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Medical ozone therapy for the inner ear acoustic trauma

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ABSTRACT

Objectives: The goal of the study was to look at the potential protective effect of ozone therapy by studying its antioxidant and vasodilatation effects against hearing loss caused by acoustic trauma.
Methods: Thirty-two male Wistar Albino rats were divided into four groups of eight. The 1st group was exposed to acoustic trauma, the 2nd group was treated with ozone initially, and was exposed to acoustic trauma 24 h later, the 3rd group received ozone without trauma, while the 4th group was the control group. The 1st and 2nd groups were exposed to acoustic trauma with 105 dB SPL white band noise for 4 h. DPOAE and ABR tests were conducted in all groups on the 1st, 5th, and 10th days after trauma.
Results: In the 1st group, the effects of acoustic trauma continued on days 1, 5 and 10. The 2nd group's DPOAE and ABR results on days 5 and 10 showed significant improvement at all frequencies compared to deterioration on day 1, and the readings were comparable to baseline measurements.
Conclusion: Acoustic trauma is a pathology that is experienced frequently and leads to many problems in terms of health and cost. Ozone was demonstrated to be a reparative substance against acoustic trauma and, in addition, it can be supplied and applied easily.
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1. Introduction

The most frequent reasons for hearing loss are age, genetic factors, medicine ototoxicity and acoustic trauma. The cellular mechanism of hearing loss due to loud noise is not clearly understood. Constant exposure to high intensity acoustic trauma results in death of the outer hair cells of the organ of Corti through apoptosis [1]. The causes of cell death due to acoustic trauma are blood flow reduction in the inner ear [2], free radicals produced due to increased metabolic activity [3,4], and cellular necrosis in the outer hair cells directly induced by mechanical trauma [5].

In loss of hearing due to acoustic trauma, the reactive oxygen radicals play the role of a primer by creating an apoptotic signal in the outer hairy cells. It has been observed that hydroxyl radicals increase up to 10 times in the cochlea of rats that are constantly exposed to acoustic trauma [3]. Other studies have also shown that the number of reactive oxygen radicals in animals exposed to acoustic trauma increase up to four times compared to animals not exposed to trauma [6,7].

Medical ozone therapy is used for the treatment of inflammation, infected wounds, chronic skin disease and advanced ischemic illnesses, including burns, due to its antioxidant, antiflammatory and antimicrobial effects. Pure ozone is not used in ozone therapy owing to ozone toxicity; it is applied in the form of an ozone/oxygen mixture [8,9]. Ozone gas (O_3) is produced from the sun using the effects of ultraviolet rays, or it is produced artificially using an ozone generator [10].

Medical ozone treatment is a method in which a gas combination containing ozone and oxygen is applied to body liquids and cavities. It has been observed that ozone treatment significantly decreases oxidative stress in experimental rat models [11,12].

It has been observed that oxidative stress is reduced with reinfusion of blood mixed with ozone since it increases nitric oxide (NO) levels and results in a reduction in hypoxia due to vasodilatation in ischemic areas, superoxide dismutase (SOD) activation and a reduction in glutathione levels [13,14]. During infusion of ozonized blood to the recipient, the majority of the endothelial cells are activated with lipid oxidation products (LOPs), and this results in increased NO, plasma S-nitrosothiol and S-nitrosohemoglobin production. Although the half-life of NO is less

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than 1 s, NO connected to protein may induce vasodilatation in the far ischemic vascular field [15].

This research has been conducted to study the antioxidant and vasodilation effects of ozone therapy against damage caused by acoustic trauma that results in reactive oxygen radicals and vasoconstriction in the inner ear.

2. Materials and methods

2.1. Animals

The study was conducted after approval (approval no. 2011/65) had been obtained from the Animal Experiments Local Ethics Board of Bezmialem Vakif University. Thirty-two healthy mature male Wistar Albino rats, weighing 200–240 g, were used in the study. All rats were evaluated otoscopically, and those with pathologic findings (serous otitis, acute otitis, adhesive otitis, etc.) were excluded from the study. All rats were housed in an environment with a temperature of 21 ± 1 °C, with a 12 h light, 12 h dark cycle, where they had free access to food and water, and where the background noise level was below 50 dB (Table 1). The rats were sacrificed on the 10th day of the study. Their malondialdehyde (MDA; μmol/L), superoxide dismutase (SOD; U/ml) and advanced oxidative protein product (AOPP; μmol/L) levels were measured using blood samples obtained before sacrification.

