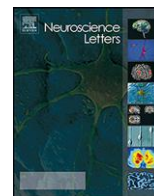




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## Hydrogen in drinking water attenuates noise-induced hearing loss in guinea pigs

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### ABSTRACT

It has been shown that molecular hydrogen acts as a therapeutic and preventive antioxidant by selectively reducing the hydroxyl radical, the most cytotoxic of the reactive oxygen species. In the present study, we tested the hypothesis that acoustic damage in guinea pigs can be attenuated by the consumption of molecular hydrogen. Guinea pigs received normal water or hydrogen-rich water for 14 days before they were exposed to 115 dB SPL 4-kHz octave band noise for 3 h. Animals in each group underwent measurements for auditory brainstem response (ABR) or distortion-product otoacoustic emissions (DPOAEs) before the treatment (baseline) and immediately, 1, 3, 7, and 14 days after noise exposure. The ABR thresholds at 2 and 4 kHz were significantly better on post-noise days 1, 3, and 14 in hydrogen-treated animals when compared to the normal water-treated controls. Compared to the controls, the hydrogen-treated animals showed greater amplitude of DPOAE input/output growth functions during the recovery process, with statistical significance detected on post-noise days 3 and 7. These findings suggest that hydrogen can facilitate the recovery of hair cell function and attenuate noise-induced temporary hearing loss.

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Exposure to loud noise may cause sensorineural hearing loss that can last for minutes, hours, days, or permanently, depending on the parameters of the acoustic overstimulation and the subject's susceptibility to noise exposure. Noise-induced temporary threshold shift (TTS) is a reversible elevation in hearing threshold that occurs after acoustic overstimulation. TTS can be an indicator of exposures that lead to permanent hearing loss after multiple, cumulative exposure events. Although the mechanisms underlying this phenomenon are not fully understood, it is widely accepted that direct mechanical damage and/or indirect metabolic alterations may be involved. Most notably, the generation of reactive oxygen species (ROS) [12], which may serve as triggers for necrosis or apoptosis, results in damage to the cochlear hair cells and the subsequent degeneration of auditory neurons. Thus, suitable antioxidants are desired to protect against oxidative damage in the inner ear. Pharmacological agents effective against TTS may have a potential clinical role in the prophylaxis of acute acoustic damage. However, most antioxidants have difficulty reaching the cochlear hair cells because of the blood–labyrinthine barrier.

Recent studies have revealed that molecular hydrogen mediates beneficial effects in different systems as an optimal antioxidant agent by selectively scavenging free hydroxyl radicals ( $\cdot\text{OH}$ ) [23,25]. Inhaled hydrogen gas can prevent or reduce pathological or biochemical changes in animal models of cerebral infarction [23], neonatal hypoxia ischemia [4], hepatic injury [9], intestinal ischemia injury [2], myocardial ischemia-reperfusion injury [11], cisplatin-induced nephrotoxicity [19], polymicrobial sepsis [26], and generalized inflammation [27]. Continuous consumption of hydrogen water can also protect against intestinal ischemia [29], neonatal hypoxia-ischemia [3], chronic allograft nephropathy [5] and acute pancreatitis [6]. It has also been shown to reduce atherosclerotic lesions in apolipoprotein E knock-out mice [24], inactivate oxidative stress in the brain of Parkinson disease rodents [7,8], and prevent stress-induced decline in learning and memory caused by chronic physical restraint [18]. Hydrogen-loaded eye drops can also protect the retina from ischemia-reperfusion injury [21]. A clinical study has shown that consuming hydrogen-rich water improves lipid and glucose metabolism in type 2 diabetes patients [14]. Furthermore, hydrogen-saturated culture medium can protect cochlear hair cells against antimycin A-induced oxidative stress *in vitro* [16].

