Effect of Enoxaparin Sodium on Experimentally-Induced Myringosclerosis in Rats

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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 thickness (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
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 visual 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 [14].

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 [13].

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

| Average±SD (min–max) | Median (min–max) | p       |
|---------------------|------------------|---------|
| Control group       | 2.10±0.99        | 2 (0–3) | <0.001 |
| ES2 group           | 0.32±0.50        | 0 (0–1) | Control vs. ES2=0.005 |
| ES4 group           | 0.2±0.42         | 0 (0–1) | Control vs. ES4=0.001 |
| ES6 group           | 0.5±0.71         | 0 (0–2) | Control vs. ES6=0.013 |

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 (min–max) | 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 (min–max) | 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 (min–max) | 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).

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