Role of tympanocentesis in the prevention of middle ear barotrauma induced by fast buoyant ascent escape from 200 m underwater

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Abstract
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Introduction: We aimed to study middle ear barotrauma caused by fast compression followed by buoyant ascent escape from 200 m underwater and its effect on the auditory system, and to validate the preventive effect of tympanocentesis on middle ear barotrauma.
Methods: Twenty Sprague Dawley rats were divided into two groups: rats in group A underwent a simulated fast buoyant ascent escape from a depth of 200 m, while those in group B underwent tympanocentesis before the procedure described for group A. Ear endoscopy, acoustic conductance, and auditory brainstem response (ABR) tests were conducted before and after the procedure to evaluate the severity of middle ear barotrauma and auditory function in both groups. Additionally, histopathological examination of the middle ear in both groups was conducted to evaluate the severity of middle ear barotrauma by observing submucosal haemorrhage.
Results: None of the ears in either group showed any abnormalities before the experiment. In group A, middle ear barotrauma was universally observed after the simulation procedure. The tympanograms of all ears were initially type A and became type B after the procedure. Further, after the simulation, the hearing thresholds at different frequencies (4, 8, 16, 24, and 32 kHz) assessed by ABR significantly increased compared to those before the procedure. In group B, no middle ear barotrauma was observed, and the hearing threshold at each frequency did not change significantly compared with post-puncturing. After dissecting the middle ear, gross pathological observations were consistent with the above results. Microscopically, blood accumulation and submucosal haemorrhage in the middle ear cavity were observed in group A but not in group B.
Conclusions: Fast buoyant ascent from 200 m underwater can cause middle ear barotrauma, resulting in hearing loss. Tympanic membrane puncture can effectively prevent middle ear barotrauma caused by the rapid buoyant ascent escape procedure.

Introduction

Submarines play an important role in the combat power of naval forces. Owing to the special sailing environment during submarine missions, shipwrecks may occur during war or peacetime. Submarine accidents have been reported in America, the former Soviet Union, Russia, and China. Therefore, countries that operate submarines value lifesaving technologies for submariners. Fast buoyant ascent escape is an internationally recognised advanced measure for submarine self-rescue. The process can be briefly described in three steps: rapid pressurisation, short stay, and fast ascent. The theoretical maximal depth for fast buoyant ascent escape ranges from 180 m to 240 m, and the greater the depth, the greater the risk. Currently, China's research on fast-ascent escape technology is at the global forefront. According to published news reports, the maximum depth of human testing in China has reached 194.6 m which to our knowledge is the deepest achieved to date.

Middle ear barotrauma (MEBt), the most common diving-related injury, occurring in > 50% of experienced divers.[1] It

is also a common injury in the fields of hyperbaric oxygen treatment and aviation.[2,3] Preventative measures have been studied. For example, one study reported that MEBt can be avoided by reducing the rate of pressure change in patients receiving hyperbaric oxygen treatment.[4] This is not an option in fast buoyant ascent escape because it increases the exposure time to high pressure and the risk of fatal decompression sickness.

Some other studies suggested that in aviation and hyperbaric treatment, the use of devices such as Cirrus ear plugs, Otovent, Ear Popper®, and pressurised masks could help open the Eustachian tube, balancing the pressure on both sides of the eardrum, and therefore reducing the incidence of MEBt.[5–7] However, these devices are not suitable for fast buoyant ascent escape because of the extremely rapid pressure change and need for additional operations.

As a simpler procedure with a favorable prognosis, tympanocentesis has been proven effective in preventing MEBt in aviation and hyperbaric oxygen treatment.[7,8] This is the most feasible method for preventing MEBt caused by fast buoyant ascent escape. However, compared to hyperbaric oxygen treatment and aviation environments, in submarine escape the degree and rate of pressure change is much greater. Thus, this study aimed to ascertain the preventive effect of tympanic membrane puncture on MEBt in this extreme-pressure-change environment.

Methods

ETHICAL APPROVAL

Ethical approval for this research project was obtained from the Ethics Committee of the PLA Naval Medical Center under the ethics number AF-HEC-041.

ANIMALS

Twenty male Sprague-Dawley rats, weighing 180–300 g, were provided by Vital River Laboratory Animal Technology Co., Ltd. Before the experiment, all rats were kept at the animal centre of the Naval Medical Centre for more than one week. The rats were evenly and randomly divided into two groups (A and B). To obtain the pathological morphology of the normal middle ear, two other rats, which did not undergo any experimental intervention, had their middle ears dissected and isolated for pathological examination, which served only as normal controls.

For group A, auditory endoscopy, acoustic impedance, and auditory brainstem response (ABR) tests were performed before and after the rats experienced simulated fast buoyant ascent escape from 200 m underwater. The effects of this procedure were evaluated by comparing the two sets of results.