Because of permeability and few side effects of molecular hydrogen, it is considered especially favorable as a component of inner-ear medicine. In the present study, therefore, we tested the

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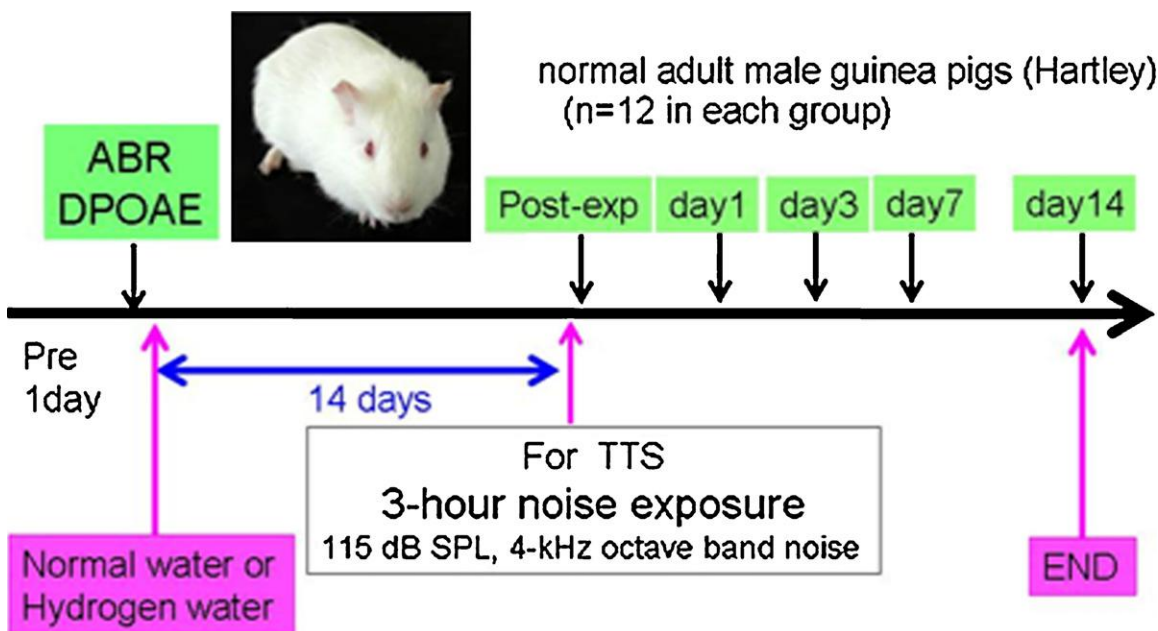


Fig. 1. Schedule of the experiment procedures.

hypothesis that continuous consumption of hydrogen water could attenuate noise-induced TTS in guinea pigs.

Thirty-four male Hartley guinea pigs weighing 250–300 g were used. Since sex differences have been associated with differing ability to detoxify ROS [13], only male guinea pigs were used. One day after arrival, their hearing was confirmed to be within the normal range (within one standard deviation of the normative laboratory baseline) with auditory brainstem response (ABR) or distortion product otoacoustic emissions (DPOAEs) measurements (Fig. 1). After the first baseline hearing tests, animals were randomly divided into normal water-treated and hydrogen water-treated experimental groups ( $n = 17$  in each group). Treatment and control solutions were administered orally with unlimited access starting 14 days before noise exposure. Each day, supersaturated hydrogen water (Blue Mercury, Tokyo, Japan) was placed in a closed glass vessel, which minimizes the leakage of hydrogen from the water and maintains the concentration to be greater than 0.4 mM one day later [24]. Weight gains and amounts of water consumed were measured daily. This study was reviewed and approved by the Committee for Ethics in Animal Experiments of the University of Tokyo and carried out under Japanese law and the Guidelines for Animal Experiments of the University of Tokyo.

Fourteen days after starting, either normal or hydrogen water treatments, the animals were subjected to a 3-h noise exposure (115 dB SPL, 4 kHz octave band noise) generated within a single-walled, sound-deadened chamber as previously reported [28]. Two separately caged animals were tested simultaneously and allowed to move freely during exposure. The sound chamber was fitted with speakers driven by a noise generator and power amplifier. A 0.5-in. Bruel and Kjaer condenser microphone and a Fast Fourier Transform analyzer were used to measure and calibrate the sound level at various locations within the chamber to ensure stimulus uniformity within  $\pm 1$  dB.

