Short Communication

Protective Effect of Neurotrophic Agent T-817MA Against Inner Ear Barotrauma in the Guinea Pig

Hitoshi Maekawa¹, Takeshi Matsunobu^{1,*}, Yasushi Satoh², Takaomi Kurioka¹, Asako Nakamura³, Noboru Iwakami³, and Akihiro Shiotani¹

¹Department of Otolaryngology, ²Department of Anesthesiology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan

³Research Laboratories, Toyama Chemical Co., Ltd., 2-4-1 Shimo-okui, Toyama 930-8508, Japan

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Abstract. T-817MA (1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}azetidin-3-ol maleate), a neurotrophic compound newly synthesized for the treatment of Alzheimer's disease, has been found to reduce oxidative stress and exert neuroprotective effects. By assessing the auditory functioning and observing the cochlear sensory epithelia, we investigated whether T-817MA protects the cochlea from inner ear barotrauma in guinea pigs treated with rapidly intense pressure change. Sustained oral administration of T-817MA significantly reduced the extent of auditory threshold shifts and outer hair cell loss, indicating that T-817MA attenuates the intense pressure-induced cochlear damage that accompanies inner ear barotrauma via antioxidative activity.

Keywords: T-817MA, inner ear barotrauma, free radical

Inner ear barotrauma (IEB) is a disorder caused by acute pressure changes such as those occurring during scuba diving, aviation, or simply blowing one's nose (1). Acute changes in pressure are among the greatest risks facing scuba divers, as they can cause IEB, which may lead to permanent sensorineural hearing loss (SNHL). However, the precise mechanism remains unknown. In our previous study of IEB, we suggested that disturbance of cochlear blood flow produced by intense pressure loading leads to lipid peroxidation of cochlear tissue and that outer hair cell (OHC) loss was seen 28 days after intense pressure loading (2).

The neurotrophic compound 1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}azetidin-3-ol maleate (T-817MA) has been developed as a therapeutic agent (Fig. 1A) for treating neurodegenerative disorders such as Alzheimer's disease (AD), by modulation of endogenous antioxidative mechanisms (3, 4). The efficacy of this agent in treating AD is currently being assessed in clinical phase II trials in the United States.

This study investigated the neuroprotective effects of T-817MA against IEB in an animal model. All the

experiments were carried out in accordance with the Guidelines for Animal Experiments of the National Defense Medical College. Thirty-six female Hartley guinea pigs (300–350 g) were randomly assigned to 1 of 3 groups (n = 12/group). Group I animals orally received water containing T-817MA (Toyama Chemical Co., Ltd., Toyama) at a concentration of 0.7 mg/mL; group II, water containing T-817MA at a concentration of 0.2 mg/mL; and group III (control group), distilled water. In our previous study (5), the doses of 0.2 and 0.7 mg/mL of T-817MA were optimal for treating noise-induced hearing loss. T-817MA treatment was initiated 10 days before pressure loading and continued until 10 days after pressure loading (Fig. 1B). The animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and medetomidine (1.0 mg/kg), and then earplugs that restrict the movement of the tympanic membrane were inserted into the external auditory canal. The animals were then placed in a chamber, and the pressure was increased from 1 to 2 atmosphere pressure absolute (ATA) for a 3-s period and decreased to 1 ATA over a 2-s period (2). A value of 2 ATA is equivalent to pressure at a depth of 10 m in water, the average depth attained by most scuba divers. The earplugs were immediately removed after this pressure loading.

Auditory functioning was assessed 10 days before, 1 h

*Corresponding author. takeshim@ndmc.ac.jp

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Fig. 1. Chemical structure of T-817MA (A), scheme of study protocol (B), and effect of pressure loading on hearing threshold shifts in groups I (T-817MA concentration: 0.7 mg/mL), II (T-817MA concentration: 0.2 mg/mL), and III (control) (C – G). The threshold shifts of the T-817MA-treated groups (I and II) were significantly smaller than those of the control group (III) 7 days after pressure loading at high frequencies (* $P < 0.05$, ** $P < 0.01$, Mann–Whitney U -test). Values are represented as mean $\pm$ S.E.M.