In group B, tympanic membrane puncture was performed using the needle of a 1 mL syringe on each rat before the simulated fast buoyant ascent escape from 200 m underwater. Auditory endoscopy and ABR tests were performed pre-puncture, post-puncture, and after the simulation procedure. The results of the three tests were compared to evaluate any preventive effect of tympanic membrane puncture on MEBt.

LABORATORY INSTRUMENTS AND EQUIPMENT

We used an animal simulation chamber for fast buoyant ascent escape (Yantai Hongyuan Oxygen Industry Co. Ltd, China). An OTOflex 100 middle ear analyser (Denmark Otometrics, Shanghai Sonbett Instrument Co. Ltd, China), a high-definition electronic ear endoscope (2.7 mm diameter, Shanghai Fiveboats Electronic Technology Co. Ltd, China) and a TDT auditory electrophysiological system (Tucker Davis Technologies, USA) were used to evaluate the middle ear.

SIMULATED FAST BUOYANT ASCENT ESCAPE FROM 200 M UNDERWATER

The rats were placed in a cabin that was quickly pressurised to 2.1 MPa (equivalent to 200 metres of seawater [msw]) using a $2^{t/4}$ exponential scheme. The rats remained at the target pressure for 4 s and were then decompressed to 0 msw at a speed of 3 $m \cdot s^{-1}$. The total decompression time was 67 s. The pressure adjustment process is illustrated in Figure 1.

OUTCOME MEASURES

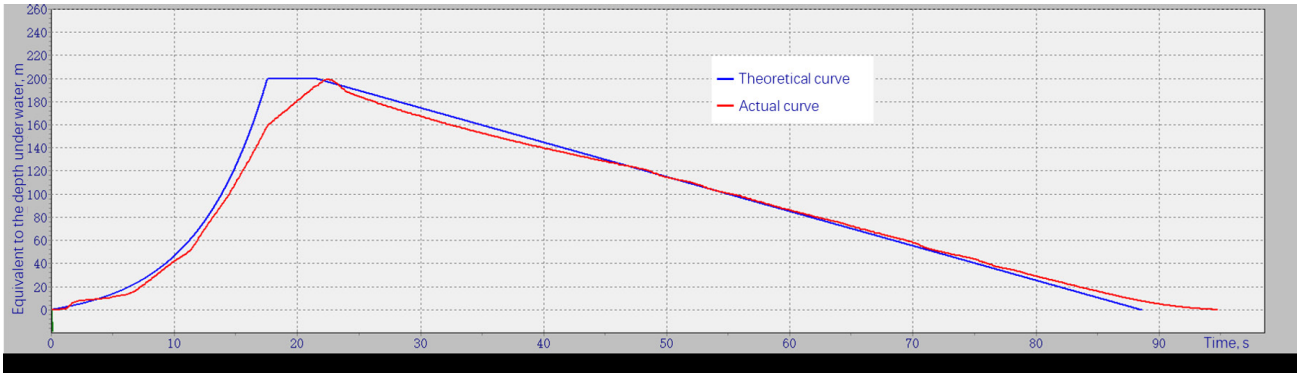
Tympanic membrane

The morphology of the tympanic membrane was observed using ear endoscopy at various stages of the experimental process, and the images were saved for subsequent analysis. The degree of MEBt was graded according to the O'Neill Injury Classification as follows. Grade 0 – no otologic signs of barotrauma on auricular endoscopy. Grade I – increased redness of the tympanic membrane compared with baseline, serous or slightly serosanguinous fluid, and/or trapped air behind the tympanic membrane. Grade II – bleeding at any location, perforation of the tympanic membrane, and/or free haemorrhage in the tympanic cavity.[3,9]

Tympanogram

An OTOflex 100 middle ear analyser was used to acquire the tympanogram of each ear before and after the simulation procedure to evaluate the conduction function of the middle ear. The middle ear analyser stimulation tone frequency was 1,000 Hz, and the pressure ranged from -400 daPa to 200 daPa at a speed of 400 $daPa \cdot s^{-1}$.

