Received: 16.01.2011 / **Accepted:** 05.04.2011
DOI: 10.5137/1019-5149.JTN.4165-11.2

Effects of Phenytoin Sodium on Dura Mater Healing in a Rat Model of CSF Leakage

BOS Kaçağı Oluşturulan Rat Modelinde Fenitoin Sodyumun Dura Mater İyileşmesi Üzerine Etkileri

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This study was presented as an electronic poster in National Congress of Turkish Neurosurgical Society, April 18-22, 2008, Antalya, Turkey.

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ABSTRACT

AIM: Cerebro-spinal fluid (CSF) leakage caused by defects on the dura mater after trauma or some neurosurgical interventions is an important issue. In this study, we investigated the effects of local and systemic use of phenytoin sodium on dural healing.

MATERIAL and METHODS: Thirty-six male Wistar rats were divided into control, local phenytoin and systemic phenytoin groups with 12 rats in each. For each group, a dura defect was created at thoracic segment. Subjects were sacrificed at following 1st and 6th weeks and damaged segments were isolated. The results were compared histopathologically by Hematoxylin-Eosin and Masson-Trichrome staining. Criteria for the rate of collagen, neovascularization, and granulation formation were assessed semi quantitatively according to the histological assessment scale modified by Ozisik et al.

RESULTS: Better healing was achieved in the systemic and local phenytoin groups than in the control group. The level of healing was significantly higher in the systemic group in both early and late periods than in other groups ($p<0.01$). The level of healing in the late-local group was also statistically significantly higher than that in the control group.

CONCLUSION: We observed that both systemic and local uses of phenytoin sodium (especially systemic) have positive effects on dura healing.

KEYWORDS: CSF leakage, Dura mater, Phenytoin sodium, Healing

ÖZ

AMAÇ: Travma veya bazı nöroşirürjikal girişimler sonrası dura materde oluşan defektlerden kaynaklanan beyin omurilik sıvısı (BOS) kaçağı önemli bir durumdur. Bu çalışmada, lokal ve sistemik fenitoin sodyum kullanımının dura iyileşmesindeki etkilerini inceledik.

YÖNTEM ve GEREÇLER: Otuz altı erkek Wistar rat, her grupta 12'şer adet olacak şekilde kontrol, lokal fenitoin ve sistemik fenitoin gruplarına ayrıldı. Her grupta torakal segmentte dura defekti oluşturuldu. Denekler, takip eden 1. ve 6. haftalarda sakrifiye edildi ve hasarlı segmentler izole edildi. Sonuçlar histopatolojik olarak Hematoksilen-Eosin ve Masson-Trikrom boyama ile karşılaştırıldı. Kollajen, neovaskülarizasyon ve granulasyon formasyonu kriterleri Özışık ve ark. tarafından modifiye edilmiş histolojik değerlendirme skalasına göre semi-kantitatif olarak değerlendirildi.

BULGULAR: Sistemik ve lokal fenitoin gruplarında kontrol grubuna göre daha iyi iyileşme elde edildi. Sistemik gruptaki iyileşme düzeyi, diğer gruplara göre hem erken hem geç dönemde belirgin olarak daha iyi idi ($P<0,01$). Lokal-geç dönem grubundaki iyileşme düzeyi de kontrol grubuna göre istatistiksel olarak belirgin derecede yüksek idi.

SONUÇ: Fenitoin sodyumun hem sistemik, hem lokal kullanımda (özellikle sistemik) dura iyileşmesinde olumlu etkileri olduğunu gözlemledik.

ANAHTAR SÖZCÜKLER: BOS kaçağı, Dura mater, Fenitoin sodyum, İyileşme

INTRODUCTION

CSF leakage after trauma and cranial or spinal operations is a big problem that may result in serious complications. Numerous attempts were made to cure this entity, but none has been effective. Materials called "dura glues" and several suture techniques were used to overcome this problem, yielding no significant success (22, 19).