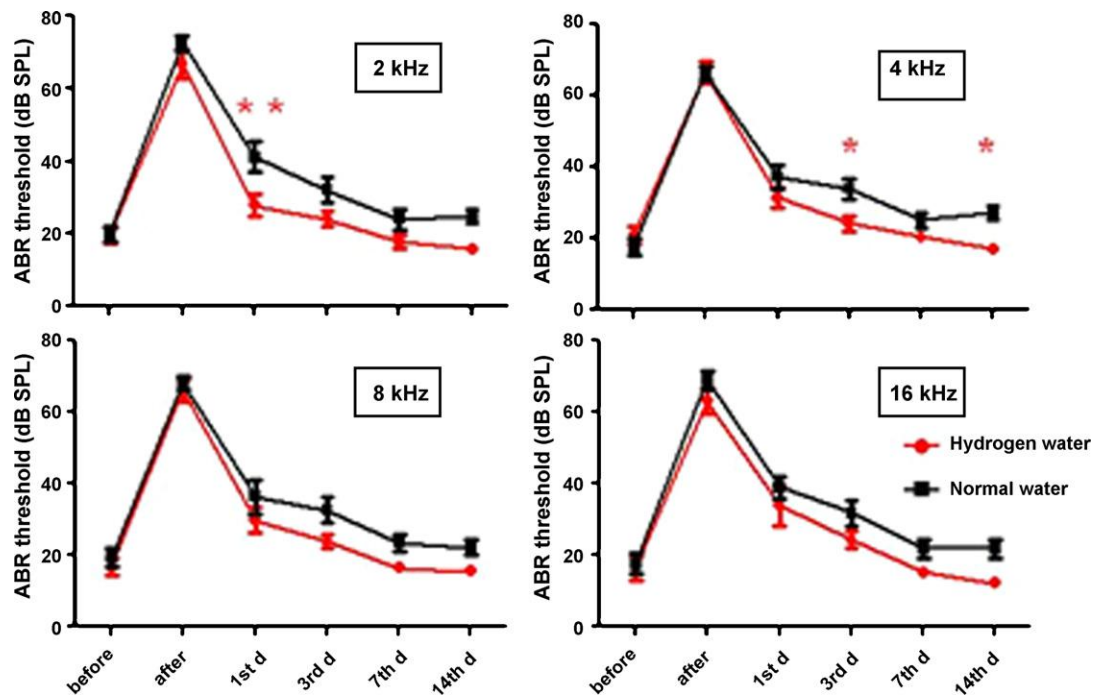
To assess the effect of hydrogen water on TTS, 24 animals ( $n = 12$  in each group) were subjected to ABR measurements immediately and at 1, 3, 7, and 14 days after noise exposure. The method of ABR measurement has been described previously [15]. In brief, animals were anesthetized intramuscularly with a mixture of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (40 mg/kg), and needle electrodes were placed subcutaneously at the vertex

(active electrode), beneath the pinna of the measured ear (reference electrode), and beneath the opposite ear (ground). The stimulus duration was 15 ms; the presentation rate, 11/s; the rise/fall time, 1 ms; and the frequencies, 2, 4, 8, and 16 kHz. Responses of 1024 sweeps were averaged at each intensity level. The sound intensity was varied in 5 dB intervals at the intensities close to the threshold, which was defined as the lowest intensity level that produced a clear reproducible waveform peak 3 or 4. In general, amplitude at threshold was approximately 0.1  $\mu$ V.

Ten animals ( $n = 5$  in each group) underwent DPOAE measurement immediately and at 1, 3, 7, and 14 days after noise exposure with an acoustic probe using the DP2000 DPOAE measurement system version 3.0 (Starkey Laboratory, Eden Prairie, MN) as described previously [20]. DP-grams comprised 2f1–f2 DPOAE amplitudes as a function of f2. The stimulus paradigm used for DPOAE input/output (I/O) growth function is constructed as follows [10]: two primary tones with a frequency ratio, f2/f1, of 1.2 were presented, with f2 in one-sixth-octave steps from 1 to 16 kHz. At each frequency pair, primary levels of L2 were incremented in 5 dB steps from 40 to 70 dB SPL with an L1–L2 value of 10 dB. DPOAE was defined to be present when its level exceeded that of the noise floor by 3 dB.

