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Audiometric Characteristics in Patients With Noise-Induced Hearing Loss After Sodium Enoxaparin Treatment

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Objectives: The aim of this study was to evaluate the effect of sodium enoxaparin treatment on patients with noise-induced hearing loss.

Methods: Sixty patients with noise-induced hearing loss were included and randomly divided into two numerically equal groups. Group A underwent therapy with sodium enoxaparin for 10 days, followed by an additional 10 days of treatment after 10 days of no treatment. Group B received placebo as a control. Before treatment, at the end of treatment, and 2 months after the end of treatment, all patients underwent evaluation by laboratory tests, pure tone audiometry, transient evoked otoacoustic emissions (TEOAEs) testing, distortion product otoacoustic emissions (DPOAEs) testing, and auditory brain stem response testing.

Results: In contrast to group B, at the end of the treatment in group A pure tone audiometry showed a significant ($p < 0.05$) improvement of the audiometric thresholds at 0.5, 1, 2, 4, and 8 kHz. Depending on the air and bone conduction thresholds, TEOAEs and DPOAEs, which had previously been absent, were evoked at the frequencies examined. These improvements were confirmed at last follow-up. We found no significant differences in auditory brain stem responses or laboratory results.

Conclusions: These preliminary data encourage further studies to collect additional evidence on the effect of sodium enoxaparin in preventing the development of noise-induced hearing loss.

Key Words: noise-induced hearing loss, sodium enoxaparin.

INTRODUCTION

Noise-induced hearing loss (NIHL) is a major cause of hearing disability in the adult population worldwide.[1] This type of sensorineural hearing loss is characterized by irreversibility and by gradual progression with risk exposure time.[2] Typically, the first sign of hearing loss from noise exposure is a notching of the audiogram at 3, 4, or 6 kHz, with recovery at 8 kHz.[3]

NIHL is thought to primarily involve damage to the sensory hair cells of the cochlea via mechanical and metabolic mechanisms. In addition to the direct mechanical trauma that loud sounds can cause to the hair cells, there are also secondary effects that can cause further damage. It is also possible that prolonged acoustic overstimulation can lead to local vascular damage and cochlear hypoxia, which in turn can cause damage to hair cells.

Several studies assessing the effectiveness of postexposure treatment after NIHL have yielded largely different results.[4] Approaches for the reversal of NIHL have included treatment with a wide variety of antioxidants, glutamate antagonists, nitric oxide synthase inhibitors, neurotrophic growth factors, steroids, vitamins (A, C, and E), calcineurin inhibitors, caspase inhibitors, etc.[5] None of these strategies has been sufficiently effective in preventing hearing loss and hair cell death.

Sodium enoxaparin is a low–molecular weight heparin (LMWH) that is endowed with a high antithrombotic activity and offers a number of clinical advantages and therapeutic effects superior to those of the other types of nonfractionated heparin. These include reduction of intracellular calcium ion release, antioxidant effects, anti-inflammatory or neurotrophic effects, and antiphospholipid antibody activity. All of these features suggest a possible neuroprotective activity of sodium enoxaparin.[6-8]

As we have previously shown the protective effect of sodium enoxaparin against various forms of sensorineural hearing loss, the aim of this study was to verify the effectiveness and applicability of sodium enoxaparin in the treatment of NIHL.[6,7]

MATERIALS AND METHODS

This protocol was reviewed and approved by the

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Genoa University Institutional Review Board. All procedures were carried out in accordance with the local ethics committee's protocol. After the patients gave informed consent, a total of 60 patients with NIHL between 35 and 45 years of age, among 108 patients eligible during the study period, were included and completed the trial, between February 2007 and October 2010. Only subjects with a documented history of at least 10 years of occupational noise exposure and absence of other possible causes of hearing loss were enrolled. Occupational noise exposure was defined as 85 to 90 dB equivalent continuous sound level at testing. All subjects were still professionally active and self-reported relative stability of their noise exposure over time and their compliance with wearing hearing protection devices. The mean reported duration of noise exposure was 18 years (range, 12 to 24 years). The mean age at first exposure to occupational noise was 21 years (range, 18 to 45 years).

The study group included only subjects affected by bilateral sensorineural hearing loss, more evident at frequencies between 2 and 8 kHz. In order to exclude subjects with other causes of degenerative hearing loss of the inner ear, only patients with an audiometric threshold above the expected-for-age International Organization for Standardization 1999 reported median values were enrolled in the study group.[9] These values presented in statistical terms the relationship between noise exposures and the "noise-induced permanent threshold shift" in people of various ages.