Phenytoin is the oldest non-sedative anticonvulsive agent commonly in use for primary and secondary epilepsy since 1938. It is effective on both generalized tonic clonic (grand mal) and complex partial (psyco-motor, temporal lobe) epilepsy as well as on epilepsy induced by neurosurgical interventions (12). Besides, it is used in the treatment of migraine, trigeminal neuralgia, some psychotic disorders, cardiac arrhythmias, digital intoxication and following

myocardial infarction (4, 7, 8, 12, 15., Phenytoin has various effects on several physiological systems. It blocks neuronal depolarization via blockade of sodium flow into cell and re-excitation of neuron via blockade of calcium flow as well. It alters the Na, K, and Ca levels, membrane potentials and concentrations of aminoacids, norepinephrine, acetylcholine and gamma-aminobutyric acid (GABA). Most significant effect is blockade of recurrent action potentials by blocking sodium channels (5, 11). The primary target of its effect is the motor cortex where the spread of convulsive activity is inhibited. Its potential positive effects on wound healing and granulation formation were first noticed in 1939 by exploration of gingival hypertrophy in patients with long-term phenytoin use (9). Since then, many studies have been conducted focusing on this effects on various tissues (periosteum, skin, cornea, gingiva, etc.) by many authors. The mechanism with which phenytoin -whose positive effects on wound healing have been substantiated - acts remains unclear. Clinical, animal and in-vitro studies show that phenytoin affects wound healing at different stages. These are acting as a collagenase inhibitor, stabilization of collagen fibers, facilitation of collagen deposition, stimulation of cell reproduction, augmentation of fibroblast activity, and augmentation of vascular epithelial growth factor and basic fibroblast growth factor. Facilitation of granulation formation, reduction of bacterial contamination and reduction of wound exudation are also noteworthy (1, 3, 23).

Effects of phenytoin use on dura mater healing have not been researched yet. In the light of these findings, we hypothesized that the phenytoin may have positive effects on dura mater healing.

In this study, we investigated the local and systemic effects of phenytoin, whose positive effects on wound healing were previously proven and which has a widespread use as an antiepileptic especially in neurosurgical patients.

MATERIAL and METHODS

Subjects:

Thirty-six male Wistar rats weighing 200± 20 gr were used for this study upon approval of the Ethics Committee of Gazi University Medical Faculty. All the subjects were kept alive under the same heat and humidity conditions with normal drinking water and standard rat chow and without any food restriction or additional special diet.

Surgical Procedure:

All the surgical interventions were performed under sterile conditions and with the animals under general anesthesia maintained by intramuscular injection of a mixture of 60-100 mg/kg Ketamine and 5 mg/kg Xylazine. The dorsal interscapular region was shaved after fixation of the subjects onto the operation table. The operation field was sterilized by topical 10% polyvinylpyrolidone-iodine. The fascia was opened following a 2 cm mid-line skin incision on the spinal processes. Paravertebral muscles were peeled subperiosteally from the spinous processes and laminae via sharp and gentle dissections. Operation field was exposed by little automatic retractors. A two-level laminectomy was achieved by thin rounger and air-drill under operating microscope. The ligamentum flavum was excised. The dura mater and nerve roots were exposed. Then, the dura mater and arachnoid mater were both incised and free CSF flow-out was seen. No additional interventions were performed on the dura mater for control group.

A 30 mg/kg dose of local phenytoin was applied on the dura mater topically for local phenytoin group.

For systemic phenytoin group, a 30 mg/kg dose of phenytoin was given intraperitoneally just after the operation.

After all these procedures, the fascia was closed by 5/0 Vicryl® sutures continuously. Then, the skin was closed primarily by 3/0 silk sutures. The operation field was sterilized again by a 10% polyvinylpyrrolidone-iodine solution. The subjects were kept in room temperature until the complete withdrawal of anesthesia and then were moved back to the cages.