Figure 1
Curves showing pressure changes; the theoretical and actual pressure curves over time are shown in blue and red, respectively



ABR threshold

Auditory function was evaluated using the ABR test. The TDT auditory electrophysiological system (Tucker Davis Technologies, Alachua, FL, USA) was used to check the auditory threshold of each ear at different frequencies (4, 8, 16, 24, and 32 kHz). A custom-made soundproof chamber was used for testing. The effects of fast buoyant ascent escape from 200 m on the ears of rats were estimated by comparing the difference in ABR thresholds pre- and post-escape. The recording electrode was placed subcutaneously on the top of the head, the reference electrode was placed subcutaneously on the side of the mastoid process, and the ground electrode was placed subcutaneously on the contralateral mastoid process. The sound pressure level (SPL) decreased to a gradient of 10 dB from 90 dB. When a regular waveform could not be elicited, the SPL increased by 10 dB and then decreased in increments of 5 dB. The SPL, where wave II can be repeatedly elicited, was taken as the ABR threshold.[10]

Histopathological examination

After the examination, the rats were euthanised, and their middle ears were dissected and isolated. Hematoxylin and eosin staining was performed on the removed middle ears after decalcification. Tympanic haematocele and submucosal haemorrhage were observed under a microscope to provide evidence of MEBt severity.

STATISTICAL ANALYSIS

The auditory threshold was described by the mean and standard deviation (SD). A paired-sample *t*-test was used to analyse the difference between the two results in group A. For group B, analysis of variance for repeated measurements was applied to analyse the difference among the three results, while the least significant difference *t*-test was used as a post hoc test to make paired comparisons among different results. Fisher's exact test was used to analyse the difference in the incidence of MEBt between the two groups. Statistical significance was set at $P < 0.05$.

Results

ANIMALS

Three rats in Group A and two in Group B died during the experiment. The cause of death was considered an overdose of anesthetic drugs. Therefore, the number of samples were 14 ears from seven rats in group A and 16 ears from eight rats in group B.

TYMPANIC MEMBRANE

There was no abnormality of the tympanic membrane or effusion of the tympanic cavity observed by the otic endoscope in either group before the experiment. In contrast, MEBt was detected in all ears in group A after the simulated fast buoyant ascent escape from 200 m underwater. According to the O'Neill injury classification, grade I injury occurred in two ears and grade II injury in 12 ears, among which two ears had tympanic membrane perforation (Figure 2). In group B, no obvious MEBt was observed after the procedure, and the tympanocentesis perforation was not enlarged (Figure 3). There was a statistically significant difference in the incidence of MEBt between the two groups (Table 1, $P < 0.05$).

TYMPANOGRAM

In group A, the tympanograms of all ears showed a type A curve with a pronounced peak before the simulation procedure. After the simulation procedure, the tympanograms of all the ears changed to type B (Figure 4), showing that the conduction function of the middle ear was impaired. The rats in group B were not subjected to this test because they underwent tympanic membrane puncture, which changed the tympanogram to type B.

Figure 2
Photos of the varying severities of MEBt in tympanic membranes in group A; A – normal tympanic membrane; B – Grade I MEBt with hyperaemia and mild tympanic effusion; C – Grade II MEBt with haematotympanum; D – Grade II MEBt with tympanic membrane perforation

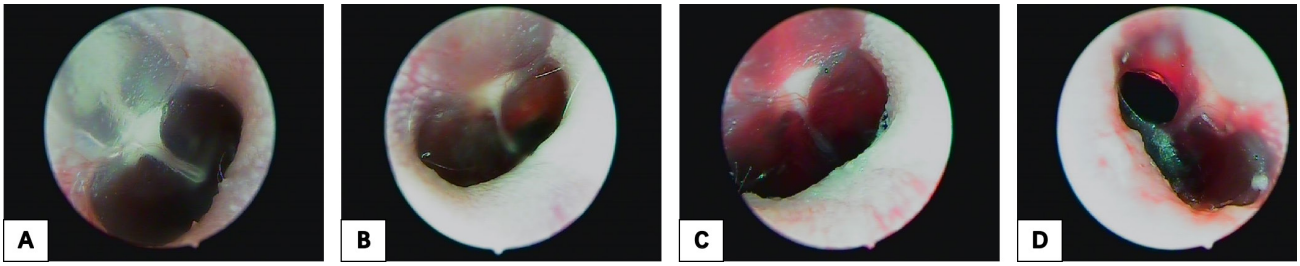


Figure 3
Photos of the tympanic membrane at different experimental stages in group B where no MEBt was observed; A – normal tympanic membrane before puncturing; B – after puncturing, the yellow arrow indicates the location of the perforation; C – after the emergency ascent simulation procedure

