The Journal of International Advanced Otology

J Int Adv Otol 2015; 11(3): 192–5 • DOI: 10.5152/iao.2015.948

Original Article

Effect of Enoxaparin Sodium on Experimentally-Induced Myringosclerosis in Rats

Murat Ayata, Zeynep Kaptan, Hakkı Uzunkulaoğlu, İlker Akyıldız, Arzu Tüzüner, Hatice Ünverdi, Hatice Karadaş

Department of Otorhinolaryngology, Ankara Training and Research Hospital, Ankara, Turkey (MA, ZK, HU, İA, AT, HK)
Department of Pathology, Ankara Training and Research Hospital, Ankara, Turkey (HÜ)

OBJECTIVE: To evaluate the effectiveness of enoxaparin sodium (ES) on experimentally-induced myringosclerosis in rats.

MATERIALS and METHODS: Twenty Wistar albino-type rats weighing up to 250–300 g each were randomized into four groups containing five rats each and were then bilaterally myringotomized. The control group (n=5) received intratympanic serum physiologic injections, whereas ES2 (n=5), ES4 (n=5), and ES6 groups (n=5) received intratympanic ES of 2000 IU, 4000 IU, and 6000 IU, respectively, for 10 days after myringotomy. Rats were sacrificed at 60 days after intratympanic application and were then prepared for histopathologic evaluation.

RESULTS: As for tympanic membrane hyaline degeneration, there were statistically significant differences among the control, ES2, ES4, and ES6 groups ($p<0.05$). As for fibrosis formation on tympanic membranes, a statistically significant difference was observed among the control and study groups; however, although not statistically significant, the formation of fibrosis was slowed down in the ES2 and ES4 groups compared with the control group. The control and study groups did not show any significant difference for calcification, hyperemia, and tympanic membrane thickening ($p>0.05$).

CONCLUSION: Although our study and control groups comprised limited number of animals, and only one parameter demonstrated a statistically significant difference between the groups, ES may have an ameliorating effect on myringosclerosis induced by myringotomy in the tympanic membranes of rats. ES proved to be effective in the prevention of hyaline disc formation. Further studies should be conducted for better understanding of the effects of low-molecular-weight heparin (LMWH) (i.e., enoxaparin) on myringosclerosis.

KEYWORDS: Tympanic membrane, myringosclerosis, enoxaparin sodium

INTRODUCTION

Myringosclerosis is an irreversible dystrophic calcification involving the fibrous layer of the tympanic membrane caused by trauma, myringotomy, ventilation tube insertion, chronic middle ear infection, chemical agents, chronic middle ear effusion, and autoimmunity [1-3]. Tympanosclerosis was first described by Zollner in 1956 [4]. Calcium deposits are mostly seen on the tympanic membrane; thus, myringosclerosis is a commonly used term during the clinical course of this disease [5]. Increased inflammatory reaction and wound healing process at the fibrous layer of the tympanic membrane induce the formation of myringosclerosis [6, 7].

Many factors affect the wound healing process. Extracellular matrix proteins and other molecules are important factors involved in the course of wound healing. Glycosaminoglycan molecules are also important molecules that play important roles during the wound healing process. Hyaluronic acid is a major glycosaminoglycan molecule, and studies have revealed that compared with spontaneous recovery, hyaluronic acid is more effective in the healing process of the tympanic membrane, particularly in the transparency of the perforated tympanic membrane. McPherson et al. [8-10] showed that heparin applied on the wound exerts a favorable effect on the wound healing process.

Heparin affects multiple levels of the coagulation system. The heparin molecule contains anti-factor Xa/anti-factor IIa at a ratio of 1/1, whereas a low-molecular-weight heparin (LMWH) molecule has a higher anti-factor Xa content, with a corresponding ratio of 3/1 ratio; consequently, it affects only factor Xa. In contrast to LMWH, heparin has a negative effect on platelet aggregation. Therefore, heparin prolongs the activated partial thromboplastin time and prothrombin time and induces undesirable bleeding episodes during the treatment period. However, LMWH affects only factor Xa; thus, compared with heparin, undesirable bleeding episodes are seen less frequently with LMWH [11, 12].

