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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.

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

identification of the superoxide radicals produced by xanthine oxidase and hypoxanthine, using tetrazolium salt. 1 unit of SOD enzyme activity is defined as the quantity of enzyme required for dismutation of 50% of the superoxide radicals. Total activity of the three types of SOD (Cu/Zn-, Mn-, Fe-SOD) was measured using this method. SOD enzyme activity was provided as U/mL.

2.5.3. Measuring serum AOPP level

AOPP formation is induced by the formation of chlorine oxidants (such as chloramines and hypochlorous acid). Thus, the AOPP concentration varies in relation to those substances. Because of this correlation, Chloramine-T in its standard form was used to measure the AOPP concentrations. The temperature of the samples was adjusted to room temperature before the study, and the samples were then processed in a Linear Cromaline Photometry device. For this purpose, 160 μL of PBS (phosphate-buffered saline) was added to 10 μL of serum and mixed, and the mixture was incubated for 25 s. 20 μL of acetic acid was then added, and the mixture was incubated for 25 s. Finally, 10 μL of KI solution was added and the mixture was incubated for another 25 s, and absorbance was read at 340 nm. Serum which exceeded the linearity limit was diluted before processing. Measured AOPP concentrations were determined with Chloramine-T in units of μmol/L. All steps were carried out at 37 °C, and time intervals were adjusted to 25 s or longer for each step.

2.6. Sacrifice and enucleation

All experimental animals were sacrificed with 200 mg/kg of intracardiac thiopental. The same method of sacrifice and enucleation was used for all animals.

2.7. Statistics

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 13.0 for Windows (SPSS Inc, Chicago, IL, USA). All quantitative variables were estimated using measures of central location (i.e. mean and median) and measures of dispersion (i.e. standard deviation (SD)). Data normality was checked using the Kolmogorov–Smirnov test for normality. The Student's *t*-test was used to compare averages in normally distributed data within the four groups. The ANOVA test was used to compare the ABR threshold values between the groups. $p < 0.05$ was accepted as statistically significant.

3. Results

3.1. DPOAE

Group 1 (trauma only): 16 ears of eight rats were evaluated in Group 1. Significant differences were observed between the DPOAE values measured before and following exposure to acoustic trauma ($p = 0.003$). No significant difference was identified between the DPOAE values measured on the 1st day after acoustic trauma, and those measured on the 5th and 10th days ($p = 0.564$; $p = 0.644$) (Fig. 1).

Group 2 (trauma + ozone): 16 ears of eight rats were evaluated in Group 2. Significant differences were observed between the DPOAE values measured before and following exposure to acoustic trauma ($p = 0.012$). Significant differences were also observed between the DPOAE values measured on the 5th and 10th days after exposure to trauma ($p = 0.02$; $p = 0.03$) (Fig. 1).

Group 3 (ozone only): 16 ears of eight rats were evaluated in Group 3. No significant difference was observed between the DPOAE values measured ozone treatment ($p = 0.140$; $p = 0.396$; $p = 0.489$) (Fig. 1).

Group 4 (control group): 16 ears of eight rats were evaluated in Group 4. No significant difference was observed between the baseline measurements and the DPOAE values measured on the 1st, 5th and 10th days ($p = 0.791$; $p = 0.965$; $p = 0.945$) (Fig. 1).

3.2. ABR

Group 1 (trauma only): The ABR threshold value increased significantly after acoustic trauma exposure ($p = 0.002$). No significant difference was identified between the ABR threshold