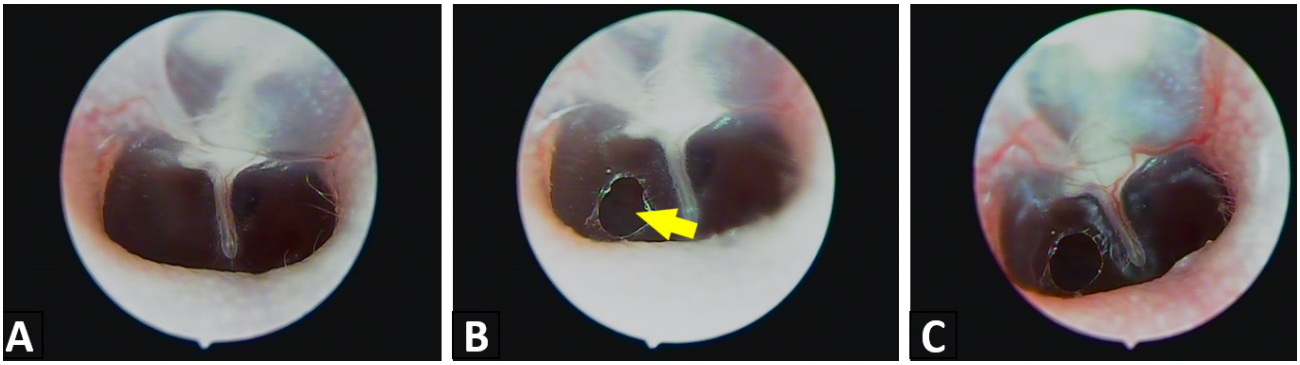


Table 1
Incidence of MEBt in two groups after simulated fast buoyant ascent escape from 200 m underwater; there was a significant difference in incidence between the two groups ($P < 0.05$)

Outcome by group	With MEBt	Without MEBt
Group B	0	16
Group A	14	0

ABR THRESHOLD

In group A, the average values of the ABR thresholds at frequencies of 4, 8, 16, 24, and 32 kHz after the simulation procedure were significantly higher than those before it, and the average ABR threshold values at each frequency before and after the procedure are shown in Table 2. A paired sample *t*-test was used to detect the difference in the ABR threshold before and after the simulation procedure, showing a statistically significant difference ($P < 0.05$). These results suggest that a simulated fast buoyant ascent escape from 200 m underwater can cause hearing loss in rats at all frequencies.

In group B, the average values of the ABR thresholds at each frequency were significantly increased after puncturing compared to pre-puncturing ($P < 0.05$), whereas there was no significant difference between post-puncturing and post-simulation procedure ($P > 0.05$). The average ABR threshold values at each frequency in Group B are shown in Table 3. These results indicate that tympanic membrane puncture causes hearing loss, while a simulated fast buoyant ascent escape from 200 m underwater after tympanocentesis does not cause further hearing loss.

HISTOPATHOLOGICAL EXAMINATION

In gross observation, as is shown in Figure 5, the middle ear samples were dark red in group A, indicating middle ear bleeding. However, similar to the normal controls, the samples in group B were white, suggesting the absence of bleeding in the middle ear cavity. The results described above were confirmed by observation at low magnification (Figure 6). Furthermore, under a high-magnification microscope, intratympanic and submucosal hemorrhages were observed in all ears in group A, regardless of perforation. No obvious submucosal bleeding was observed in group B or the standard control group. Interestingly, in group A, intratympanic haemorrhage was less severe, but submucosal haemorrhage was more severe in ears with

Figure 4

Tympanograms of experimental rats in group A; A – Type A curve before the simulation procedure; B – Type B curve after the simulation procedure

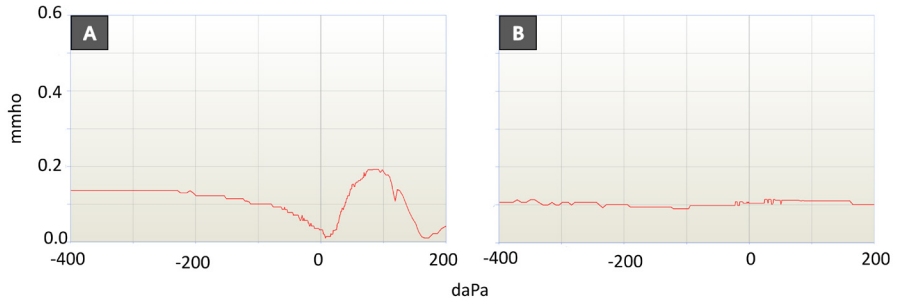


Table 2

Results of pre- and post-simulation ABR thresholds of rats in group A; data are mean (SD) dB; there was a significant difference pre- and post-simulation (fast buoyant ascent escape from 200 m underwater) at every frequency ($P < 0.05$)

Parameter	4 kHz	8 kHz	16 kHz	24 kHz	32 kHz
Pre-simulation	31.1 (5.3)	30.4 (6.0)	29.6 (6.0)	32.5 (4.3)	36.8 (4.2)
Post-simulation	71.1 (8.1)	63.2 (9.3)	68.6 (6.9)	71.8 (7.2)	72.9 (4.7)

Table 3

Results of pre- and post-simulation ABR thresholds of rats in group B; data are mean (SD) dB; * – statistically significant difference between pre-puncturing and post-puncturing at every frequency ($P < 0.05$); # – no statistically significant difference between post-puncturing and post-simulation (fast buoyant ascent escape from 200 m underwater) at every frequency ($P > 0.05$)