2.2. Hearing assessment

At the beginning of the study, the pinna reflex test was performed for hearing assessment of all rats. Ketamine 45 mg/kg i.m. was used to induce sedation, after which all rats were examined otoscopically. Any obstacles which might impede the tests, such as earwax, were removed. Then, the basal Distortion-Product Otoacoustic Emission (DPOAE) and Auditory Brainstem Response (ABR) measurements were performed on all rats.

2.2.1. DPOAE

A GSI Audera otoacoustic emission instrument was used for DPOAE measurements. The smallest size tympanometry probe was attached to the tip of the device. Measurements were carried out in a noise-treated cabin. The measurement process was initiated after observing that the ear probe of the device was in the appropriate measurement position with proper configuration of its probe indicator and stimulation waveform. DPOAEs were measured using stimulations with different frequencies and intensities. Primary signal levels were adjusted to L1 = 65 dB, L2 = 55 dB for DPgram measurements. Frequencies of the primary signals were set as 1.2. DPgram measurements were carried out at 2997, 4002, 5004, 6002, 7001, 8003, 9006, 10005, 11000 and 12000 Hz frequencies as a function of f2. The detection threshold was defined as the primary signal level at which the DPOAE was just distinguishable, at 3 dB above the noise floor. In all measurements, the responses were recorded up to the highest level and the test was concluded.

2.2.2. ABR

A Viasys Medelec Synergy instrument was used for ABR measurements. Measurements were performed on both ears of the anesthetized rats in a noise-treated cabin. ABR responses were recorded using needle electrodes placed under the skin. The electrodes were positioned as follows: active electrode in the vertex, ground electrode in the contralateral mastoid and reference electrode in the ipsilateral mastoid. The stimuli were provided through insert earphones using 8 kHz tone-burst sounds with alternating polarities. The filter was adjusted to 30–3000 Hz, the repetition rate was 21/s and the time window was adjusted to 10 ms. A total of 1024 stimuli were given for signal averaging.

The threshold was defined as the lowest intensity level that could be observed and repeated. Each test started by applying stimuli at 80 dB nHL level and the intensity was reduced in 20 dB steps until the threshold value was passed. As we approached the threshold, we preferred to increase the intensity by 10 dB each time until we reached the threshold value. At least two traces were created for each measurement, and attempts were made to repeat the threshold to cross-check it. The ABR threshold was defined as the lowest intensity level in which wave III of ABR was observed. Baseline ABR measurements carried out before acoustic trauma were compared with ABR measurements on the 1st, 5th and 10th days after trauma.

Table 1
Experimental groups.

Groups	Procedure	No. of rats
Group 1	Acoustic trauma	8
Group 2	Acoustic trauma and ozone therapy	8
Group 3	Ozone therapy	8
Group 4	Control group	8

2.3. Noise exposure and procedures

The first two groups of rats were exposed to acoustic trauma, using 105 dB SPL (sound pressure level) white noise for 4 h. The rats were sedated with an intramuscular injection of ketamine 45 mg/kg, and DPOAE and ABR measurements were carried out on the 1st, 5th and 10th days following acoustic trauma. The measurements were carried out in a room where the noise level did not exceed 50 dB.

2.4. Ozone application

Ozone (O_3) gas at 0.7 mg/kg was applied to Group 2 and 3 rats through intraperitoneal injection 24 h before the trauma and was continued for 10 days after the acoustic trauma. For this purpose, the concentration of ozone produced by the ozone generator (Ozonosan photonik, Dr J. Hänsler GmbH, Iffezheim, Germany) was adjusted to 75 μg/ml. The average volume of the oxygen/ozone gas mixture containing O_3 gas with 0.7 mg/kg dosage was identified to be 5 ml for rats weighing between 200 and 240 g. An equivalent volume (5 ml) of saline was intraperitoneally injected into the control group rats.

2.5. Biochemical parameters

Blood samples extracted intracardiacally from the rats on the 10th day were centrifuged at 3500 cycles/min and the serum was separated. The serum samples were stored at −80 °C in Eppendorf tubes with closed caps, and tagged with numbers until biochemical analyses were conducted. On the day of the analysis, serum samples were left at room temperature until they had melted, after which MDA, SOD and AOPP enzyme activities were measured.

2.5.1. Measuring MDA Level

For MDA measurement, citrated blood plasma was separated from the tube and stored at −80 °C. The MDA levels were determined using a color spectrophotometer after thiobarbituric acid had reacted with MDA at a wavelength of 532 nm. The values obtained through this measurement were provided in nmol/ml.

2.5.2. Measuring superoxide dismutase activity (SOD)

A superoxide dismutase measurement kit (Cayman Superoxide Dismutase Assay Kit, Cayman Chemical Company, USA) was used for this purpose. The principle of the method is based on

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