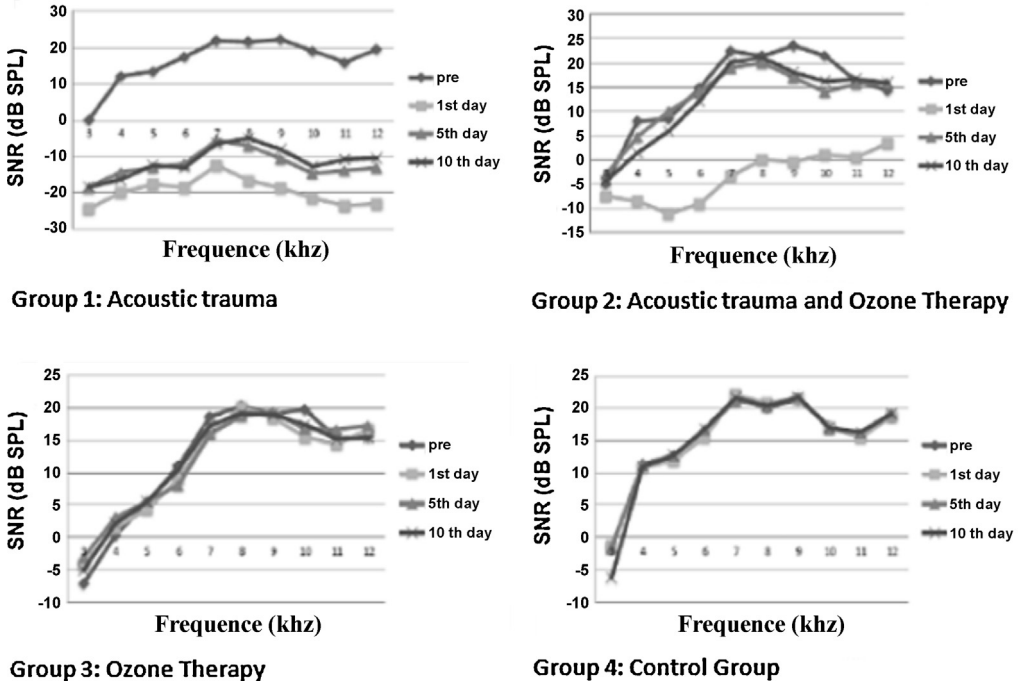

Fig. 1. DPgrams of all groups.

Table 2
Thresholds of auditory brainstem response measured before acoustic trauma and 1, 5, and 10 days after trauma.

Groups	Before	1st day	5th day	10th day
Group 1 (trauma)	26.8	43.6 ($p=0.002$)[¥]	42.5 ($p=0.526$)[Ψ]	40.2 ($p=0.672$)[Ψ]
Group 2 (trauma + ozone)	28.9	44.3 ($p=0.003$)[¥]	32.5 ($p=0.003$)[¥]	31.3 ($p=0.003$)[¥]
Group 3 (ozone)	27.1	27.5 ($p=0.462$)[Ψ]	28.7 ($p=0.965$)[Ψ]	26.2 ($p=0.532$)[Ψ]
Group 4 (control)	29.1	31.3 ($p=0.972$)[Ψ]	29.3 ($p=0.947$)[Ψ]	29.4 ($p=0.432$)[Ψ]

Student's *t*-test for independent samples ($p<0.05$).
[¥] Significance level obtained.
[Ψ] Insignificant level obtained.

values measured on the 1st day after acoustic trauma, and those measured on the 5th and 10th days ($p = 0.526$; $p = 0.672$) (Table 2).

Group 2 (trauma + ozone): The ABR threshold value increased significantly after acoustic trauma exposure ($p = 0.003$). A significant difference was also observed between the ABR threshold values measured before trauma and those measured on the 5th and 10th days after exposure to trauma ($p = 0.003$; $p = 0.003$) (Table 2).

Group 3 (ozone only): No significant difference was observed between the ABR threshold values measured before ozone treatment and those measured on the 1st, 5th and 10th days of treatment ($p = 0.462$; $p = 0.965$; $p = 0.532$) (Table 2).

Group 4 (control group): No significant difference was observed between the baseline measurements and the ABR threshold values measured on the 1st, 5th and 10th days in the control group treated with saline ($p = 0.972$; $p = 0.947$; $p = 0.432$) (Table 2).

3.3. Comparison of ABR thresholds among the groups

No significant difference was observed between the ABR thresholds of the 1st and 2nd groups on the 1st day after acoustic trauma ($p = 0.423$) (Fig. 2). A significant difference was detected between the ABR thresholds of the 1st and 2nd groups measured on the 5th and 10th days after exposure to acoustic trauma ($p = 0.003$; $p = 0.003$) (Fig. 2).

A significant difference was present between Groups 1 and 3 also Groups 1 and 4 for the ABR threshold values on the 1st, 5th and 10th days ($p = 0.002$) (Fig. 2). The difference was not significant between Groups 3 and 4 on the 1st, 5th and 10th days ($p = 0.950$; $p = 0.593$; $p = 0.946$) (Fig. 2).

A significant difference was present between Groups 2 and 3 also Groups 2 and 4 for the ABR threshold values on the 1st day ($p = 0.004$; $p = 0.004$) (Fig. 2). The difference was not significant between Groups 2 and 3 also Groups 2 and 4 for the ABR threshold values on the 5th and 10th days ($p = 0.850$; $p = 0.850$) (Fig. 2).