after (day 0), and at 3, 7, 14, and 35 days after pressure loading by measurement of auditory brainstem response (ABR) thresholds at frequencies of 4, 8, 12, 16, and 20 kHz. The ABR threshold was determined by measurement of wave III or IV, which reflect the neural activity of the cochlear nucleus and the superior olivary complex in response to sound stimulation, respectively. In our previous study, we found that the threshold shift remains nearly unchanged 35 days after pressure loading in cases of IEB (2). For histological assessment, 9 animals were randomly assigned to 1 of 3 groups ($n = 3/\text{group}$): T-817MA-treated group (T-817MA concentration, 0.2 mg/mL), non-treated (distilled water) control group, and normal group. The T-817MA-treated and non-treated control animals were decapitated 4 weeks after pressure

loading. The cochleae were locally perfused with 4% paraformaldehyde and allowed to remain in the fixative overnight. The organ of Corti was stained with 1% rhodamine-phalloidin (Invitrogen, Carlsbad, CA, USA) and processed for surface preparation. The sections were examined with a fluorescence microscope and the number of the missing OHCs was counted. To clarify if T-817MA attenuates oxidative stress, 6 animals were randomly assigned to 1 of the 3 groups ($n = 2/\text{group}$) described before. The T-817MA-treated and non-treated control animals were decapitated 1 h after pressure loading, and the expression of 8-hydroxy-2 deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was examined immunohistochemically. This method precisely follows that described in a previous report (2).

The Baseline ABR thresholds did not differ significantly among the groups. As can be observed in Fig. 1, C – G, the threshold shifts of both groups of T-817MA-treated animals significantly decreased at 12 kHz at 14 days; at 16 kHz at 7, 14, and 35 days; and at 20 kHz at 7, 14, and 35 days after pressure loading ($*P < 0.05$, $**P < 0.01$, Mann–Whitney *U*-test). Although OHC loss was significantly reduced in the T-817MA-treated group in the basal turn compared with the non-treated control group, no significant difference was observed between the untreated and treated groups in the middle and apical turns (Fig. 2: A – L; $P < 0.05$, Mann–Whitney *U*-test). In the immunohistochemistry for 8-OHdG, the animals were decapitated 1 h after pressure loading and the cochleae were removed. Strong immunoreactivity was observed in the OHC nuclei of the non-treated control group, whereas weak immunoreactivity was observed in the normal group. Lesser amounts of immunoreactivity were observed in the same region of the T-817MA-treated group as compared with that observed in the non-treated control group (Fig. 3: A – C).

We previously reported that oxidative stress is produced in the cochlea in response to acute pressure change

Fig. 3. Immunohistochemical detection of 8-hydroxy-2 deoxyguanosine (8-OHdG). The non-treated control group (A), the T-817MA (0.2 mg/mL)-treated group (B), and the normal group (C). The T-817MA-treated and non-treated control groups were decapitated 1 h after pressure loading. Whole mount preparations of the organ of Corti were presented. Scale bar = 50 μ m.

Fig. 2. Percentage of outer hair cell (OHC) loss (A – C) and wide-field fluorescence microscopic images of OHCs stained with rhodamine–phalloidin 4 weeks after pressure loading (D – L). Scale bar = 50 μ m. The T-817MA (0.2 mg/mL)-treated group experienced significantly less OHC loss in the basal turn than did the control group ($*P < 0.05$, Mann–Whitney *U*-test). Values are represented as mean $\pm$ S.E.M.

and that 3-methyl 1-phenyl-2-pyrazolin-5-one (edaravone), a potent free radical scavenger, has a therapeutic effect on IEB-induced SNHL. We also suggested that intense pressure loading induced circulatory disturbances and produces reactive oxygen species (6), which leads to biochemical damage of cochlear cells and functional damage in hearing. Because T-817MA suppresses a decrease of glutathione (7) and reduces hydrogen peroxide-induced neuronal death (8), we hypothesized that T-817MA could be effective for treating neurodegenerative disorders associated with oxidative stress such as IEB.

In the present study, administration of T-817MA reduced the oxidative stress in the cochlear cells, especially in the basal turn, which is associated with high-frequency hearing. The tendency that the basal turn is more severely injured than the other turns is similar to that in noise-induced hearing loss (9). The difference in vulnerability could be due to not only the anatomical but also the biochemical gradient through the turns. Although the precise mechanism of the neuroprotective effect of T-817MA remains unknown, several studies have reported it. T-817MA promotes neurogenesis in cultured neuronal cells (8) and functions as a neurotrophic factor, such as insulin-like growth factor-1, which protects the cochlear tissue from oxidative stress (10). T-817MA could act as a neurotrophic factor in the cochlea. Furthermore, we have shown that the neuroprotective effect of T-817MA is related to the protein kinase C (PKC) pathway because translocation of PKC ϵ was observed in T-817MA-treated neuronal cultured cells (11). Translocation of PKC isoforms was observed in the phorbol 12-myristate 13-acetate-stimulated OHCs in the cochlea (12); thus, T-817MA might demonstrate a protective effect through activation of PKC in the cochlea. Although the pharmacological details remain to be elucidated, the findings in the present study indicate that T-817MA could be a new candidate drug for the protection or treatment of acute SNHL.

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