The exclusion criteria were genetic and congenital conditions; neoplasms; smoking; acute contemporary bacterial and/or viral upper respiratory tract infections; any past or present external and/or middle ear infection; history of treatment with ototoxic drugs; previous treatment for hearing loss and/or tinnitus; history of sudden sensorineural hearing loss; experience with firearms; systemic disease such as diabetes; a history of thrombocytopenia following heparin treatment; hemorrhagic manifestations or tendencies due to disorders of hemostasis that are not heparin-dependent or related to consumption coagulopathy; organic injuries at risk of bleeding; renal failure; acute infectious endocarditis (excluding endocarditis due to mechanical prostheses); hemorrhagic cerebrovascular events; allergy to sodium enoxaparin; and concurrent use of ticlopidine, salicylate, or nonsteroidal anti-inflammatory drugs with sodium enoxaparin or platelet anticoagulants (such as dipyridamole and sulfinpyrazone). Patients with any of the exclusion criteria were excluded from the study.

At the beginning of the study, the subjects were tested at least 16 hours after occupational exposure to noise so that the temporary threshold shift did not diminish the real pure tone audiometry values.

Before treatment and at the end of the treatment, all patients underwent the following instrumental examinations: laboratory tests (prothrombin and fibrinogen levels), pure tone audiometry, transient evoked otoacoustic emission (TEOAE) testing, distortion product otoacoustic emission (DPOAE) testing, and auditory brain stem response (ABR) testing with an MK 12-ABR screener with Natus-ALGO2e (Amplifon, Milan, Italy). To evaluate the efficacy of the therapy, we had each patient return 2 months after the end of treatment for laboratory tests (prothrombin and fibrinogen levels), liminal tonal audiometry, and TEOAEs, DPOAEs, and ABR testing.

Conventional pure tone audiometry was carried out in a soundproof room. Each subject was evaluated by means of pure tone audiometry for the octave frequencies from 0.5 kHz through 8 kHz, inclusive. TEOAEs and DPOAEs were recorded in a sound-attenuated chamber with an ILO-92 instrument (Amplifon).

The patients were equally divided, at random, into two numerically equal groups (A and B); the random allocation sequence was done through numbered containers. Group A included 30 patients between 35 and 44 years of age (average, 40.1 years); group B included 30 patients between 36 and 45 years of age (average, 41.2 years).

Patients in group A had subcutaneous administration of sodium enoxaparin at a dose of 4,000 IU daily for 10 days, followed by an additional 10 days of treatment after 10 days of no treatment. The group B (control) patients received placebo (0.2 mL of physiological saline solution) by the same method of administration.

Statistical analysis was performed with a *t*-test; p values of less than 0.05 were regarded as significant.

RESULTS

At the end of the treatment, in group A, the pure tone audiometry showed an improvement of the mean air and bone conduction thresholds in all of the subjects. The improvement in the mean air and bone conduction thresholds for both ears was statistically significant ($p < 0.05$) at the frequencies of 0.5, 1, 2, 4, and 8 kHz (Table 1). The improvement was confirmed 2 months after the end of the treatment at the frequencies of 0.5, 1, 2, 4, and 8 kHz

TABLE 1. PURE TONE AUDIOMETRIC RESULTS IN DECIBELS (MEAN ± SD)