The overall effects of the hydrogen treatment were examined using a two-way factorial analysis of variance with Bonferroni post-tests (SPSS software).  $p$  values of less than 0.05 were considered to be statistically significant. Values are expressed as the mean (standard deviation).

Weight gain and the amount of water consumed were not statistically different between the 2 groups (data not shown). Chronological alterations in the ABR threshold shifts at 2, 4, 8, and 16 kHz before and after noise exposure with the application of hydrogen-rich or normal water are shown in Fig. 2. ABR thresholds before noise exposure were essentially equivalent between the 2 groups. In normal water-treated controls, ABR thresholds were moderately increased by approximately 45 dB at all frequencies immediately after noise exposure. Subsequently the ABR thresholds showed gradual recovery, returning to pre-exposure baseline thresholds 14 days later, indicating that the noise exposure induced TTS. Hydrogen-treated animals showed similar but smaller ABR threshold shifts after noise exposure, as compared to the controls.



**Fig. 2.** Thresholds of auditory brainstem response (mean  $\pm$  S.D.) measured before, immediately after noise exposure and post-noise 1st day, 3rd days, 7th days, 14th days in normal water-treated controls and hydrogen-treated animals ( $n = 12$  in each group). There is a statistical significance at all frequencies (two-way ANOVA) and post-noise 1st day for 2 kHz, 3rd day and 14th day for 4 kHz with Bonferroni post-tests (\*\* $p < 0.01$ , \* $p < 0.05$ ).

The overall effect of hydrogen significantly attenuated the TTS across the measurement period for all the tested frequencies ( $p < 0.05$ ). Compared to the controls, the hydrogen-treated animals showed significantly smaller ABR thresholds at 2 kHz on day 1 day after noise exposure ( $p < 0.01$ ) and at 4 kHz on day 3 and 14 after noise exposure ( $p < 0.05$ ).

Fig. 3 shows the mean DPOAE input/output (I/O) growth functions at 16 kHz before and immediately, 1, 3, 7, and 14 days after noise exposure. There was no statistically significant difference between the 2 groups considering the amplitude of DPOAE I/O function. Both the groups showed a severe decrease in DPOAE amplitude immediately after noise exposure. Compared to the controls, the hydrogen-treated animals showed greater amplitudes during the recovery process. The overall effect of hydrogen water application was statistically significant 3 and 7 days after noise exposure ( $p < 0.01$ ), although both groups exhibited almost normal I/O function 14 days after noise exposure.