Six subjects from each group were sacrificed on the 7th day (early group) and the remaining 6 subjects were sacrificed on the 6th week (late group) by injection of 100 mg/kg pentobarbital intraperitoneally; thus, early and late groups were formed. A ~ 4 cm long midline skin incision was made throughout the previous scar on the dorsal interscapular region. Then, the vertebral column was cut transversally by a size 20 scalpel, 0.5 cm below and above the laminectomy region and was en-bloc extirpated.

Histopathological examination:

Block tissue samples were collected in 10% neutral formaldehyde fixation solution. The specimens were moved into a decalcification solution including EDTA at the end of 72-hour fixation for decalcification of bony structures. The tissues were fixated again after two weeks of decalcification period. Then, they were embedded into paraffin and sections of 5 µ were moved onto slides. The slides were stained with Hematoxylin-Eosin and Masson-Trichrome. Criteria for the rate of collagen, neovascularization, and granulation formation were assessed according to the histological assessment scale described by Ozisik et al for each of the early (1 week) and late (6 weeks) groups (Table I) (17). Tables were created for each criterion. Microscopic examinations were performed by a blinded histopathologist.

Statistical Analysis:

All the findings were analyzed and compared with Kruskal Wallis and Wilcoxon tests.

A P value of <0.01 was considered statistically significant.

RESULTS

At the end of the 1st week, the rate of collagen, neovascularization and granulation formation were statistically significantly increased for dura mater healing in all the groups. The

Table I: Grading System for Quantifying Histopathological Findings. (Ozisik et al.)

Criteria / Score	+1	+2	+3	+4	+5
Cell types	No cells / few inflammatory cells	Inflammatory cells and few fibroblasts	Moderate numbers of fibroblast and inflammatory cells	Fibroblast dominancy	Few fibroblasts
Granulation	None	Thin layer	Moderate thickness	Thick	Thick
Collagen deposit	None	Few fibers	Moderate number of fibers	Intensive fibers	Densely organized fibers
Vascularization	None	Few new capillaries	Moderate number of capillaries	Dense capillaries	Dense capillary network

comparisons of the criteria for wound healing between 1st and 6th weeks by Wilcoxon test showed significantly higher rates of collagen, neovascularization, and granulation formation in all the groups in the 6th week than in the 1st week.

The comparisons of the groups for wound healing in the 1st and 6th weeks by Wilcoxon test showed no statistically significant differences in granulation formation for the control group (P=0.063). Similarly, no significant differences were found in the local group for the rates of collagen and granulation formation (P=0.180 and 0.059 respectively). Neovascularization was significantly increased in the 6th week than in the 1st week. The values of all the parameters were statistically significantly higher in 6th week than in the 1st week in the systemic phenytoin group based on the results of Wilcoxon test as well as Kruskal Wallis test.

The comparisons of the control, local, and systemic groups in the 1st week by Kruskal Wallis test showed the highest increase in the rate of collagen formation in the systemic group and the least increase in the control group. Moreover, the rate of neovascularization was the highest in the systemic group and the lowest in the control group. Finally, the increase in granulation formation was the highest in the systemic group and the lowest in the control group (Figure 1,2,3).

In the comparisons of the control, local, and systemic groups in the 6th week by Kruskal Wallis test, maximum increase was determined for each parameter including the rates of collagen, neovascularisation, and granulation formation in the systemic group and minimal increase, for each parameter in the control group.

In the control group, the comparisons of the rates of collagen, neovascularization, and granulation formation in the 1st week showed the least increase in neovascularization and granulation formation and the most increase in the rate of collagen. In the 6th week, the same increase rates were found in collagen and neovascularization; however, the lowest increase rate was measured for granulation formation. These findings suggest that there was more increase in neovascularization than in the other parameters after the 1st week.

