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Post-exposure administration of edaravone attenuates noise-induced hearing loss

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Abstract

We investigated the effects of the antioxidant edaravone against acoustic trauma in guinea pigs. Edaravone (1.722×10^{-2} M) was infused into the right ear by an osmotic pump, and the left ear was untreated for control. Animals received edaravone 9 h before (−9 h group, $n=7$) and 9 h (+9 h group, $n=8$), 21 h (+21 h group, $n=7$) and 33 h (+33 h group, $n=4$) after 3-h exposure to 130-dB noise. Seven days after noise exposure, we examined the shift in auditory brainstem response thresholds and histopathologic characteristics of the sensory epithelia. The smallest shift in auditory brainstem response threshold and smallest proportion of missing outer hair cells were observed in the +9 h group. This result was supported by immunohistochemical analysis of 4-hydroxy-2-nonenal. Our data suggest that edaravone may be clinically effective in the treatment of acoustic trauma, especially if given within 21 h of noise exposure.
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Keywords: Edaravone; Cochlear protection; Antioxidant; Reactive oxygen species; Acoustic trauma; 4-HNE(4-hydroxy-2-nonenal)

1. Introduction

Noise exposure leads to increased levels of reactive oxygen species in the cochlea (Yamane et al., 1995), and noise-induced hearing loss can be reduced by treatment with antioxidants (Yamasoba et al., 1999; Kopke et al., 2000; Ohinata et al., 2000, 2003; McFadden et al., 2001; Lynch et al., 2004). However, these drugs have not been used in clinical settings.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-1) is the first free-radical scavenger used in clinical practice in Japan, where it is used to treat acute cerebral infarction (Yamamoto et al., 1997). There are some studies showing an effect of edaravone on the inner ear (Horiike et al., 2003, 2004; Maetani et al., 2003; Takemoto et al., 2004). Takemoto et al. (2004) reported that pre-exposure perilymphatic application of edaravone reduced noise-induced hearing loss in guinea pigs. Thus, in the present study, we administered edaravone to guinea pigs before and after noise exposure, and investigated the optimal timing for administration of edaravone to protect the cochlea. In addition, at various time points after noise exposure we measured levels of 4-hydroxy-2-nonenal (4-HNE), which is a phospholipid peroxidation product generated by the reaction of free radicals with the plasma membrane.

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2. Materials and methods

2.1. Animals

We used 34 male Hartley guinea pigs (300–450 g each; Chiyoda, Tokyo, Japan) with normal Preyer's reflexes and normal tympanic membranes. Twenty-six animals were divided into four treatment groups as described below, and 8 animals were used for immunohistochemical analysis of 4-HNE, also described below. This experiment was reviewed by the Committee for the Ethics of Animal Experiments in Yamaguchi University School of Medicine and carried out according to the Guideline for Animal Experiments of Yamaguchi University School of Medicine and The Law (No. 105) and Notification No. 6 of the Japanese Government.

2.2. Pump implantation

An osmotic pump (Model 2002, Alza Co., Palo Alto, CA, USA) filled with saline was implanted in the right ear of 26 guinea pigs, and the left ear was kept intact as a control. The pigs were anesthetized with a mixture of ketamine (16 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.). After hypodermic injection of 1.5 ml 1% lidocaine, the temporal bone was exposed via postauricular incision. The mastoid bulla was opened with a 4-mm diamond burr to allow visualization of the round window. A tiny hole was made with a needle at a distance of 1 mm from the round window. The tip of the catheter was inserted into the hole, and saline was infused into the perilymphatic space of the cochlea. The polyethylene catheter was fixed to the mastoid bulla with dental cement (GC Fuji I, GC Co., Tokyo, Japan). The skin incision was closed and treated with antibiotic. The flow rate of the pump was 0.5 l/h. The osmotic pump was connected to a 10-cm polyethylene catheter (inner diameter = 0.28 mm, outer diameter = 0.61 mm; Becton Dickinson, Franklin Lakes, NJ, USA) and a 1-mm Teflon catheter (inner diameter = 0.18 mm, outer diameter = 0.3 mm; Unique Medical, Tokyo, Japan). The pump and catheter were filled with saline.