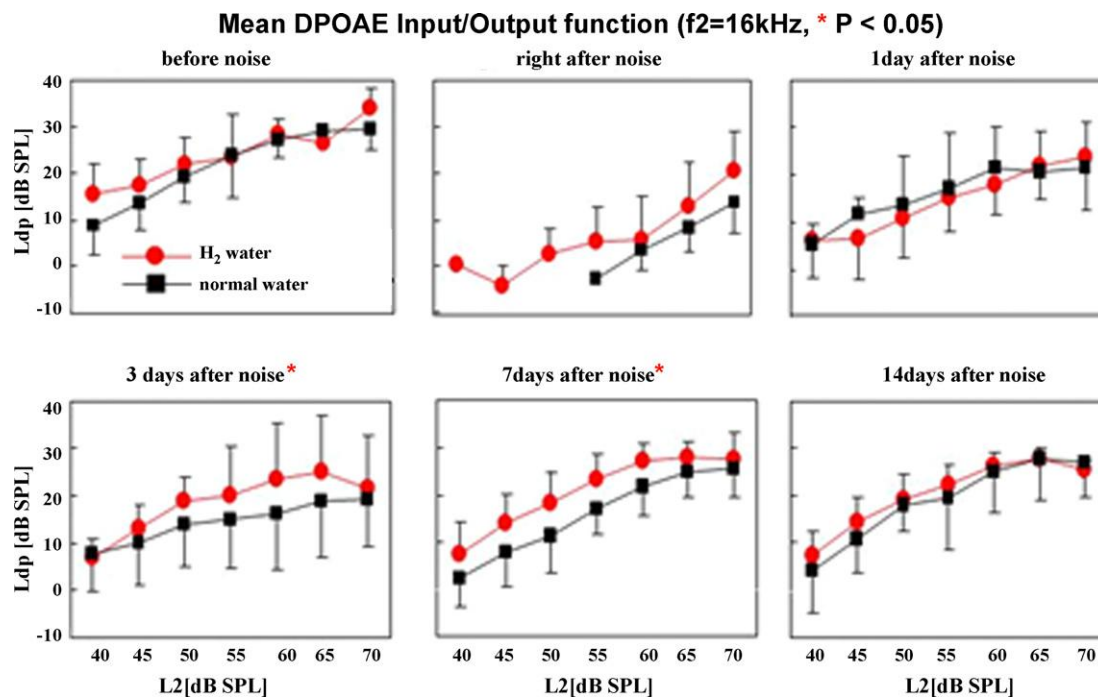
The present study showed that hydrogen attenuated noise-induced TTS and accelerated the recovery of DPOAE. It is likely that hydrogen facilitates the recovery of hearing function because of its antioxidant property [1]. A previous *in vitro* study has also demonstrated the potential of hydrogen to protect both the inner hair cells and outer hair cells from oxidant damage induced by different concentrations of antimycin A [16]. Incubation with a hydrogen-saturated medium significantly reduced ROS generation and subsequent lipid peroxidation in the auditory epithelia, leading to increased survival of hair cells. Hydrogen selectively alleviates hydroxyl radicals ( $\cdot\text{OH}$ ) and peroxynitrite radical ( $\text{ONOO}^-$ )-induced cytotoxicity without affecting other ROS, such as superoxide ( $\text{O}_2^{\cdot-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), or nitric oxide ( $\text{NO}^{\cdot}$ ) [23]. Thus, it is unlikely that hydrogen disturbs metabolic oxidation–reduction reactions or disrupts ROS involved in cell signaling. This characteristic of hydrogen is advantageous in medical treatments because the use of hydrogen should not cause serious unwanted side effects.

In the current study, we did not examine the morphological changes in the cochlea because the abnormalities in ABR and DPOAE

measurements were minimal 14 days after noise exposure. The physiological findings, however, suggest that the noise level used in the current study induced only subtle morphological changes such as bleb formation, but not severe degeneration such as apoptosis of the outer hair cells. No significant permanent morphological changes have been shown in the hair cells in previous studies using a similar protocol of noise exposure [22]. In contrast, it has been shown that in guinea pigs, the afferent dendrites beneath the inner hair cells become swollen immediately after exposure to similar noise causing TTS [28]. Kujawa and Liberman [17] have reported that acoustic overexposures causing moderate, but completely reversible threshold elevation leave cochlear sensory cells intact but cause acute loss of the afferent nerve terminals and delayed degeneration of the cochlear nerve in mice. Although the difference of ABR thresholds immediately after noise was small between hydrogen-treated animals and controls, therefore, it is considered intriguing to examine if hydrogen attenuates such acute and chronic changes of the neural elements.

The efficacy of any single antioxidant may be limited by several factors, including limited access to cellular compartments, action against only a few forms of ROS, interference with redox-based signaling, or a tendency to throw innate ROS protections out of balance [22]. Thus, despite its mild effect, molecular hydrogen is still an optimal choice. It reacts only with the strongest oxidants. Besides, it can penetrate biological membranes by gaseous rapid diffusion and target organelles like the mitochondria and nucleus, which makes it highly effective for reducing cytotoxic radicals. This unique feature of molecular hydrogen is especially favorable for drug delivery to the inner ear compared to other antioxidants, because the blood–labyrinthine barrier blocks many therapeutic compounds and does not allow them to reach cochlear hair cells.

The present study shows that hydrogen can promote hearing recovery from acoustic trauma-induced TTS and can attenuate TTS. This improvement likely reflects hydrogen’s scavenging of detrimental ROS. Since hydrogen has already been used in humans clinically to treat decompression sickness in divers with few or no side effects, our findings reinforce the potential clinical utility of



**Fig. 3.** Mean DPOAE input–output function at different time points at f<sub>2</sub> = 16 kHz in normal water-treated controls and hydrogen-rich water-treated animals (n = 5 in each group). There is a statistical significance on post-noise days 3 and 7 days. \*p < 0.05.

hydrogen as an adjuvant agent for noise-induced hearing loss in humans.