	Group A			*Group B*		
	Before Treatment	*At End of Treatment**	*2 mo After End of Treatment†*	*Before Treatment*	*At End of Treatment‡*	*2 mo After End of Treatment§*
0.5 kHz						
Right ear						
AC	34.9 ± 2.58	21.6 ± 1.68	22.1 ± 1.71	35.1 ± 2.63	34.8 ± 2.62	34.9 ± 2.62
BC	32.1 ± 2.55	19.7 ± 1.53	21.3 ± 1.61	32.4 ± 2.57	32.1 ± 2.56	32.3 ± 2.57
Left ear						
AC	32.8 ± 2.56	22.3 ± 1.73	22.5 ± 1.74	33.6 ± 2.60	33.3 ± 2.59	33.5 ± 2.59
BC	30.3 ± 2.48	20.4 ± 1.57	21.1 ± 1.59	31.4 ± 2.53	31.1 ± 2.52	31.2 ± 2.53
1 kHz						
Right ear						
AC	35.6 ± 2.69	22.6 ± 1.76	23.1 ± 1.79	35.2 ± 2.64	34.9 ± 2.62	35.1 ± 2.63
BC	34.2 ± 2.64	21.2 ± 1.64	21.8 ± 1.69	34.4 ± 2.61	34.1 ± 2.60	34.3 ± 2.61
Left ear						
AC	35.2 ± 2.63	22.8 ± 1.81	23.0 ± 1.78	35.4 ± 2.64	35.0 ± 2.63	35.2 ± 2.63
BC	34.1 ± 2.53	22.1 ± 1.71	22.6 ± 1.75	33.2 ± 2.58	33.1 ± 2.58	33.2 ± 2.58
2 kHz						
Right ear						
AC	40.6 ± 2.78	31.4 ± 2.49	31.6 ± 2.51	40.4 ± 2.77	40.1 ± 2.76	40.3 ± 2.77
BC	38.4 ± 2.71	28.9 ± 2.35	29.4 ± 2.37	38.2 ± 2.71	38.1 ± 2.71	38.2 ± 2.71
Left ear						
AC	41.1 ± 2.81	29.6 ± 2.41	30.2 ± 2.44	40.1 ± 2.75	39.7 ± 2.74	39.9 ± 2.75
BC	39.7 ± 2.76	28.2 ± 2.31	28.8 ± 2.34	38.1 ± 2.70	38.0 ± 2.70	38.1 ± 2.70
4 kHz						
Right ear						
AC	50.2 ± 2.91	38.1 ± 2.69	38.4 ± 2.71	51.3 ± 2.92	49.9 ± 2.89	51.1 ± 2.91
BC	48.9 ± 2.84	37.0 ± 2.64	37.5 ± 2.66	49.1 ± 2.86	48.6 ± 2.84	48.9 ± 2.85
Left ear						
AC	51.7 ± 2.93	39.1 ± 2.72	39.4 ± 2.78	51.5 ± 2.93	51.1 ± 2.91	51.3 ± 2.92
BC	49.7 ± 2.87	37.3 ± 2.66	37.8 ± 2.67	49.6 ± 2.87	49.2 ± 2.86	49.4 ± 2.87
8 kHz						
Right ear						
AC	45.1 ± 2.81	36.4 ± 2.65	37.1 ± 2.66	45.6 ± 2.84	45.1 ± 2.81	45.5 ± 2.83
Left ear						
AC	45.2 ± 2.82	35.7 ± 2.64	36.3 ± 2.65	45.5 ± 2.83	45.2 ± 2.81	45.3 ± 2.83

Group A received sodium enoxaparin, and group B received placebo. AC — audiometric air conduction threshold; BC — audiometric bone conduction threshold.

*Versus "Before Treatment," all comparisons $p < 0.05$ (significant).

†Versus "At End of Treatment," all comparisons $p > 0.05$ (not significant).

‡Versus "Before Treatment," all comparisons $p > 0.05$ (not significant).

§Versus "At End of Treatment," all comparisons $p > 0.05$ (not significant).

(Table 1).

Depending on the air and bone conduction thresholds, in all of the patients with hearing improvement the TEOAEs revealed an improvement from "fail" to "pass." Moreover, the DPOAEs, which were previously absent, were evoked at frequencies of the tonal field normally examined (0.5, 1, 4, and 8 kHz; Table 2).

In group B, at the end of treatment and 2 months after the end of the treatment, the pure tone audiometry showed no statistically significant improvement ($p > 0.05$) of the mean air and bone conduction thresholds at any of the frequencies (Table 1). For the TEOAEs and DPOAEs, no differences were found at 0.5, 1, 4, and 8 kHz, before, after, and 2 months after the end of the treatment (Table 2).

No significant changes in the other examined parameters (ABR and laboratory tests) were discovered in the two groups during the study. In both groups, ABR testing indicated that the I/V amplitude ratio, the latency values of wave V, and the I-V intervals

TABLE 2. TEOAEs AND DPOAEs

			Before Treatment	*At End of Treatment*	*p**	*2 mo After End of Treatment*	*p†*
Group A	TEOAEs	Present	12	30	<0.05	30	>0.05
		Absent	18	0		0	
	DPOAEs	Present	16	30	<0.05	30	>0.05
		Absent	14	0		0	
Group B	TEOAEs	Present	11	11	>0.05	10	>0.05
		Absent	19	19		20	
	DPOAEs	Present	14	11	>0.05	13	>0.05
		Absent	16	16		17	

Data are numbers of subjects. TEOAEs — transient evoked otoacoustic emissions; DPOAEs — distortion product otoacoustic emissions.

*"Before Treatment" versus "At End of Treatment."

†"At End of Treatment" versus "2 mo After End of Treatment."

fell within the normal range in all ears. Prothrombin and fibrinogen levels were normal in all of the subjects of the two groups. None of the patients experienced side effects.

DISCUSSION

Research on NIHL has produced two basic theories on the underlying cause.[10] One is that intense noise can damage the cochlea mechanically by vibrating the organ of Corti beyond its structural limits[11]; the second is that metabolic stress triggers hair cell death.[10,12] These two theories are not mutually exclusive, and different mechanisms may operate at higher and lower intensities of noise exposure.