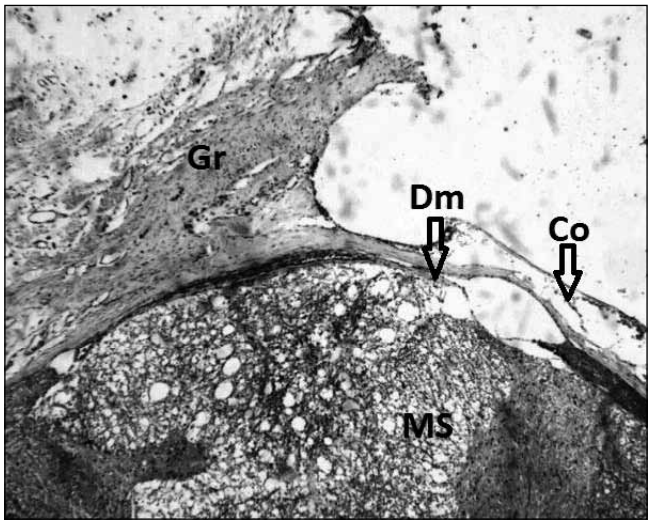

Figure 1: Control group, 6th week. Showing incomplete dural healing. (Stained with mason-trichrome, x40) **Gr:** Granulation tissue, **Dm:** Dura mater, **MS:** Medulla Spinalis, **Co:** Collagen fibers.

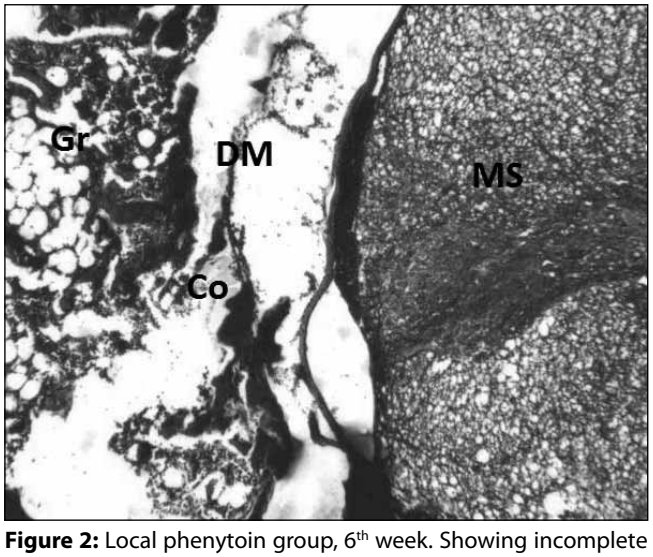

Figure 2: Local phenytoin group, 6th week. Showing incomplete dural healing. (Stained with mason-trichrome, x100) **Gr:** Granulation tissue, **Dm:** Dura mater, **MS:** Medulla Spinalis, **Co:** Collagen fibers.

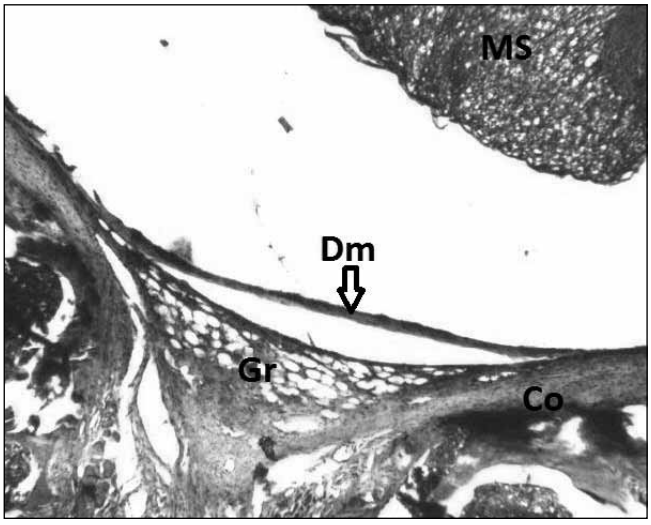

Figure 3: Systemic phenytoin group, 6th week. Showing complete and regular dural healing. (Stained with mason-trichrome, x40) **Gr:** Granulation tissue, **Dm:** Dura mater, **MS:** Medulla Spinalis, **Co:** Collagen fibers.