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#### References

[1] J.H. Abraini, M.C. Gardette-Chauffour, E. Martinez, J.C. Rostain, C. Lemaire, Psychophysiological reactions in humans during an open sea dive to 500 m with a hydrogen-helium-oxygen mixture, *J. Appl. Physiol.* 76 (1994) 1113–1118.  
 [2] B.M. Buchholz, D.J. Kaczorowski, R. Sugimoto, R. Yang, Y. Wang, T.R. Billiar, K.R. McCurry, A.J. Bauer, A. Nakao, Hydrogen inhalation ameliorates oxidative stress in transplantation induced intestinal graft injury, *Am. J. Transplant.* 8 (2008) 2015–2024.  
 [3] J. Cai, Z. Kang, K. Liu, W. Liu, R. Li, J.H. Zhang, X. Luo, X. Sun, Neuroprotective effects of hydrogen saline in neonatal hypoxia-ischemia rat model, *Brain Res.* 1256 (2009) 129–137.  
 [4] J. Cai, Z. Kang, W.W. Liu, X. Luo, S. Qiang, J.H. Zhang, S. Ohta, X. Sun, W. Xu, H. Tao, R. Li, Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model, *Neurosci. Lett.* 441 (2008) 167–172.  
 [5] J.S. Cardinal, J. Zhan, Y. Wang, R. Sugimoto, A. Tsung, K.R. McCurry, T.R. Billiar, A. Nakao, Oral hydrogen water prevents chronic allograft nephropathy in rats, *Kidney Int.* 77 (2009) 101–109.  
 [6] H. Chen, Y.P. Sun, Y. Li, W.W. Liu, H.G. Xiang, L.Y. Fan, Q. Sun, X.Y. Xu, J.M. Cai, C.P. Ruan, N. Su, R.L. Yan, X.J. Sun, Q. Wang, Hydrogen-rich saline ameliorates the severity of L-arginine-induced acute pancreatitis in rats, *Biochem. Biophys. Res. Commun.* 393 (2010) 308–313.  
 [7] Y. Fu, M. Ito, Y. Fujita, M. Ito, M. Ichihara, A. Masuda, Y. Suzuki, S. Maesawa, Y. Kajita, M. Hirayama, I. Ohsawa, S. Ohta, K. Ohno, Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease, *Neurosci. Lett.* 453 (2009) 81–85.  
 [8] K. Fujita, T. Seike, N. Yutsudo, M. Ohno, H. Yamada, H. Yamaguchi, K. Sakumi, Y. Yamakawa, M.A. Kido, A. Takaki, T. Katafuchi, Y. Tanaka, Y. Nakabeppu, M. Noda, Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, *PLoS One* 4 (2009) e7247.