Parameter	4 kHz	8 kHz	16 kHz	24 kHz	32 kHz
Pre-puncturing*	31.9 (4.4)	31.6 (4.7)	28.8 (4.7)	31.9 (6.3)	33.4 (4.0)
Post-puncturing*#	50.9 (5.8)	52.2 (6.0)	50.3 (9.7)	47.5 (9.5)	47.5 (9.5)
Post-simulation#	49.7 (5.0)	53.4 (4.7)	49.4 (7.0)	49.4 (7.9)	50.3 (9.4)

perforation than in those without perforation (Figure 7). The most likely reason for this is that blood in the tympanic chamber flows out through the perforation, although perforation indicates a more serious injury.

Discussion

In this study, we assessed MEBt caused by fast buoyant ascent escape from 200 m underwater and its effect on the auditory system and evaluated the preventive effect of tympanocentesis on MEBt. The results showed that the incidence of MEBt in rats was 100% in group A, and most of them were seriously injured. However, prior tympanocentesis effectively protected rats from MEBt in group B.

The Eustachian tube connects the tympanic cavity to the external environment. Owing to the physiological characteristics of the 'unidirectional valve' of the eustachian tube,[11] it is necessary to actively open the pharyngeal orifices of the tube to balance the pressure inside and outside the tympanic cavity when environmental pressure changes rapidly. When the pressure difference reaches a level equivalent to 3 m of seawater depth (approximately 30 kPa), the eustachian tube closes completely. Once the eustachian tube is closed completely, it seldom reopens with the usual equalisation techniques, such as the Valsalva manoeuvre.[3] As the ambient pressure continues to increase, the pressure difference between the inside and outside of the tympanic cavity becomes more pronounced, resulting in MEBt.

Studies have shown a positive correlation between the duration of exposure to high pressure and the incidence of cardiopulmonary decompression sickness, which may lead to death in severe cases.[12,13] Therefore, it is necessary to shorten the exposure time to high pressures as much as

Figure 5
Gross specimen of middle ears in different groups; A – MEBt in group A without tympanic membrane perforation; B – MEBt in group A with tympanic membrane perforation; C – No MEBt in group B; D – Middle ear of normal control

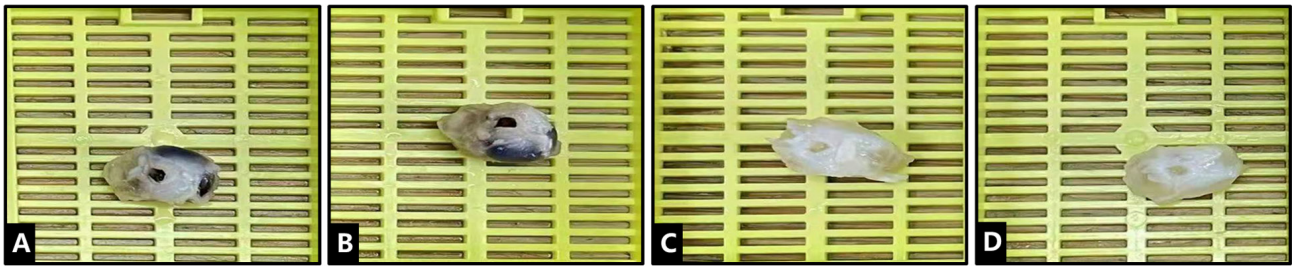


Figure 6
Hematoxylin and eosin staining of the mucosa of the tympanic cavity in different groups observed at low magnification (20×); A – moderate submucosal haemorrhage detected in group A without tympanic membrane perforation; B – obvious submucosal haemorrhage detected in group A with tympanic membrane perforation; C – no submucosal haemorrhage detected in group B without barotrauma; D – no submucosal haemorrhage detected in the middle ear of normal control