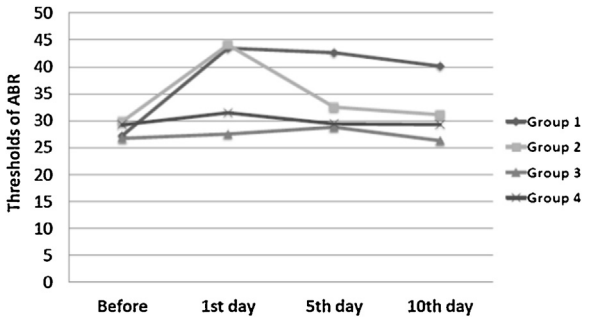

Fig. 2. Comparison of ABR thresholds among the groups. While there is no statistically significant difference between Groups 1 and 2 after trauma ($p > 0.01$), there is a significant difference on the 5th and 10th days for ABR threshold values ($p < 0.01$).

3.4. Biochemical parameters

The MDA values of the blood tests of Group 2 (trauma + ozone) were significantly less than those of Group 1 (trauma only), and the SOD values of Group 2 were significantly higher ($p < 0.05$). No significant difference was observed between Group 3 (ozone only) and Group 4 (control group) for MDA values ($p = 0.512$), and SOD values ($p = 0.112$). No significant difference was observed between Group 1 (trauma only) and Group 2 (trauma + ozone) for AOPP values ($p = 0.06$) (Fig. 3).

4. Discussion

The pathogenesis of acoustic trauma is multifactorial. Acoustic trauma may result in damage to hair cells, disorders of stereocilia, collapse of support cells, rupture of dendrites and structural and metabolic damage such as strial edema [16,17]. Bray and associates have studied 23 disk jockeys. The noise level was at least 108 dB in their study environment and they observed that 70% complained of loss of hearing and 74% complained of tinnitus [18]. This study was conducted to show the potential protective effect of ozone by examining the effects of antioxidants and vasodilatation against hearing loss due to acoustic trauma.

Broadband noise (white noise), with a constant amplitude over the whole frequency spectrum, causes homogeneous damage throughout the cochlea [19]. In our study, we applied 105 dB broadband noise to the first two groups of rats for 4 h to create acoustic trauma. ABR and DPOAE measurements were carried out on the 1st day after trauma in the 1st and 2nd groups; these values, when compared with the baseline values, showed a significant difference (ABR: Group 1 $p = 0.002$, Group 2 $p = 0.003$; DPOAE: Group 1 $p = 0.003$, Group 2 $p = 0.012$), which confirmed that acoustic trauma was successful in inducing hearing loss.

No significant difference was observed between Group 1 and Group 2 on the 1st day after acoustic trauma ($p > 0.5$), which shows that initially, ozone treatment did not have a preventive effect on acoustic trauma. However, a significant difference was observed between the ABR threshold values of Groups 1 and 2 measured on the 5th and 10th days after exposure to acoustic

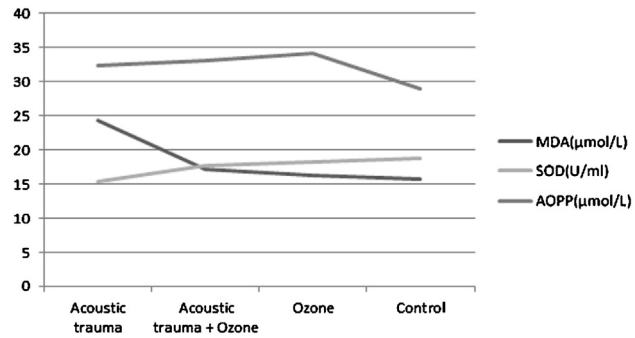

Fig. 3. Biochemical parameters.

trauma ($p = 0.003$; $p = 0.003$). In Group 2, with ozone given prophylactically, the ABR and DPOAE values on the 5th and 10th days were restored to the baseline values, and the effects of the established acoustic trauma were completely removed and corrected. These findings show that, after acoustic trauma, ozone repairs the damage in the inner ear. In our study, no significant difference was identified between Groups 3 and 4 on the 1st, 5th and 10th days ($p = 0.950$; $p = 0.593$; $p = 0.946$). This demonstrates that ozone has no negative effect on hearing. This is the first study in the medical literature to investigate the relationship between ozone given prophylactically and its ototoxicity, and which shows the rapid reparative effect of ozone to damage caused by acoustic trauma.