[9] K. Fukuda, S. Asoh, M. Ishikawa, Y. Yamamoto, I. Ohsawa, S. Ohta, Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress, *Biochem. Biophys. Res. Commun.* 361 (2007) 670–674.  
 [10] D.D. Gehr, T. Janssen, C.E. Michaelis, K. Deingruber, K. Lamm, Middle ear and cochlear disorders result in different DPOAE growth behaviour: implications for the differentiation of sound conductive and cochlear hearing loss, *Hear. Res.* 193 (2004) 9–19.  
 [11] K. Hayashida, M. Sano, I. Ohsawa, K. Shinmura, K. Tamaki, K. Kimura, J. Endo, T. Katayama, A. Kawamura, S. Kohsaka, S. Makino, S. Ohta, S. Ogawa, K. Fukuda, Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury, *Biochem. Biophys. Res. Commun.* 373 (2008) 30–35.  
 [12] D. Henderson, E.C. Bielefeld, K.C. Harris, B.H. Hu, The role of oxidative stress in noise-induced hearing loss, *Ear Hear.* 27 (2006) 1–19.  
 [13] R.H. Julicher, L. Sterrenberg, G.R. Haenen, A. Bast, J. Noordhoek, Sex differences in the cellular defense system against free radicals from oxygen or drug metabolites in rat, *Arch. Toxicol.* 56 (1984) 83–86.  
 [14] S. Kajiyama, G. Hasegawa, M. Asano, H. Hosoda, M. Fukui, N. Nakamura, J. Kitawaki, S. Imai, K. Nakano, M. Ohta, T. Adachi, H. Obayashi, T. Yoshikawa, Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance, *Nutr. Res.* 28 (2008) 137–143.  
 [15] A. Kashio, T. Sakamoto, K. Suzukawa, S. Asoh, S. Ohta, T. Yamasoba, A protein derived from the fusion of TAT peptide and FNK, a Bcl-x(L) derivative, prevents cochlear hair cell death from aminoglycoside ototoxicity in vivo, *J. Neurosci. Res.* 85 (2007) 1403–1412.  
 [16] Y.S. Kikkawa, T. Nakagawa, R.T. Horie, J. Ito, Hydrogen protects auditory hair cells from free radicals, *Neuroreport* 20 (2009) 689–694.  
 [17] S.G. Kujawa, M.C. Liberman, Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss, *J. Neurosci.* 29 (2009) 14077–14085.  
 [18] K. Nagata, N. Nakashima-Kamimura, T. Mikami, I. Ohsawa, S. Ohta, Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice, *Neuropsychopharmacology* 34 (2009) 501–508.  
 [19] N. Nakashima-Kamimura, T. Mori, I. Ohsawa, S. Asoh, S. Ohta, Molecular hydrogen alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising anti-tumor activity in mice, *Cancer Chemother. Pharmacol.* 64 (2009) 753–761.  
 [20] Y. Noguchi, K. Kurima, T. Makishima, M.H. de Angelis, H. Fuchs, G. Frolenkov, K. Kitamura, A.J. Griffith, Multiple quantitative trait loci modify cochlear hair cell degeneration in the Beethoven (Tmc1Bth) mouse model of progressive hearing loss DFNA36, *Genetics* 173 (2006) 2111–2119.  
 [21] H. Oharazawa, T. Igarashi, T. Yokota, H. Fujii, H. Suzuki, M. Machide, H. Takahashi, S. Ohta, I. Ohsawa, Protection of the retina by rapid diffusion of hydrogen: administration of hydrogen-loaded eye drops in retinal ischemia-reperfusion injury, *Invest. Ophthalmol. Vis. Sci.* 51 (2010) 487–492.

- [22] K.K. Ohlemiller, Recent findings and emerging questions in cochlear noise injury, *Hear. Res.* 245 (2008) 5–17.
- [23] I. Ohsawa, M. Ishikawa, K. Takahashi, M. Watanabe, K. Nishimaki, K. Yamagata, K. Katsura, Y. Katayama, S. Asoh, S. Ohta, Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals, *Nat. Med.* 13 (2007) 688–694.
- [24] I. Ohsawa, K. Nishimaki, K. Yamagata, M. Ishikawa, S. Ohta, Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice, *Biochem. Biophys. Res. Commun.* 377 (2008) 1195–1198.
- [25] Y. Sato, S. Kajiyama, A. Amano, Y. Kondo, T. Sasaki, S. Handa, R. Takahashi, M. Fukui, G. Hasegawa, N. Nakamura, H. Fujinawa, T. Mori, M. Ohta, H. Obayashi, N. Maruyama, A. Ishigami, Hydrogen-rich pure water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice, *Biochem. Biophys. Res. Commun.* 375 (2008) 346–350.
- [26] K. Xie, Y. Yu, Y. Pei, L. Hou, S. Chen, L. Xiong, G. Wang, Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release, *Shock* 34 (2010) 90–97.
- [27] K. Xie, Y. Yu, Z. Zhang, W. Liu, Y. Pei, L. Xiong, L. Hou, G. Wang, Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model, *Shock* (2010), doi:10.1097/SHK.0b013e3181def9aa.
- [28] T. Yamasoba, A. Pourbakht, T. Sakamoto, M. Suzuki, Ebselen prevents noise-induced excitotoxicity and temporary threshold shift, *Neurosci. Lett.* 380 (2005) 234–238.
- [29] X. Zheng, Y. Mao, J. Cai, Y. Li, W. Liu, P. Sun, J.H. Zhang, X. Sun, H. Yuan, Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats, *Free Radic. Res.* 43 (2009) 478–484.