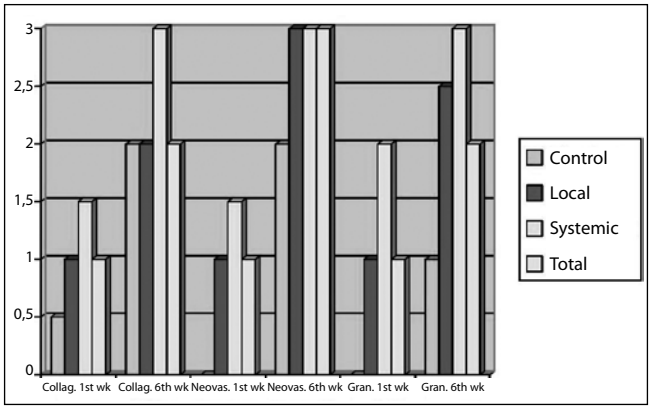

Figure 4: Graph showing the comparisons of the median values of all the groups for collagen fibers, neovascularization and granulation formation according to the grading system of Ozisik et al.

In the local phenytoin group, all the parameters were concurrently increased in the 1st week. The maximum increase was noted in neovascularization and the minimal increase, in collagen in the 6th week. Contrary to the findings of previous studies, the major increase in neovascularization with a mild increase in the rate of collagen was striking despite the expectation of local phenytoin to induce a considerable increase in the rate of collagen. The maximum increase was observed in granulation formation in the 1st week for the systemic phenytoin group with a less but similar increase in the rates of collagen and neovascularization. In the 6th week, the same values were obtained, but they were markedly high. We also observed that this increase was higher in the systemic phenytoin group than in both control and local groups (Figure 4).

Evaluation of these results showed that the local and systemic uses of phenytoin had statistically significant positive effects on dura mater healing, particularly in the in systemic phenytoin group than in the local phenytoin group.

DISCUSSION

The dura mater, which constructs the outermost layer of meninges covering the central nervous system, is a collagenous connective tissue consisting of numerous collagen fibers, fibroblasts, and few elastic fibers built in parallel form.. It may be injured or excised in traumas, intracranial surgeries, or spinal surgeries. Insufficient repair of the dura mater may result in numerous complications including CSF leakage, pneumocephalus, headache, nausea, vomiting, subdural hemorrhage, pseudomeningocele, and meningitis. For these reasons, accurate repair of the dura mater is very important. Various methods have been developed for an accurate repair of the dura mater after cranial or spinal surgical interventions. Some of these methods are closure of the dura mater with different suture techniques and use of duraplasty materials, fibrin glues, absorbable gelatin substitutes, and synthetic surgical materials. Unfortunately, at the present time, none of these techniques provides an accurate repair of the dura mater.

Phenytoin, which was first presented in 1938, is the oldest non-sedative antiepileptic drug. It has a widespread use for grand-mal and psychomotor epilepsy. Kimball first noticed the gingival hypertrophy in patients treated with phenytoin in 1939. In the light of this observation, studies on its effects on wound healing were undertaken. Shapiro observed less inflammation and pain concomitant with more favorable healing in periodontology patients who were pre-treated with phenytoin in 1958. (21) In the phase one studies, 28 patients were investigated with a double-blinded and placebo controlled method, and oral phenytoin was found to be useful in cutaneous wound healing in the treatment of venous stasis ulcers (3).