In the light of this knowledge, we aimed to evaluate the effectiveness of enoxaparin sodium (ES), a type of LMWH, on experimentally-induced myringosclerosis in rats.

Corresponding Address: İlker Akyıldız, E-mail: ilkerakyildiz@yahoo.com
Submitted: 05.06.2015 **Revision received:** 07.09.2015 **Accepted:** 22.10.2015

MATERIALS and METHODS

Experimental Design

All animal studies were conducted with the approval of the Institutional Animal Care and Use Committee (23.02.2012/0007). The animals were housed under constant temperature (20–22°C) and humidity (50%–60%) with 12-h light–dark cycles. They were allowed free access to water and standard rat chow. Twenty Wistar albino-type rats weighing up to 250–300 g each were randomized into four groups containing five rats each. All rats underwent bilateral myringotomy at the anterior inferior quadrant using a sterile pick under 50 mg/kg intraperitoneal ketamine hydrochloride anesthesia. The control group (n=5) received intratympanic physiologic serum, whereas ES2 (n=5), ES4 (n=5), and ES6 groups (n=5) received intratympanic ES of 2000 IU, 4000 IU, and 6000 IU, respectively, for 10 days after myringotomy. No chemoprophylaxis was administered to the rats after myringotomy. Rats were sacrificed using 80 mg/kg intraperitoneal pentothal, decapitated, and prepared for histopathologic evaluation at 60 days after the intratympanic application of ES.

Outcome Parameters

Tympanic membranes were evaluated for hyaline disc formation, fibrosis, hyperemia, calcification, epithelial layer dissociation, and thickening. A visal analogue scale (VAS) scale ranging between 0 and 3 points was used for every histopathologic evaluation (Figures 1, 2).

Statistical Analysis

Data were analyzed using the (IBM Statistical Package for Social Sciences v21 SPSS Inc.; Chicago, IL, USA). Average values standard deviation and median values were calculated and the Kruskal–Wallis test was used to analyze the statistical difference between the different groups. All the differences associated with a chance probability of 0.05 or less were considered statistically significant.

RESULTS

Permanent tympanic membrane perforation was determined in one rat in the ES2 group. Differences between the groups according to tympanic membrane perforation were not significant.

As for tympanic membrane hyaline degeneration and fibrosis formation, statistically significant differences were observed among the control and ES2, ES4, and ES6 groups ($p<0.05$) (Table 1). Although not statistically significant, fibrosis formation was decreased in the ES2 and ES4 groups relative to the control group (Table 2). Significant differences could not be demonstrated between the control and the study groups for calcification, hyperemia, and tympanic membrane thickening ($p>0.05$) (Tables 3, 4).

DISCUSSION

Tympanic membrane perforation affects 1-3% of the population in the US. Infectious diseases, myringotomy, ventilation tube insertion, and traumas are the frequent causes of tympanic membrane perforation [12-15].

Acute otitis media is a common childhood disease, which affects 85% of children under five years old. Most of patients recover spontaneously, while in 5% of them, the disease has a chronic clinical course, which eventually progresses to tympanic membrane perforation [16].

Temporal bone traumas induce hyaline disc formation, calcification, and hyaline degeneration of the tympanic membrane, which involve the fibrous layer of the tympanic membrane.

Chronic middle ear infections also induce myringosclerosis and tympanosclerosis, which lead to hearing impairment in 7-33% of patients [3].

Myringosclerosis may be seen during otomicroscopic examination of the tympanic membrane as white calcification plaques. On histopathologic examination, irregular collagen fibers and calcium phosphate aggregation are observed on the myringosclerotic tympanic membrane [17, 18].

Flint et al. [19] proved that heparin has a positive effect on wound healing by activating organization of the fibroblastic cells.
Heparin induces the activation of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) by increasing their dwelling times around the wound, leading to glycosaminoglycan synthesis, cell proliferation, and angiogenesis [20, 21].