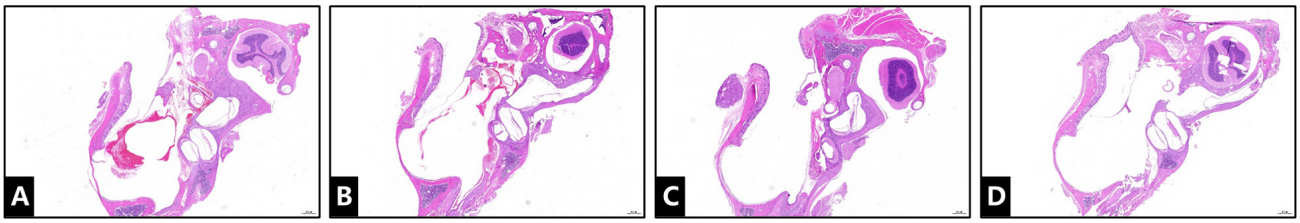
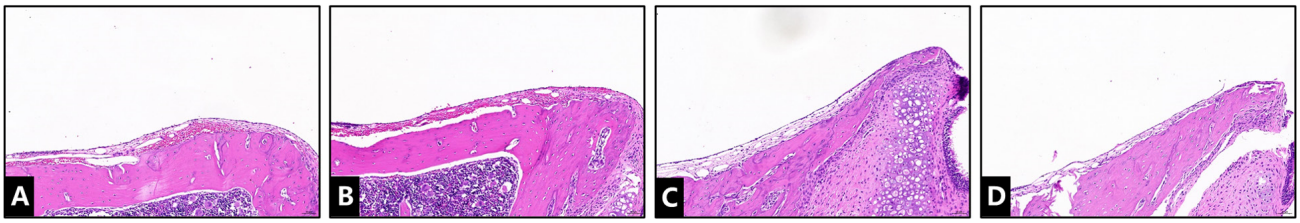


Figure 7
Hematoxylin and eosin staining of the mucosa of the tympanic cavity in different groups observed at high magnification (200×); A – moderate submucosal haemorrhage detected in group A without tympanic membrane perforation; B – obvious submucosal haemorrhage detected in group A with tympanic membrane perforation; C – no submucosal haemorrhage detected in group B without barotrauma; D – no submucosal haemorrhage detected in the middle ear of normal control



possible, which means that escaping submariners need to undergo pressurisation and initiate decompression extremely quickly. Studies have suggested that the severity of MEBt positively correlates with the rate of pressure change. Thus, during the procedure of fast buoyant ascent escape, if someone cannot open the Eustachian tube quickly, whether due to tube dysfunction or poor technique, MEBt would occur accompanied with symptoms such as pain, tinnitus, vertigo, and hearing loss.[3,5,14] Under these conditions, the safe operation of the escape equipment is affected, and the escape process may be compromised, perhaps catastrophically so.

The morphology, anatomy, and physiology of a rat's middle ear and Eustachian tube do not significantly differ from those of humans; therefore, rats are a suitable model for otological studies.[15] In clinical practice, 226 Hz tympanometry is the most commonly used method for adults. However, the anatomical structures of the ears in rats are similar to those of human babies.[16] Due to the characteristics of a small volume of the tympanic cavity and soft external auditory canal with good compliance, in our preliminary experiment, stable and repeatable tympanograms were not obtained from rats using 226 Hz tympanometry, consistent with the findings of others.[17]

It is generally accepted that 226 Hz tympanometry is not reliable for assessing the middle ear functional status of infants under seven months.[18,19] Several studies have shown that 1,000 Hz tympanometry is more accurate

than 226 Hz tympanometry for middle ear dysfunction in newborn babies.[20–22] The diagnostic consistency of 1,000 Hz tympanometry and temporal bone CT examinations in infants has been confirmed to be significantly higher than that of 226 Hz tympanometry.[23] Similarly, the diagnostic consistency of 1,000 Hz tympanometry and temporal bone magnetic resonance imaging in infants is significantly higher than that of 226 Hz tympanometry.[24] Therefore, in this study, 1,000 Hz tympanometry was used to evaluate the functional status of the middle ear to obtain stable and reliable test results.

For group A, our findings revealed that after undergoing a simulation of fast buoyant ascent escape from 200 m underwater, all experimental SD rats suffered obvious MEBt with symptoms of tympanic membrane hyperaemia or haemorrhage, haematotympanum, or tympanic membrane perforation. After the simulation procedure, the tympanograms of all ears changed from type A to type B, suggesting that the conduction function of the middle ear was impaired. Additionally, the ABR threshold in different frequencies increased significantly after the procedure, indicating that hearing loss occurred in experimental rats after simulated fast buoyant ascent escape from 200 m underwater.

In group B, tympanic membrane puncture effectively prevented MEBt caused by the simulation procedure. However, the hearing abilities of the rats decreased significantly after the needle puncture (approximately 0.4 mm in diameter). Based on clinical experience, the commonly used puncture needle for tympanocentesis is about 0.7–0.9 mm in diameter, and such perforation does not cause significant hearing loss in patients. The results of animal experiments are inconsistent with clinical experience. Studies have shown that the degree of hearing loss caused by tympanic membrane perforation is positively correlated with the size of tympanic membrane perforation and negatively correlated with the volume of the mastoid air chamber in the middle ear.[25,26] Some scholars have also pointed out that the degree of hearing loss caused by tympanic membrane perforation positively correlates with the percentage of perforated areas in the total tympanic membrane.[27] ImageJ software was used to measure the size of the tympanic membrane and perforation. The percentage of the perforated area was then calculated. In our study, the measurements and calculations showed that the perforated area accounted for approximately 10% of the tympanic membrane area. As is known, the area of the tympanic membrane of the human ear is about 55 mm^2, and the diameter of the tympanic membrane puncture needle usually used in clinical practice is about 0.7–0.9 mm. The percentage of the tympanic membrane perforation area caused by the needle in clinical practice is approximately 0.7–1.2%. The percentage of the tympanic membrane area of rats accounted for by tympanic membrane perforation in this study was much larger than that of human eardrum perforation caused by tympanic membrane puncture in clinical work, which may explain the significant hearing loss in rats caused by puncture (tympanocentesis) in this study.

