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ANL-2366; No. of Pages 6

ARTICLE IN PRESS

Auris Nasus Larynx xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Influence of Ginkgo Biloba extract (EGb 761) on expression of IL-1 Beta, IL-6, TNF-alfa, HSP-70, HSF-1 and COX-2 after noise exposure in the rat cochlea

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ARTICLE INFO

Article history:
Received 6 August 2017
Accepted 21 September 2017
Available online xxx

Keywords:
Cochlea
Cytokines
Ginkgo Biloba
Heat shock proteins
Noise induced

ABSTRACT

Objective: The objective of this study was to investigate the influence of Ginkgo Biloba in early treatment of noise induced hearing loss on expression of IL-6, IL-1 Beta, TNF-alfa, HSP-70, HSF-1 and COX-2 in the rat cochlea.
Methods: Thirty two female rats were randomly divided into four groups (Acoustic Trauma, Ginkgo Biloba, Acoustic Trauma + Ginkgo Biloba, Non Treatment). Auditory brainstem response (ABR) was applied in all the groups. At the end of the study, IL-1Beta, IL-6, TNF-alpha, HSP-70, HSF-1 and COX-2 were studied in cochlear tissue with ELISA and Western blot analysis.
Results: There were significant increases in ABR values measured at days 1 and 7 compared to baseline values in Group 3. IL-1 Beta, IL-6 and TNF-alpha values were significantly higher in Group 1 than in the other groups. Whereas HSP-70 and HSF-1 values were found to be significantly lower in Group 1 compared to those in Group 2 and Group 3. COX-2 of Group 1 was significantly higher than the other groups.
Conclusion: Ginkgo Biloba is helpful in the treatment of noise induced hearing loss and exerts its effect by inhibiting expression of IL-1 Beta, IL-6, TNF-alpha and COX-2 and increasing HSP-70 and HSF-1 values in rat cochlea.

1. Introduction

Noise induced hearing loss is a public health problem occurring during everyday life, which is initially ignored, but may cause severe morbidity as it progresses. Noise induced hearing loss (NIHL) is a complex disorder that may be influenced by environmental and genetic factors [1]. The same intensity of noise with the same duration may create different levels of hearing loss in different persons.

Although numerous studies have been conducted to explain the influence of noise on the inner ear, its pathophysiology has not been fully enlightened. However, the most frequently emphasized mechanism is that, increased oxidative stress by free oxygen radicals that are increased after noise, produce necrosis and apoptosis in the outer hair cells of the cochlea [2]. Numerous antioxidant agents have been tested in

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https://doi.org/10.1016/j.anl.2017.09.015
0385-8146/

Please cite this article in press as: Dogan R, et al. Influence of Ginkgo Biloba extract (EGb 761) on expression of IL-1 Beta, IL-6, TNF-alfa, HSP-70, HSF-1 and COX-2 after noise exposure in the rat cochlea. Auris Nasus Larynx (2017), https://doi.org/10.1016/j.anl.2017.09.015

prevention of noise induced hearing loss, yet there is not a generally recognized treatment modality.

Ginkgo Biloba extract (EGb 761) has been shown to be effective on the inner ear, neural pathways and in the treatment of vascular pathologies [3,4]. EGb 761 anti inflammatory, antioxidant (by inhibition of oxidative stress, inhibition of cyclic nucleotide phosphodiesterase, membrane stabilizes), antitumoral and antiapoptotic effects, increase cognitive functions [5,6]. Influences of EGb 761 on the inner ear and neural structures have been demonstrated, although there is only limited information about its effect on the changes occurring in the inner ear due to noise.

Studies conducted to demonstrate the damage which occurs in the outer hair cells due to noise have shown that, the levels of proinflammatory cytokines (IL-1 Beta, IL-6, TNF-alpha) in the inner ear are increased after noise [7,8]. Additionally, studies have reported significant changes in expression of HSF-1, HSP 70 and COX-2 in noise induced hearing loss [9,10]. Based on this information, it was thought that the agents used in prevention and treatment of noise induced hearing loss might affect the structures expressed in the inner ear.

In this study, we aimed to evaluate the influence of EGb 761 which is a potent antioxidant, antiinflammatory agent in prevention of noise induced hearing loss, on the levels of IL-6, IL-1 Beta, TNF-alfa, HSP-70, HSF-1 and COX-2 that are expressed in the inner ear by noise impact.