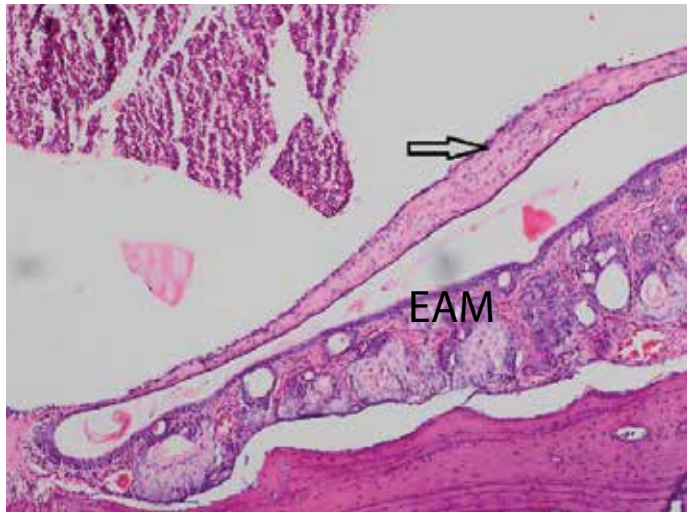

Figure 1. Tympanic membrane thickening because of calcification in the control group (Arrow) (EAM: external acoustic meatus)

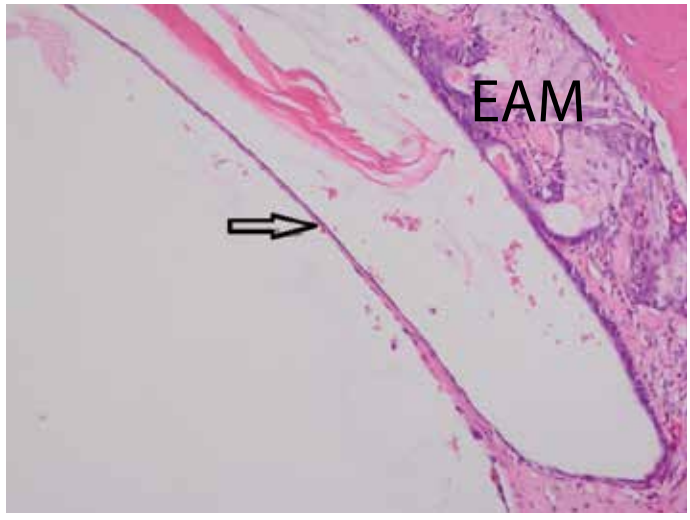

Figure 2. Normal thickness of the tympanic membrane in the ES4 group (EAM: external acoustic meatus)

Table 1. Comparison of tympanic membrane hyaline disc formation among the four groups

	Average±SD	Median (min–max)	p	
Control group	2.10±0.99	2 (0–3)	<0.001	Control vs. ES2=0.005
ES2 group	0.33±0.50	0 (0–1)		Control vs. ES4=0.001
ES4 group	0.2±0.42	0 (0–1)		Control vs. ES6=0.013
ES6 group	0.5±0.71	0 (0–2)		

SD: standard deviation; ES2: 2000 IU of enoxaparin sodium; ES4: 4000 IU of enoxaparin sodium; ES6: 6000 IU of enoxaparin sodium

Table 2. Comparison of tympanic membrane fibrosis formation among the four groups

	Average±SD	Median (min–max)	p
Control group	1.90±0.88	2 (1–3)	0.052
ES2 group	1.67±1.23	2 (0–3)	
ES4 group	0.80±0.79	1 (0–2)	
ES6 group	1.10±0.57	1 (0–2)	

SD: standard deviation; ES2: 2000 IU of enoxaparin sodium; ES4: 4000 IU of enoxaparin sodium; ES6: 6000 IU of enoxaparin sodium