Current theories of metabolic damage center on the formation of free radicals, evoked by excessive noise stimulation, followed by activation of signaling pathways that lead to cell death. In addition, free radicals in the form of reactive nitrogen species derived from nitric oxide are also present. Peroxynitrite, generated by the combination of nitric oxide and free radicals, has been found in the cochlea several days after noise exposure, underscoring the case for oxidant stress contributing to hair cell death.[13] In animal studies, heparin was found to attenuate inducible nitric oxide synthase expression and nitric oxide release after cytokine activation in brain microvascular endothelial cells.[14] Although no results with sodium enoxaparin have been reported, the hypothesis of an interaction between sodium enoxaparin and free radicals could also be considered.

Noise exposure leads to dramatic physiological and anatomic changes within the central auditory pathway, besides the well-known cochlear damage. A consequence of noise exposure is an increase of free calcium ions in outer hair cells immediately after acoustic overstimulation: a calcium ion/calmodulin–independent protein phosphatase is activated after noise exposure and can, in turn, activate mitochondria-mediated cell death pathways.[15] Noise exposure induces apoptosis-related pathophysiological changes within the central auditory pathway in a time-dependent manner.[16] Apoptosis is a tightly regulated process that involves changes in the expression of a distinct set of proteins. Two of the major proteins responsible for regulating apoptotic cell death are bcl-2 and caspase-3. Under experimental conditions, Aβ25-35 has caused neuronal apoptosis; LMWH can block the increase of caspase-3 expression and the decrease of bcl-2 expression.[17] An Aβ-induced increase in intracellular calcium ion concentration has been proposed as one of the mechanisms involved in Aβ-induced apoptosis in cortical neurons.[18] Recent animal studies have highlighted that LMWH decreases intracellular calcium ion levels.[19] Thus, LMWH might inhibit calcium ion–activated pathways through suppression of the elevation of intracellular calcium ions, which was beneficial in preventing apoptosis. However, the underlying mechanism is not yet clear.[19]

These experiences confirm a possible interaction between sodium enoxaparin and intracellular calcium ion concentrations and suggest a possible neuroprotective activity of sodium enoxaparin.

Another factor associated with excessive noise is decreased cochlear blood flow, suggested to be caused by vasoactive lipid peroxidation products.[20] Past experience suggests that sodium enoxaparin may directly block the binding of antibodies to phospholipids and facilitate the clearance of antiphospholipid antibody in vivo.[6,7] These findings may suggest a positive effect of sodium enoxaparin on cochlear blood flow.

These acute neurodegenerative diseases initiate a deleterious cascade, which includes, at the least, excitotoxicity, edema, inflammatory processes, oxidative stress, and finally, neuronal cell death, including necrosis and apoptosis.[21,22] In the inflamma-

tory reaction, adherent neutrophils may exacerbate neural injury by participating in the no-reflow phenomenon. Factor Xa could act as a proinflammatory agent. Sodium enoxaparin induces a significant and persistent plasma anti-Xa activity.[23] Also, the complement system has been implicated in neural injury: complement inhibition provides neuroprotection in models of brain injury from stroke.[24] Different studies have shown that sodium enoxaparin inhibits complement pathways of activation.[24]

Sodium enoxaparin interacts with the heparin binding growth factor family. These proteins are stabilized by sodium enoxaparin heparin. Because it has been suggested that heparin binding growth factors could play an endogenous neuroprotective function, we believe that sodium enoxaparin may have a role in neural protection.[25]

Our audiometric data highlight the restoration of hearing. This may involve the activation of possible compensation mechanisms. Recent investigations have suggested that the central nervous system has a role in the changes that occur in auditory function after acoustic trauma caused by noise exposure. Noise exposure, resulting in a loss of cochlear sensitivity, can lead to elevated levels of spontaneous activity, ie, hyperactivity, in central nuclei such as the cochlear nucleus, inferior colliculus, and cortex. The current view is that this hyperactivity is centrally generated as a result of altered input[26]: the hyperexcitability is a change in the intrinsic properties of neuronal membranes and/or synapses that renders the neurons more likely to produce action potentials in response to a given level of input. This hyperexcitability is able to recruit new synapses; results of animal studies suggest that associative plasticity at these synapses is a central mechanism that improves signal resulting from a low peripheral stimulation in the central nervous system.[26] These plastic changes may justify the observed improvements in the study group.

Other animal studies show the absence of an inflammatory reaction and brain hemorrhages after prolonged treatment with the LMWH enoxaparin, suggesting that this long-term treatment is well tolerated.[27] The possible side effects of treatment with sodium enoxaparin are slight hemorrhaging, usually due to preexisting risk factors; thrombocytopenia; sometimes-serious cutaneous necrosis near the injection site; cutaneous or systemic allergy; and increased transaminase levels.[21,22]

In addition, sodium enoxaparin shows an anti-inflammatory action with subcutaneous administration, so we consider this type of administration to be efficacious in this treatment.[6,7]

Although further studies are necessary, the data presented in this study add evidence for a positive effect of sodium enoxaparin in the treatment of NIHS.

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