There was no significant difference in the ABR thresholds of rats in group B after the simulated fast buoyant ascent escape from 200 m underwater compared to that after puncturing, indicating that after the tympanic puncture, the simulated fast buoyant ascent escape procedure did not lead to further hearing loss in rats. In other words, tympanic puncture had a protective effect against hearing loss caused by the simulation procedure.

Histopathological examination showed intratympanic and submucosal haemorrhages in all ears with MEBt in group A. However, the intratympanic haemorrhage was less severe, but submucosal haemorrhage was more severe in ears where barotraumatic perforation occurred than in those without. Eardrum perforation indicates more severe barotrauma; however, blood in the tympanic chamber flows out through the perforation, whereas submucosal blood does not. Therefore, the extent of submucosal tympanic haemorrhage can be used as an objective reference index to evaluate the severity of MEBt.

An invasive operation such as a tympanic membrane puncture should have the smallest diameter while ensuring safety and minimising injury. Therefore, the critical minimum diameter value should be determined in future studies. Second, there may be an incidence of harm associated with myringotomy by casual operators in a subsunk situation. Therefore, it is necessary to explore how to perform tympanic membrane puncture handily, promptly and safely in emergency cases. The above problems are the focus of our further research.

Conclusions

Fast buoyant ascent escape from deep depths can lead to MEBt and hearing loss. Barotrauma occurred in all animals not submitted to tympanocentesis, whereas tympanocentesis prevented ear injury.

References

1 Covington D, Pitkin A. Underwater nasal decongestant use: a novel approach to middle ear equalization. Undersea Hyperb Med. 2018;45:679–82. PMID: 31158935.

2 Varughese L, O'Neill O, Marker J, Smykowski E, Dayya D. The effect of compression rate and slope on the incidence of symptomatic Eustachian tube dysfunction leading to middle ear barotrauma: a Phase I prospective study. Undersea Hyperb Med. 2019;46:95–100. PMID: 31051053.

3 ONeill OJ, Brett K, Frank AJ. Middle ear barotrauma. Treasure Island (FL): StatPearls Publishing LLC. 2021. PMID: 29763026.