Table 3. Comparison of tympanic membrane thickening among the four groups

	Average±SD	Median (min–max)	p
Control group	1.80±1.03	2 (1–3)	0.126
ES2 group	1.67±1.23	0 (0–1)	
ES4 group	0.80±1.03	1 (0–3)	
ES6 group	1.40±0.52	1 (1–2)	

SD: standard deviation; ES2: 2000 IU of enoxaparin sodium; ES4: 4000 IU of enoxaparin sodium; ES6: 6000 IU of enoxaparin sodium

Table 4. Comparison of tympanic membrane calcification among the four groups

	Average±SD	Median (min–max)	p
Control group	0.00±0.00	0 (1–3)	0.343
ES2 group	0.11±0.33	0 (0–1)	
ES4 group	0.00±0.00	0 (0–0)	
ES6 group	0.00±0.00	0 (0–0)	

SD: standard deviation; ES2: 2000 IU of enoxaparin sodium; ES4: 4000 IU of enoxaparin sodium; ES6: 6000 IU of enoxaparin sodium

Folkman et al. [21] demonstrated the wound healing effect of heparin on the tympanic membranes of rats.

In the control group, hyaline plaque formation was seen in nine out of ten tympanic membrane specimens of five rats. Three, two, and four hyaline plaque formations were seen in the 2000 IU ES, 4000IU ES, and 6000IU ES groups, respectively. Differences between the control group and ES2-ES4 groups were statistically significant, whereas the difference between the control group and ES6 was not significant and the authors thought that this was due to the irritative effect of high dose ES.

Since time to myringosclerosis formation changes based on various factors, decapitation times after myringotomy of the rats varied among researchers. Histopathological evaluations were performed at variable intervals (i.e., Akdagli et al. [22] 14 days and Güneş et al. [23] 15 days) after myringotomy. In our study, we waited 60 days after myringotomy for histopathologic evaluation in order to see the effects of myringosclerosis. We used a minimal number of rats in accordance with the regulations for the protection of experimental animals enforced by The Ministry of Food, Agriculture and Livestock.

Although our study and control groups consisted of limited number of animals and only one parameter demonstrated a statistically significant difference between groups, ES may have an ameliorating effect on myringosclerosis induced with myringotomy in the tympanic membranes of rats. ES proved to be effective in the prevention of hyaline disc formation. Further studies should be designed for better understanding the effects of LMWH (i.e., enoxaparin) on myringosclerosis.

Ethics Committee Approval: Ethics committee approval was received for this study from animal care and use committee of Ankara Training and Research Hospital (23.02.2012-0007).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.K.; Design - Z.K.; Supervision - H.K.; Resources - Z.K.; Materials - H.U.; Data Collection and/or Processing - M.A., A.T.; Analysis and/or Interpretation - H.Ü.; Literature Search - İ.A.; Writing Manuscript - İ.A.; Critical Review - İ.A., H.K.; Other - (Animal Study) A.T., M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Mattsson C, Marklund SL, Hellstrom S. Application of oxygen free radical scavengers to diminish the occurrence of myringosclerosis. Ann Otol Rhinol Laryngol 1997; 106: 513-8. [CrossRef]
2. Dursun G, Acar A, Turgay M, Calgüner M. Human leucocyte antigens in tympanosclerosis. Clin Otolaryngol Allied Sci 1997; 22: 62-4. [CrossRef]
3. Asiri S, Hasham A, al Anazy F, Zakzouk S, Banjar A. Tympanosclerosis: review of literature and incidence among patients with middle-ear infection. J Laryngol Otol 1999; 113: 1076-80. [CrossRef]
4. Zollner F. Tympanosclerosis. J Laryngol Otol 1956; 70: 77-85. [CrossRef]
5. Akbas Y, Pata YS, Görür K, Polat G, Polat A, Ozcan C, et al. The effect of L-carnitine on the prevention of experimentally induced myringosclerosis in rats. Hear Res 2003; 184: 107-12. [CrossRef]
6. Spratley JE, Hellström SO, Mattsson C, Pais-Clemente M. Topical ascorbic acid reduces myringosclerosis in perforated tympanic membranes. A study in rat. Ann Otol Rhinol Laryngol 2001; 110: 585-91. [CrossRef]
7. Özcan C, Görür K, Cinel L, Talas DU, Unal M, Cinel I. The inhibitory effect of topical N-acetylcysteine application on myringosclerosis in perforated rat tympanic membrane. Int J Pediatr Otorhinolaryngol 2002; 63: 179-84. [CrossRef]
8. Laurent TC, Fraser JR. Hyalorunan. FASEB J 1992; 6: 2397-404.
9. Laurent C, Hellström S, Fellenius E. Hyalorunan improves the healing of experimental tympanic membrane perforations: A comparision of preparations with different rheologic properties. Arch Otolaryngol Head Neck Surg 1988; 114: 1435-41. [CrossRef]