2.3. Pump exchange

The implanted osmotic pump was replaced by another pump filled with 1.722×10^{-2} M edaravone (Mitsubishi Pharma Co., Osaka, Japan), the concentration used in a previous in vivo study (Yamamoto et al., 1997). Edaravone was dissolved in 1 mol/l NaOH and water just prior to use, and the pH was adjusted to 7 with 1 mol/l HCl. The pumps were replaced under xylazine and ketamine general anesthesia. After hypodermic injection of 1.5 ml 1% lidocaine, an incision was made on each animal's back, and the saline-filled osmotic pump was replaced by an edaravone-filled pump. After the incision was closed, antibiotic ointment was applied to the incision site. The catheter was designed to begin administration of edaravone to the cochlear perilymph 12 h after pump exchange. In one group of animals, pumps were changed 21 h before noise exposure, and edaravone administration was started 9 h before noise exposure (−9 h group, $n = 7$). In another group, pumps were changed immediately before noise exposure, and edaravone administration was started 9 h after noise exposure (+9 h group, $n = 8$). In a third group and a fourth group, pumps were changed 9 and 21 h, respectively, after noise exposure, and edaravone administration was started 21 and 33 h, respectively, after noise exposure (+21 h group, $n = 7$, and +33 h group, $n = 4$, respectively).

2.4. Noise exposure

Guinea pigs under pentobarbital anesthesia (33 mg/kg, i.p.) were exposed to intense (130 dB sound pressure level) band noise centered at 4 kHz for 3 h. The noise we used was designed to cause permanent threshold shifts and to damage cochlear hair cells at the basal end of the second turn (Yamasoba et al., 1999). Each animal was immobilized, and a speaker was centered over the animal's head at a distance of 15 cm. The sound intensity was monitored with a sound-level meter (NA-60, Rion, Tokyo, Japan) positioned near the external auditory canal.

2.5. Auditory brainstem responses

The auditory brainstem response threshold was examined in guinea pigs under xylazine (16 mg/kg, i.p.) and ketamine (16 mg/kg, i.p.) anesthesia 3 days after pump implantation and 7 days after noise exposure. Responses were recorded between subcutaneous stainless steel electrodes located at the vertex (positive) and antinion (negative); the lower back served as the ground. The sound stimuli consisted of 2-, 4-and 8-kHz tone bursts (rise–fall time 2 ms, duration 4 ms). Stimuli were presented through a 10-cm-long tube that connected an earphone to the external auditory canal. The stimulus intensity was evaluated with a NA-60 sound-level meter adjacent to the tip of the tube. Responses to 500 stimuli were recorded with a signal processor (Synax 1100, NEC Co., Tokyo, Japan). The auditory brainstem response threshold was defined as the lowest stimulus intensity that produced a reliable waveform of 3–5 peaks.

2.6. Histopathology

One week after noise exposure, auditory brainstem response thresholds were recorded, and the 26 guinea pigs were killed with an overdose of pentobarbital. Both temporal bones of each animal were removed. The cochlea was opened at the apex, base and oval window and perfused with fixative (4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS), pH 7.3) gently for 12 h. The cochlea was rinsed in PBS, and the organ of Corti in the second turn of the cochlea was removed. The specimen was permeabilized with 0.3% Triton X-100 (Katayama Chemical Inc., Osaka, Japan) for 10 min and subsequently incubated with fluorescein isothiocyanate-conjugated phalloidin (1 : 50 dilution; Sigma, St. Louis, MO, USA) at room temperature for 1 h. The specimen was rinsed in PBS and then mounted using a SlowFade Light Antifade Kit (Molecular Probes, Eugene, OR, USA). The surface structure of each specimen was observed under a fluorescence microscope (Zeiss, Jena, Germany). The missing inner hair cells and outer hair cells in the second turn of the cochlea were counted, and the percentage of missing outer hair cells was recorded.

2.7. Immunohistochemistry for 4-HNE

We examined 4-HNE production in animals that did not receive edaravone. Animals were exposed to the same experimental noise conditions and killed at three different time points (immediately, 9, and 21 h) after noise exposure ($n = 2$ each group). Two animals that were unexposed served as controls ($n = 2$). The temporal bones were removed under deep pentobarbital anesthesia and transferred to 4% paraformaldehyde as described above and kept in fixative overnight. The tissue was decalcified in 5% ethylene diamine tetraacetic acid (pH 7.2) for approximately 14 days. The lateral walls of the cochleae were removed with a micromanipulator.

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