the left ear were continued until sacrification of the animal. Six rabbits were randomly sacrificed on day 3, then 6 more were sacrificed on days 7 and 14 respectively in order to examine the vascularization, inflammation and fibroblastic reaction process in different stage. Results: There was no significant difference between the phenytoin group and control group in terms of inflammation, neovascularization and fibroblastic reaction.

Conclusion: Topical phenytoin application to an acute traumatic tympanic membrane perforation does not have a significant effect in this study.

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Introduction

Many different types of trauma result in tympanic membrane perforation. Slapping, barotrauma, temporal bone fractures, self-inflicted injuries, iatrogenic injuries and penetrating trauma are common causes. Hearing loss and tinnitus are the main symptoms. Traumatic tympanic membrane perforations remain a controversial subject, with treatment varying from conservative management, cauterization of the edges with chemicals to some form of grafting. Epithelial migration, enhanced fibroblastic activity and vascular proliferation are the main components of the complex healing process. Most patients with acute traumatic tympanic membrane perforations heal spontaneously. Nevertheless, some patients appear to be unable to recover spontaneously and often require surgical interventions.

The exact reason for the persistence of a traumatic tympanic membrane perforation remains unclear. Recently topical application of hyaluronic acid,

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insulin, epidermal growth factor, transforming growth factor-β1, basic fibroblast growth factor or pentoxifyllin have been tried to improve the healing process.[1-5]

The use of phenytoin as an antiseizure medication has resulted in gingival hyperplasia as a side effect in many patients and so the concerns were concentrated on ways to use the drug to promote wound healing.[6,7] Shapiro carried out the first controlled clinical trial examining the effects of oral phenytoin on periodontal wounds and reported that phenytoin accelerated wound healing and reduced pain and inflammation.[8] Recent studies in other surgical and non- surgical specialties indicated that topical phenytoin generally improves wound healing.[6,7,9] However, studies concerning the effect of topical phenytoin on healing of tympanic membrane perforations are lacking.

To address the potential role of topical application of phenytoin in the healing process, it is crucial to construe an animal traumatic tympanic membrane perforation model. Therefore, we conducted an experimental study to determine the effect of topical phenytoin on healing of tympanic membrane in rabbits.

Materials and Methods

The study protocol was approved by the Local Ethics Committee for Animal Experiments of Gülhane Military Medical Academy (KS-09/51K, June.2009). Eighteen healthy adult New Zealand white rabbits were used in this study. The weights of the animals ranged btw 2.5-3.0 kg. Before the study, the animals had been kept under uniform conditions for 1 week and animals with any underlying diseases were excluded. During the experiment, each rabbit was separately caged and had free access to standard food pellets and water. The animals received care according to the NIH Guide for The Care and Use of Laboratory Animals and the study protocol was approved by the animal research ethics committee. All animals were anesthetized with intraperitoneal ketamine hydrochloride (35mg/kg) and intramuscular xylazine hydrochloride (5mg/kg) injections. Ears of all rabbits were washed with povidone iodine and examined under operating microscope and the animals that had normal tympanic membranes and no infections were included in the study. Under otomicroscopy, through an aural speculum, a 4 mm perforation was created in the posterosuperior quadrants in both ears with a calibrated perforator. Absorbable gelatin sponge (Spongostan, Johnson&Johnson Medical Ltd., Gargrave, Skipton, UK) was applied to close the perforation of tympanic membranes of the animals. The sponge was not replaced during the study. Place of the sponge was checked with a microscope every other day. Then 20 mg/ml phenytoin solution was prepared from the drug's powder and 0.9% NaCl. Daily applications of the freshly prepared topical phenytoin solution (0.5ml) to the right and isotonic saline (0.5 ml) to the left ear were continued until sacrification of the animal.

Six rabbits were randomly sacrificed on day 3, and 6 more on days 7 and 14 respectively in order to examine the histological process in different stages. After the rabbits were sacrificed by overdose of intraperitoneal pentobarbital, middle ear bullas were carefully dissected and removed for histopathological examination.

Histopathologic examinations: The specimens were fixed overnight in 10% formaldehyde and decalcified with 10% formic acid solution. After decalcification, tissue specimens were prepared in an Autotechnicon tissue processor, embedded in paraffin and sectioned in 5mm slices and stained with hemotoxylin-eosin. The same senior pathologist evaluated all the sections under the light microscope as for the presence of inflammation, neovascularization and fibroblastic reaction.

The scoring system used for inflammation, neovascularization and fibroblastic reaction was adopted from Ramalho et al. and Guneri et al.[1,10] For each of these parameters a score ranging from 0 to 3 was assigned (0=absent; 1=mild; 2=moderate; and 3=marked).