10. McPherson JM, Ledger PW, Ksander G, Sawamura SJ, Conti A, Kincaid S, et al. The influence of heparin on the wound healing response to collagen implants in vivo. Collagen Rel Res 1988; 8: 83-100. [CrossRef]
11. Sharrock NE, Salvati EA. Hypotensive epidural anesthesia for total hip artroplasty: a review. Acta Orthop Scand 1996; 67: 91-107. [CrossRef]
12. Haines ST, Bussey HI. Thrombosis and the pharmacology of antithrombotic agents. Ann Pharmacother 1995; 29: 892-905.
13. Somers T, Goovaerts G, Schelfhout L, Peeters S, Goovaerts PJ, Offeciers E, et al. Growth factors in tympanic membrane perforations. Am J Otol 1998; 19: 428-34.
14. Ibekwe TS, Ijaduola GT, Nwaorgu OG. Tympanic membrane perforation among adults in West Africa. Otol Neurotol 2007; 28: 348-52. [CrossRef]
15. Voss SE, Rosowski JJ, Merchant SN, peake WT. Non-ossicular signal transmission in human middle ears: Experimental assessment of the "acoustic route" with perforated tympanic membranes. J Acoust Soc Am 2007; 122: 2135-53. [CrossRef]
16. Roberts JE, Burchinal MR, Collier AM, Ramey CT, Koch MA, Henderson FW. Otitis media in early childhood and cognitive, academic and classroom performance of the school-aged child. Pediatrics 1989; 83: 477-85.
17. Selcuk A, Ensari S, Sargin AK, Can B, Dere H. Histopathological classification of tympanosclerotic plaques. Eur Arch Otorhinolaryngol 2008; 265: 409-13. [CrossRef]
18. Doner F, Yariktaş M, Doğru H, Uzun H, Aydin S, Delibaş N, et al. The biochemical analysis of tympanosclerotic plaques. Otolaryngol Head Neck Surg 2003; 128: 742-5. [CrossRef]
19. Flint M. Interrelationships of mucopolysaccharide and collagen in connective tissue remodelling. J Embryol Exp Morphol 1972; 27: 481-95.
20. Terranova VP, Diflorio R, Lyall RM, Hic S, Friesel R, Maciag T. Human endothelial cells are chemotactic to endothelial cell growth factor and heparin J Cell Biol 1985; 101: 2330-4. [CrossRef]
21. Folkman J: Regulation of angiogenesis: A new funstion of heparin. Biochem Pharmacol 1985; 34: 905-9. [CrossRef]
22. Akdagli S, Tuzuner A, Demirci S, Karadas H, Tulaci KG, Dogan M, et al. Do all antioxidant supplements have the same potential effect on preventing myringosclerosis? Clin Exp Otorhinolaryngol 2015; 8: 1-6. [CrossRef]
23. Güneş A, Mutlu M, Akın İ, Köybaşioğlu F, Güvey A, Karasu MF, et al. The impact of systemic and local administration of ascorbic acid on traumatic perforation of tympanic membrane and myringosclerosis. J Int Adv Otol 2015; 11: 48-52. [CrossRef]