We measured serum MDA, SOD and AOPP levels to examine oxidative stress related to acoustic trauma. MDA values in Group 2 (trauma + ozone) were significantly lower than in Group 1 (trauma only) ($p < 0.05$), and SOD values in Group 2 were significantly higher than in Group 1 ($p < 0.05$). No statistically significant difference was observed between the AOPP values of Groups 1 and 2. Our study is again the first in the medical literature to investigate the relationship between AOPP and acoustic trauma. The finding that, while oxidative parameters increased after exposure to acoustic trauma, no permanent hearing loss was seen in the long term, making it necessary to conduct further research on other factors (antioxidant enzymes and heat shock proteins) which may lead to hearing loss or which may limit the effects of oxidative stress.

The treatment effect of ozone is, in particular, due to reactive oxygen products (ROP), hydrogen peroxide and Lipid oxidation products (LOP). When ozonized blood is re-infused to the blood circulation, it activates endothelial cells by LOP, and nitric oxide, plasma S-nitrosothiol and S-nitrosohemoglobin are produced. The half-life of nitric oxide is less than 1 s, but the half-life of nitric oxide connected to protein is much longer, and this may cause vasodilatation in distant regions [9]. It has been proven that low dose ozone is useful in inducing antioxidant enzymes, nitric oxide canal and other cellular activities [20].

Ozone therapy applied to an ischemic organ results in the creation of erythrocytes, increased oxygen absorption in these erythrocytes and emission of nitric oxide, carbon monoxide and growth factors from endothelial cells and thrombocytes, which increases oxygenation further [9]. Although the amount of oxygen that enters the body with infusion of blood ozonized with an oxygen/ozone mixture may be negligible, ozone changes the normal oxygen distribution level by triggering a series of biologic events, and it shows its effects in this way. As a result, ozone has been used for treatment, since it has been thought that it can prevent ischemia-reperfusion damage [21,22].

Ozone treatment leads to free radical production such as hydrogen peroxide (H_2O_2), reactive oxygen radicals (ROS), and lipid oxidation products (LOP). Antioxidation enzymes that provide system defense (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-P_x)) are stimulated toward these increased free radicals. In studies using ozone, this stimulation is referred to as oxidative preconditioning. The scope of these studies is to create ischemia-like conditions so that the body defense system is stimulated [9,20]. It has been shown that controlled ozone therapy can decrease the damage induced by reactive oxygen production by providing adaptation to oxidative stress [23]. Ozone treatment with preconditioning by creating ischemia-like conditions is a viable method in the treatment of ischemic vascular diseases by stimulating the synthesis of antioxidation enzymes [24]. In our study, antioxidation enzyme synthesis was increased with stress adaptation by starting ozone treatment one day before acoustic trauma and generating preconditioning using ozone free radicals.

In recent studies, it was shown that ozone preconditioning treatment is an effective way to prevent ischemia perfusion damage in various organs such as the liver, lungs, and kidneys. Ajamieh et al. [21] have demonstrated by histopathologic examination that ozone preconditioning aids recovery in hepatic ischemia. In another study, it was shown that creating oxidative preconditioning with ozone can stimulate the endogen antioxidation system against liver damage by creating oxidative stress [24]. Barber et al. [22] have reported that ozone preconditioning before renal transplantation decreased renal damage in rats with renal ischemia. Stadlbauer et al. have applied intraperitoneal ozone to both donor and receiver before cardiac transplantation and demonstrated by histologic and biochemical outcomes that this ozone application led to a reduction in ischemia reperfusion damage occurring during transplantation [25]. Moreover, Borrego et al. [26] have shown that ozone has an effect on the antioxidation system in rats and reduces the damage caused by induced renal ischemia with cisplatin. Accompanying these studies, in our study, we have not come across any pathologic finding in the liver, lung, and kidney during autopsy after sacrifice and because of this, additional examination was not required. However, further studies should be performed to clarify any possible side effects of ozone on other systems and organs.

It has been suggested that ozone therapy may be able to protect antioxidant systems and keep other indicators of endothelial cell damage, which are related to diabetic complications, at physiologic levels since there is a known relationship between diabetes mellitus and oxidative stress [27].

H1-receptor antagonists, corticosteroids, vasodilators, anticoagulants, volume expanders, antioxidant agents such as vitamin A, C and E, and many other agents such as Mg and hyperbaric oxygen, have been employed in the treatment of acoustic trauma, either isolated or in various combinations [28]. In our study, we believe that Ozone's benefits were observed in as short a time as 5 days, due to its biphasic effect which activates vasodilator agents and the endogenous antioxidant system.