Statistical analysis: Data analysis was performed by using SPSS for windows, version 11.5 (SPSS Inc., Chicago, IL, US). Median (minimum-maximum) scores of inflammation, vascularization, and fibroblast were recorded. Statistical significance of differences (if any) between daily scores was evaluated by Kruskal Wallis test with Bonferroni correction. A *p* value less than 0.025 was considered statistically significant. When the p-value determined using Kruskal Wallis test was statistically significant, Mann Whitney U test with Bonferroni correction was used to evaluate the extent of significance. A *p* value less than 0.0083 was considered statistically significant. The differences between saline and phenytoin groups were analyzed by Wilcoxon signed- rank test with Bonferroni correction. A *p* value less than 0.017 was considered statistically significant.

Results and Analysis

Inflammation: Inflammation was subjectively scored as 0, 1, 2 and 3. No significant differences were observed between the saline and the phenytoin groups with respect to the inflammation scores on days 3 (p=0.025), 7 (p=0.157) and 14 (p=0.785). In addition, no statistically significant difference was observed among days within the saline (p=0.309) and the phenytoin groups (p=0.034) (Table 1).

Neovascularization: Neovascularization was subjectively scored as 0, 1, 2 and 3. No significant differences were observed between the saline and the phenytoin groups with respect to the neovascularization scores on days 3 (p=0.083), 7 (p=0.046) and 14 (p=0.317). Also no statistical difference was observed among days within the saline (p=0.195) and the phenytoin groups (p=0.027) (Table 2).

Fibroblastic reaction: Fibroblastic reaction was subjectively scored as 0, 1, 2 and 3. No significant differences were observed between the saline and the phenytoin groups with respect to the fibroblastic reaction scores on days 3 (p= 0.083), 7 (p=0.046) and 14 (p=0.083). There were significant daily differences in the fibroblastic reaction of the phenytoin group (p<0,001) (Table 3).

Table 1. Inflammation scores

Day	Saline	Phenytoin	$p^{a,b}$
3	2 (2-2)	3 (2-3)	0,025
7	2 (1-2)	2 (2-2)	0,157
14	1 (1-3)	2 (1-3)	0,785
$p^{c,d}$	0,309	0,034	

Values are given in median and range (minimum-maximum)

a= Comparisions of saline and phenytoin groups in days 3, 7 and 14; b= Bonferroni Adjusted Wilcoxon Sign Rank test (A p value less than 0.017 was considered statistically significant); c= Comparision of saline and phenytoin among days; d= Bonferroni Adjusted Kruskal Wallis test (A p value less than 0.025 was considered statistically significant).

Table 2. Vascularization scores

Day	Saline	Phenytoin	$p^{a,b}$
3	2 (1-2)	1 (1-2)	0,083
7	1 (1-2)	2 (2-2)	0,046
14	1 (1-2)	2 (1-2)	0,317
$p^{c,d}$	0,195	0,027	

Values are given in median and range (minimum-maximum)

a= Comparisons of saline and phenytoin groups in days 3, 7 and 14; b= Bonferroni Adjusted Wilcoxon Sign Rank test (A p value less than 0.017 was considered statistically significant); c= Comparison of saline and phenytoin among days; d= Bonferroni Adjusted Kruskal Wallis test (A p value less than 0.025 was considered statistically significant).

Table 3. Fibroblastic reaction scores

Day	Saline	Phenytoin	$p^{a,b}$
3	2 (1-2)	1 (1-1)e,f	0,083
7	1 (1-2)	2 (2-2)e	0,046
14	1 (1-2)	2 (2-2)f	0,083
pc,d	0,607	<0,001	

Values are given in median and range (minimum-maximum)

a= Comparisons of saline and phenytoin groups in days 3, 7 and 14; b= Bonferroni Adjusted Wilcoxon Sign Rank test (A p value less than 0.017 was considered statistically significant); c= Comparison of saline and phenytoin among days d= Bonferroni Adjusted Kruskal Wallis test (A p value less than 0.025 was considered statistically significant); e= Statistical significance between the day 3 and 7 (p=0,004); f= Statistical significance between the day 3 and 14 (p=0,004).

Discussion

In this study, topical phenytoin showed no difference when compared to isotonic saline for traumatic tympanic membrane perforation in terms of inflammation, vascularization and fibroblastic reaction in a rabbit model. Several studies in other specialties have shown proven usefulness of topical application of phenytoin sodium for a wide variety of soft tissue wounds such as trophic ulcers, decubitus ulcers, diabetic foot ulcers, burns, traumatic wounds, war related missile wounds, venous stasis ulcers and abscess and the present study is based on previous reports of enhanced wound healing with phenytoin as the topical treatment agent. Most reports are about wound closure and wound healing over time. Many authors reported that phenytoin has a positive healing effect,[6] whereas others found no significant difference between phenytoin and control groups.[11] This discrepancy may arise from different methods used to deliver phenytoin. Topical administration of phenytoin may prevent systemic absorption that might lead to development of side effects. The best method of topical administration of phenytoin remains unclear. Gel, cream and powder of the drug contain the active compound phenytoin for topical use. To avoid damage to the skin, injectible phenytoin should not be used topically. An experimental animal study of wound healing performed with rats claimed that powder formulation can be more promising[12]. Therefore, in most studies, a thin uniform layer of phenytoin powder which can be extracted from pure phenytoin, phenytoin sodium and phenytoin capsules have been applied directly on wounds. The administration of phenytoin with NaCl (0.9%) and applying a medicated gauze on the wound prevents the white eschar like coating caused by the powder from the capsules.[12] Thus, in the present study, phenytoin solution was absorbed to the gelatin sponge,and applied on the perforation, which is safe and easy to use for such experiment.

