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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.

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 (•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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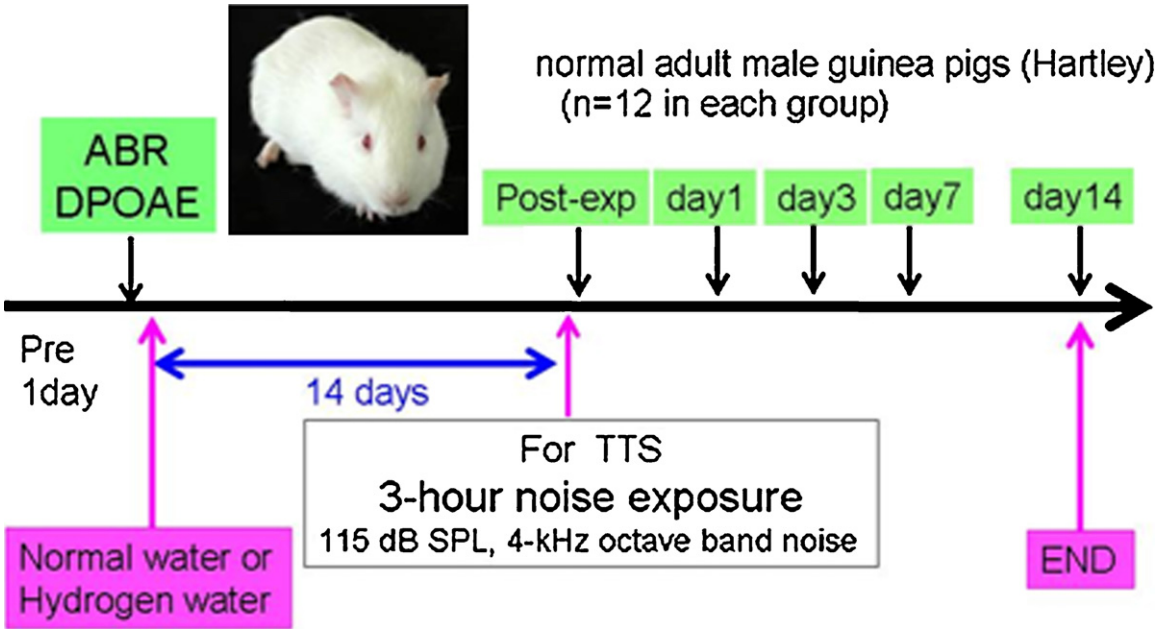

Fig. 1. Schedule of the experiment procedures.

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 ±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 μV.

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.