In papers showing the relationship between ketamine and neuronal toxicity, it was demonstrated that ketamine causes neuronal cell death if it is administered as six or seven injections of 20 mg/kg. A lower number injections did not cause any neuronal damage [29,30]. In addition, another study showed that a single dose of ketamine (25, 50, or 75 mg/kg) did not cause any neuronal degeneration. However, repeated doses of 25 mg/kg ketamine at 90 min intervals over 9 h increased neuron degeneration [31]. Moreover, ketamine dose for rats is stated as 40–80 mg/kg through IM at rodent anesthesia and analgesia guideline prepared by University of Pennsylvania [32].

For this reason, we have used a single dose of 45 mg/kg ketamine in our study, and have not observed any neuronal pathology affecting the results.

5. Conclusion

Acoustic trauma is currently a frequently encountered pathology, which leads to economic costs and health problems. Ozone given pre-exposure may prevent permanent hearing loss from acoustic trauma, due to its biphasic effect, while by itself, ozone has antioxidant and vasodilatory properties. This study demonstrated that ozone may be regarded as an alternative therapy that is conveniently accessible, and is an easily applicable otoprotective substance repairing inner ear damage induced by acoustic trauma in rats.

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Conflict of interest

None.

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References

[1] B.H. Hu, D. Henderson, T.M. Nicotera, Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise, Hear Res. 166 (2002) 62–71.
[2] J.M. Miller, T.Y. Ren, H.A. Dengerink, A.L. Nuttall, Cochlear blood flow changes with short sound stimulation, in: A. Axelsson, H.M. Borchgrevink, R.P. Hamernik, P.A. Hellstrom, D. Henderson, R.J. Salvi (Eds.), Scientific Basis of Noise-Induced Hearing Loss, Thieme Medical, New York, 1996, pp. 95–109.
[3] K.K. Ohlemiller, S.L. McFadden, D.L. Ding, P.M. Lear, Y.S. Ho, Targeted mutation of the gene for cellular glutathione peroxidase (gpx1) increases noise-induced hearing loss in mice, J. Assoc. Res. Otolaryngol. 1 (2000) 243–254.
[4] K.K. Ohlemiller, S.L. McFadden, D.L. Ding, D.G. Flood, A.G. Reaume, E.K. Hoffman, et al., Targeted deletion of the cytosolic Cu/Zn-superoxide dismutase gene (sod1) increases susceptibility to noise-induced hearing loss, Audiol. Neurootol. 4 (1999) 237–246.
[5] J.C. Saunders, S.P. Dear, M.E. Schneider, The anatomical consequences of acoustic injury: a review and tutorial, J. Acoust. Soc. Am. 78 (1985) 833–860.
[6] K.K. Ohlemiller, J.S. Wright, L.L. Dugan, Early elevation of cochlear reactive oxygen species following noise exposure, Audiol. Neurootol. 4 (1999) 229–236.
[7] K.K. Ohlemiller, L.L. Dugan, Elevation of reactive oxygen species following ischemia-reperfusion in mouse cochlea observed in vivo, Audiol. Neurootol. 4 (1999) 219–228.
[8] V. Bocci, Physical–chemical properties of ozone – natural production of ozone: the toxicology of ozone, in: Ozone. A New Medical Drug, Springer, Netherlands, 2005, pp. 5–8.
[9] V.A. Bocci, Scientific and medical aspects of ozone therapy. State of the art, Arch. Med. Res. 37 (2006) 425–435.
[10] M.A. Mehlman, C. Borek, Toxicity and biochemical mechanisms of ozone, Environ. Res. 42 (1987) 36–53.
[11] A. Guven, G. Gundogdu, S. Vurucu, B. Uysal, E. Oztas, H. Ozturk, et al., Medical ozone therapy reduces oxidative stress and intestinal damage in an experimental model of necrotizing enterocolitis in neonatal rats, J. Pediatr. Surg. 44 (2009) 1730–1735.
[12] A. Guven, G. Gundogdu, S. Sadir, T. Topal, E. Erdogan, A. Korkmaz, et al., The efficacy of ozone therapy in experimental caustic esophageal burn, J. Pediatr. Surg. 43 (2008) 1679–1684.