4 Hwang L, Song M, Lee Y, Shin T. Methods for preventing middle ear barotrauma in computer-controlled pressurization

of monoplace hyperbaric chambers. Undersea Hyperb Med. 2019;46:107–16. PMID: 31051055.
5 Mirza S, Richardson H. Otic barotrauma from air travel. J Laryngol Otol. 2005;119:366–70. doi: 10.1258/0022215053945723. PMID: 15949100.
6 O'Neill O, Smykowski E, Marker J, Perez L, Gurash S, Sullivan J. Proof of concept study using a modified Politzer inflation device as a rescue modality for treating Eustachian tube dysfunction during hyperbaric oxygen treatment in a multiplace (Class A) chamber. Undersea Hyperb Med. 2019;46:55–61. PMID: 31154685.
7 Wu J, Zheng Z, Wang X, Mao H, Cheng P. The prevention of the ear barotrauma by positive pressure on nasopharynx. Lin Chuang Er Bi Yan Hou Ke Za Zhi. 2006;20:878–9. PMID: 17168113. Chinese.
8 Jiang D, Zhang X, Sun J, Liu Y. Experimental investigation of tympanostomy for preventing barotrauma. Chin J Aerospace Med. 2009;20:283–6. doi: 10.3760/cma.j.issn.1007-6239.2009.04.013.
9 Hamilton-Farrell M, Bhattacharyya A. Barotrauma. Injury. 2004;35:359–70. doi: 10.1016/j.injury.2003.08.020. PMID: 15037370.
10 Gedik Ö, Doğan R, Babademez M, Karataş E, Aydın M, Koçyiğit A, et al. Therapeutic effects of metformin for noise induced hearing loss. Am J Otolaryngol. 2020;41:102328. doi: 10.1016/j.amjoto.2019.102328. PMID: 31732304.
11 Yule C. The mechanism of opening and closing the Eustachian Tube. J Anat Physiol. 1873;8:127–32.1. PMID: 17230993. PMCID: PMC1319011.
12 Yiqun F, Pu Y, Haitao W, Xiaochen B, Jun M, Shi Z, et al. Metabonomic potential plasma biomarkers in abnormal fast buoyancy ascent escape-induced decompression sickness model and the protective effects of pyrrolidine dithiocarbamic acid. Undersea Hyperb Med. 2017;44:109–19. doi: 10.22462/3.4.2017.4. PMID: 28777901.
13 Klapa S, Meyne J, Kähler W, Tillmans F, Werr H, Binder A, et al. Decompression illness with hypovolemic shock and neurological failure symptoms after two risky dives: a case report. Physiol Rep. 2017;5:e13094. doi: 10.14814/phy2.13094. PMID: 28325788. PMCID: PMC5371546.
14 Lynch JH, Deaton TG. Barotrauma with extreme pressures in sport: from scuba to skydiving. Curr Sports Med Rep. 2014;13:107–12. doi: 10.1249/jsr.0000000000000039. PMID: 24614424.
15 Van Heerbeek N, Tonnaer ELGM, Ingels KJAO, Curfs JHAJ, Cremers CWRJ. Effect of exogenous surfactant on ventilatory and clearance function of the rat's eustachian tube. Otol Neurotol. 2003;24:6–10. doi: 10.1097/00129492-200301000-00003. PMID: 12544020.
16 Daniel H, Fulghum R, Brinn J, Barrett K. Comparative anatomy of eustachian tube and middle ear cavity in animal models for otitis media. Ann Otol Rhinol Laryngol. 1982;91:82–9. doi: 10.1177/000348948209100118. PMID: 7073182.
17 Zheng H, Li X, Yu L. Study of 1000 Hz probe tone tympanometry on examination of fluid accumulation in tympanic cavity. Chin Arch Otolaryngol Head Neck Surg. 2008;15:79–81. doi: 10.3969/j.issn.1672-7002.2008.02.006.
18 Holte L, Margolis R, Cavanaugh R. Developmental changes in multifrequency tympanograms. Audiology. 1991;30:1–24. doi: 10.3109/00206099109072866. PMID: 2059166.
19 McKinley A, Grose J, Roush J. Multifrequency tympanometry and evoked otoacoustic emissions in neonates during the first 24 hours of life. J Am Acad Audiol. 1997;8:218–23. PMID: 9188078.
20 Meyer S, Jardine C, Deverson W. Developmental changes in tympanometry: A case study. Br J Audiol. 1997;31:189–95. doi: 10.3109/03005364000000021. PMID: 9276101.
21 Kei J, Allison-Levick J, Dockray J, Harrys R, Kirkegard C, Wong J, et al. High-frequency (1000 Hz) tympanometry in normal neonates. J Am Acad Audiol. 2003;14:20–8. doi: 10.3766/jaaa.14.1.4. PMID: 12833925.
22 Margolis R, Bass-Ringdahl S, Hanks W, Holte L, Zapala D. Tympanometry in newborn infants – 1 kHz norms. J Am Acad Audiol. 2003;14:383–92. PMID: 14620612.
23 Zhiqi L, Kun Y, Zhiwu H. Tympanometry in infants with middle ear effusion having been identified using spiral computerized tomography. Am J Otolaryngol. 2010;31:96–103. doi: 10.1016/j.amjoto.2008.11.008. PMID: 20015724.
24 Yang K, Liu Z. Comparison of 1000 Hz-, 226 Hz-probe tone tympanometry and magnetic resonance imaging in evaluating the function of middle ear in infants. Int J Pediatr Otorhinolaryngol. 2020;136:110135. doi: 10.1016/j.ijporl.2020.110135. PMID: 32544643.
25 Aslıer M, Özay H, Gürkan S, Kırkım G, Güneri E. The effect of tympanic membrane perforation site, size and middle ear volume on hearing loss. Turk Arch Otorhinolaryngol. 2019;57:86–90. doi: 10.5152/tao.2019.4015. PMID: 31360926. PMCID: PMC6640663.
26 Mehta R, Rosowski J, Voss S, O'Neil E, Merchant S. Determinants of hearing loss in perforations of the tympanic membrane. Otol Neurotol. 2006;27:136–43. doi: 10.1097/01.mao.0000176177.17636.53. PMID: 16436981. PMCID: PMC2918411.
27 Choffor-Nchinda E, Djomou F, Meva'a Biouele R, Mindja D, Bola A, Kewe I, et al. Determinants of hearing loss severity in tympanic membrane perforations in a sub-Saharan African setting. J Laryngol Otol. 2018;132:1013–7. doi: 10.1017/s0022215118001962. PMID: 30409241.

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