Bansal and Mukul have compared topical phenytoin and sodium chloride for tropical leprosy ulcers and found more and earlier granulation tissue formation in phenytoin group (2). El Zayat has compared phenytoin, chlorhexidine and hydrogen peroxide for complex decubitus ulcers and missile wounds in 15 patients. While wound healing was achieved in phenytoin group within 1-3 weeks, it was achieved within 5-6 weeks in the other groups (6). Lodha et al have compared phenytoin, eusole, and urea solution for gluteal abscesses. Healing was achieved in phenytoin group within 10 days, whereas it was achieved within 20 days in the other groups (10). Nevertheless, Pendse et al have compared Phenytoin with sodium chloride for various chronic wounds (burn, cellulitis, trauma amputation, etc.) and Oluwatosin et al have also compared phenytoin with honey for chronic leg ulcers; both studies have suggested better effects of phenytoin on wound healing (16, 18). Phenytoin was found to be more effective than silver-sulfodiazine in treatment of 2nd and 3rd degree burns. In addition, it has been used in the course of

clear surgical wound healing and has been reported to be more effective than Opsite and topical soframycin (24).

Topical phenytoin was also used during Iraq-Iran war. It was found to be effective for the treatment of decubitus ulcers caused by war, pain control, reduction of exudation, reduction of bacterial contamination, facilitation of granulation formation, and fast recovery (13). With these uses, phenytoin has been considered more advantageous for countries with limited resources. All these studies show that the phenytoin affects the healing course favorably.

Biopsy of open wounds, which were previously treated with phenytoin, has also revealed neovascularization, collagenization and reduced polymorphonuclear and eosinophilic cell infiltration.

Shafer has observed the stimulating effect of phenytoin sodium on normal and neoplastic cell clusters. Phenytoin has augmented the proliferation by 50-90% in binary fibroblast cell clusters. Additionally, a difference has also been observed in cytokine and growth factor activities that may be effective on inflammatory cells (20).

Moy et al have determined that the effects of phenytoin on human skin fibroblasts are dependent on concentration and duration. They observed that the lower concentrations (5 mg/L) and shorter incubation time (3 hrs) increased proliferation significantly; however, higher concentrations (25 mg/L) and longer incubation time (25 hrs and more) decreased fibroblast proliferation. Phenytoin may reduce collagenase activity by reduction of synthesis centrally throughout the pituitary-adrenal axis or by antagonizing glucocorticoid receptors competitively – not by direct enzyme inhibition (14). Besides, various studies have showed that phenytoin also reduces the bacterial load of the wound. Topical use of phenytoin eliminates Staphylococcus aureus, E. coli, Clebsiella spp and Pseudomonas spp in the wound within 7 to 9 days (13) and provides pain control, and this entity may be explained by its membrane stabilizing property and suppression of inflammatory response. Furthermore, nerve regeneration facilitating effect of phenytoin has also been observed. (23).

Considering the collagen-rich structure and fibroblast content of the dura mater and well-known stimulating effects of phenytoin on these structures, it can be said that phenytoin potentially has favorable effects on the dura mater healing. We investigated the effects of local and systemic use of phenytoin on dura mater healing considering its benefits on the rates of collagen, neovascularization and granulation formation.

This study showed that the systemic use of low-cost phenytoin as an antiepileptic before and after neurosurgical operations have favorable effects on dura mater healing. Fast and accurate achievement of dura mater healing after neurosurgical interventions and trauma is very important with respect to possible mortality and morbidity caused by CSF leakage. Unfortunately, current literature lacks information on agents effective on dura mater healing that are also practical to use.

Significant rise in neovascularisation increases the local blood supply as well as the increase in migration and adhesion of cells, which play a great role in wound healing. Increased neovascularization in especially systemic and other experiment groups is an important parameter showing the fortification of dura mater healing by phenytoin.

In this study, increases in the amount of granulation tissue were particularly notable in the systemic phenytoin group. Although granulation tissue is not functional, it is a precursor of scar tissue in wound healing. Furthermore, the most important point is its pioneering role in prevention of CSF leakage from the dura mater. The results of this study show the significant positive effects of phenytoin, which is currently used widespread in neurosurgical patients, on dura mater healing, but further clinical studies are needed to determine the effective doses and duration of use.

Various pharmacological agents with modulating effects have been researched for wound healing. However, our study may be a model for future research on this issue by showing the effectiveness of phenytoin on regeneration of the dura mater.

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