[13] H.H. Ajamieh, S. Menéndez, G. Martínez-Sánchez, E. Candelario-Jalil, L. Re, A. Giuliani, et al., Effects of ozone oxidative preconditioning on nitric oxide generation and cellular redox balance in a rat model of hepatic ischaemiareperfusion, Liver Int. 24 (2004) 55–62.
[14] C. Peralta, O.S. Leon, C. Xaus, N. Prats, E.C. Jalil, E.S. Planell, et al., Protective effect of ozone treatment on the injury associated with hepatic ischemia reperfusion: antioxidant-prooxidant balance, Free Radic. Res. 31 (1999) 191–196.
[15] E.J. Frehm, J. Bonaventura, A.J. Gow, S-nitrosohemoglobin: an allosteric mediator of NO group function in mammalian vasculature, Free Radic. Biol. Med. 37 (2004) 442–453.
[16] Y. Wang, K. Hirose, M.C. Liberman, Dynamics of noise-induced cellular injury and repair in the mouse cochlea, J. Assoc. Res. Otolaryngol. 3 (2002) 248–268.
[17] S. Plontke, H.P. Zenner, Current aspects of hearing loss from occupational and leisure noise, Curr. Top. Otorhinolaryngol. Head Neck Surg. 3 (2004) Doc06.
[18] A. Bray, M. Szymanski, R. Mills, Noise induced hearing loss in dance music disc jokeys and an examination of sound levels in nigthclubs, J. Larygol. Otol. 118 (2004) 123–128.
[19] M.A. Bogoyevitch, I. Boehm, A. Oakley, A.J. Ketterman, R.K. Barr, Targeting the JNK MAPK cascade for inhibition: basic science and therapeutic potential, Biochim. Biophys. Acta 1697 (2004) 89–101.
[20] L. Re, M.N. Mawsouf, S. Menendez, O.S. Leon, G.M. Sanchez, F. Hernandez, Ozone therapy: clinical and basic evidence of its therapeutic potential, Arch. Med. Res. 39 (2008) 17–26.
[21] H.H. Ajamieh, S. Menéndez, N. Merino, G. Martínez-Sánchez, L. Re, O. León, Ischemic and ozone oxidative preconditioning in the protection against hepatic ischemic-reperfusion ınjury, Ozone Sci. Eng. 25 (2003) 241–250.
[22] E. Barber, S. Menéndez, O.S. Leon, M.O. Barber, N. Merino, J.L. Calunga, et al., Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia, Mediat. Inflamm. 8 (1999) 37–41.
[23] O.S. Leon, S. Menendez, N. Merino, R. Castillo, S. Sam, L. Perez, et al., Ozone oxidative preconditioning: a protection against cellular damage by free radicals, Mediat. Inflamm. 7 (1998) 289–294.
[24] J. Chen, R. Simon, Ischemic tolerance in the brain, Neurology 48 (1997) 306–311.
[25] T.H. Stadlbauer, A. Eisele, M.C. Heidt, H.H. Tillmanns, S. Schulz, Preconditioning with ozone abrogates acute rejection and prolongs cardiac allograft survival in rats, Transplant Proc. 40 (2008) 974–977.
[26] A. Borrego, Z.B. Zamora, R. Gonzalez, C. Romay, S. Menendez, F. Hernandez, et al., Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats, Mediat. Inflamm. 13 (2004) 13–19.
[27] S.M. Al Dalain, G. Martinez, E.C. Jalil, S. Menéndez, L. Re, A. Giuliani, et al., Ozone treatment reduces markers of oxidative and endothelial damage in an experimental diabetes model in rats, Pharmacol Res. 44 (2001) 391–396.
[28] C.G. Le Prell, L.F. Hughes, J.M. Miller, Free radical scavengers vitamins A C, and E plus magnesium reduce noise trauma, Free Radic. Biol. Med. 42 (2007) 1454–1463.
[29] F. Liu, M.G. Paule, S. Ali, C. Wang, Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain, Curr. Neuropharmacol. 9 (2011) 256–261.
[30] A.C. Scallet, L.C. Schmued, W. Slikker Jr., N. Grunberg, P.J. Faustino, H. Davis, et al., Developmental neurotoxicity of ketamine: morphometric confirmation, exposure parameters, and multiple fluorescent labeling of apoptotic neurons, Toxicol. Sci. 81 (2004) 364–370.
[31] H. Hayashi, P. Dikkes, S.G. Soriano, Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain, Paediatr. Anaesth. 12 (2002) 770–774.
[32] D.J. Gaertner, T.M. Hallman, F.C. Hankenson, M.A. Batchhelder, Anestesia and Analgesia in Rodents. Anesthesia and Analgesia in Laboratory Animals, 2nd ed., Academc Press, CA, 2008.