2. Material and methods

2.1. Study design

The study was initiated after receiving approval from Experimental Animals Local Ethics Committee. A total of 32 healthy female Sprague Dawley rats weiging between 200–240 g were included in the study. Ears of all rats were endoscopically examined and those having pathology (serous otitis media, acute otitis media, adhesive otitis media, plugs) were excluded. All animals were housed under appropriate environmental conditions including 21 ± 1 °C temperature and 12-h light-dark cycle, food and water ad libitum and a background noise level under 50 dB. Thirty-two rats were equally randomized into four groups.

Group 1 (Acoustic Trauma = AT) (n = 8): Rats in this group were exposed to white noise at 100 dB SPL for 8 h at the beginning of the study. Afterwards, no medication was administered.

Group 2 (Ginko Biloba = GB) (n = 8): Rats received Ginko Biloba extract (EGb761) at a dose of 100 mg/kg/day with gavage over a period of 21 days from initiation of the study.

Group 3 (Acoustic Trauma + Ginko Biloba = AT + GB) (n = 8): Rats in this group were exposed to white noise at 100 dB SPL for 8 h at the beginning of the study. In addition, Ginko Biloba extract (EGb761) was given at a dose of 100 mg/kg/day with gavage over a period of 21 days from initiation of the study.

Group 4 (Non Treatment = NT) (n = 8) was accepted as the control group and received no application.

2.2. Evaluation parameters

2.2.1. ABR

Animals were sedated with an intraperitoneal injection of Ketamine 45 mg/kg and Xylazine (5 mg/kg) for audiological evaluation. First, audiological evaluation (ABR) was carried out before initiation of the study in order to determine hearing levels of rats. Groups 1 and 3 were subjected to audiological evaluation (ABR) on days 1, 7, 14 and 21 of the study. Whereas Groups 2 and 4 underwent audiological evaluation (ABR) on days 7, 14 and 21. This difference resulted from the acoustic trauma induced in Groups 1 and 3 at the beginning of the study. ABR measures were carried out in an anechoic room with Viasys Medelec Synergy using subcutaneous needle (Technomed Europe) electrodes. The stimuli were presented in alternating polarity via ER 3A insert headphones using a tone-burst stimulus of 8 kHz. Filter was set at 30–1500 Hz, repetition rate was set at 21/s, and the time window was set at 25 ms. A total of 1024 samples were taken for signal averaging. Stimuli were initially given at 80 dB normal hearing level intensity and the intensity level was reduced in 20-dB intervals until near-threshold values were reached. Then the intensity level was reduced in 10-dB intervals to determine the threshold. At least 2 tracks were produced for each measurement to test reproducibility of behavior, and the threshold was cross checked. The ABR threshold was defined as the lowest intensity level where the III wave was monitored.

2.2.2. ELISA

After audiological evaluations were complete on day 21 of the study, intracardiac blood samples were collected. The levels of TNF-alpha, IL-6 and IL-1 beta were studied from the blood samples collected with enzyme linked immunosorbent assays (ELISA) method (eBioscience,Inc. San Diego, USA) as specified in the protocol by the manufacturer.

2.2.3. Western blots

After rats were sacrified on day 21 of the study, the bulla was opened under microscope. Following the excision of bony tissue in the rat cochlea, soft tissue in the cochlea was excised for Western blot analysis. Western blot was used to analyze the expression of HSF-1, HSP-70 and COX-2 proteins. In this method, cellular proteins were prepared from the cochlear cells found in the middle ear. Total sample volume was 25 μl with a concentration of 2 μg/μl. All cellular proteins were electrophoresed in 4–12% SDS-PAGE acrylamide gel and transferred into polyvinylidene fluoride membranes (PVDF). The membranes were incubated in tris-buffered saline for one hour and rinsed with 5% Tween (TBS-T) containing 5% skim milk (Sigma). The membranes were incubated at 4 °C overnight and then among primary antibody agents; HSF-1 (Santa Cruz 1:1000), HSP70 (Santa Cruz, 1:1000) and COX-2 (Santa Cruz 1:1000) were rinsed in TBS-T. HRP conjugates were incubated at room temperature with anti-rabbit antibody (Santa Cruz Biotechnology, 1:5000) for one hour. Development of the membrans occurred with SuperSignal West Pico HRP substrate kit (Pierce), each membrane was measured through a