Approximately more than 80% of the traumatic perforations heal spontaneously within 7-10 days.[13,14] Although many unfavourable factors have been proposed, Infection, presence of foreign bodies and large size wounds are the main risk factors which delay spontaneous healing.[5,14,15] Immediately after traumatic injury, a pattern of local reactions is launched. Homeostasis and inflammation are the common initial stages for all types of wound healing. The latter stages of the tympanic membrane healing are unique and occur in a reverse manner.[13] After early stages, epidermis of the tympanic membrane proliferates 2 mm away from the point of injury to cover the perforation with a keratin spur. Keratin spur functions as a scaffold for the migratory epithelium to seal the perforation. After that, reformation of the connective tissue layer occurs.[5] This sharply contrasts with all other types of wound healing in which connective tissue ingrowth reaction occurs before epithelization.[1] Mucosal layer may have an inconsiderable influence on healing of the tympanic membrane.[5] Studies have shown that small congested capillaries and fibrous tissue begin to take place in the healing process 2 days after epidermal reaction which is often incomplete.

A wait-and-see policy, with adequate advice and regular follow up, will often suffice for the patients with uncomplicated traumatic tympanic membrane perforations. Paper patching, mechanical or chemical debridement of the perforation margin are the office based traditional minor procedures with some success. Currently, research has focused on better tympanic membrane closure by guiding regeneration or inducing cellular proliferation. As a scaffold for the regeneration of the tympanic membrane, a water insoluble chitosan patch demonstrated a favourable difference in the healing speed only, but not the healing rate.[16] Merogel is a sponge like material produced from esterified hyaluronic asid which has been supposed to regulate the healing pattern of the fibrous layer by preventing dehydratation of the perforation margins and increasing the quality of the healed tympanic membrane.[4,15] Pentoxifylline, a pharmaceutical agent with hemorrheological and antithrombotic properties, did not improve either rate

of healing or quality of the tympanic membrane.[5,10] Growth factors have been also shown to play role in wound healing, stimulating proliferation and chemotaxis. Topical application of epidermal growth factor, basic fibroblast growth factor and platelet-derived growth factor accelerates epithelization, improves angiogenesis and modulates collagen metabolism to enhance the healing tympanic membrane.[4] However transforming growth factor b-1 has a dose dependent effect and repeated application rather than a single application accelerates the healing process of tympanic membrane perforations.[3,4]

There are multiple proposed mechanisms put forth for phenytoin induced wound healing. Phenytoin has been suggested to stimulate fibroblast proliferation, facilitate collagen deposition, and glucocorticoid antagonism.[11] In addition, phenytoin appears to reduce edema, wound exudates, transudates and inflammation.[7] About 7 days after injury, phenytoin reduces bacterial load particularly by eliminating *S.aureus, E.coli, Klebsiella spp* and pseudomonas spp from wounds. This animal model demonstrated that phenytoin has an inhibitory effect on inflammation on day 7. Although it is statistically insignificant, it may be clinically important to decrease the bacterial load and need for antibiotic therapy. The concentration of topical phenytoin may be a reason for statistical insignificance. It is postulated that lower concentrations are more effective.[11]

Phenytoin is emerging as a potential agent to modify the regeneration of fibrous component. Although the primary site of action is in the motor cortex, phenytoin has a stimulatory effect on connective tissue.[7] Our results show that both isotonic saline and phenytoin have similar effects on fibroblastic reaction, however favourable effects of topical phenytoin become more prominent with time. This result implies that continuous application of phenytoin induces connective tissue healing, which, in turn, promotes a more stable tympanic membrane.

In addition, topical phenytoin has been shown to accelerate angiogenesis in wounds.[7] However, according to the results of our study, neovascularization scores showed no statistical significance, and phenytoin group had a tendency to stimulate angiogenesis between 3-7 days postapplication. This finding supported the results from other researches in that neovascularization is accelerated by phenytoin.[7] Nonetheless the effect that phenytoin exerts on endothelial cells is not well understood.

Conclusion

In conclusion, topical phenytoin application to an acute traumatic tympanic membrane perforation does not have a significant effect on inflammation, vascularization and fibroblastic reaction at concentrations and modes of application used in this study. Further studies using different concentrations would provide additional information in order to clarify the effect of topical phenytoin on the healing of tympanic membrane.

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