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European Journal of Pharmacology 487 (2004) 113–116
www.elsevier.com/locate/ejphar

The clinical free radical scavenger, edaravone, protects cochlear hair cells from acoustic trauma

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Received 4 September 2003; received in revised form 15 January 2004; accepted 21 January 2004

Abstract

It is known that reactive oxygen species have toxicity to the cochlea. We investigated the effect of edaravone, a free radical scavenger for clinical use, on the cochleae of guinea pigs subjected to acoustic trauma. We assessed auditory brainstem response (ABR) thresholds to evaluate cochlear function and observed the sensory epithelium. After noise exposure (130 dB SPL, 3 h), we observed that the auditory brainstem response threshold shift in edaravone-treated ears was significantly less than that in untreated ears. This result suggests that edaravone protected the cochleae from acoustic trauma.

Keywords: Edaravone; Reactive oxygen species; Cochlear protection; Noise trauma

1. Introduction

Substantial evidence suggests that reactive oxygen species are derived from acoustic trauma and are a cause of cochlear damage (Kaygusuz et al., 2001; Kopke et al., 2000; Yamasoba et al., 1999). Some studies reported that free radical scavengers administered systemically or topically played an important role in protecting the cochlea (Tabuchi et al., 1998, 2001). Although many studies show that free radical scavengers protect tissues from oxidative stress, these drugs have not been used in clinical settings. Edaravone is the first free radical scavenger to be used clinically in Japan, where it is used for treatment of cerebral infarction in the acute phase (Yamamoto et al., 1997). There have been some studies about the effect of edaravone on the central nervous system. About the effect of edaravone on the inner ear, Horiike et al. (2003) reported that edaravone suppressed streptomycin-induced vestibulotoxicity in the guinea pig. But there is no report of its effects on the cochlear function. Thus, we conducted an animal study assessing the protective effect of edaravone on the cochlea.

2. Materials and methods

2.1. Animals

Six Hartley guinea pigs (350–400 g) with normal Preyer's reflexes and normal tympanic membranes were used in this study. The experimental protocol was reviewed by the Yamaguchi University School of Medicine. Experiments were carried out in accordance with these guidelines and Japanese Federal Law (no. 105) and Notification No. 6 of the Japanese Government.

2.2. Pump implantation

Osmotic pumps (Model 2002; Alza, Palo Alto, CA, USA) were implanted in the right ears of all animals, and the left ears were kept intact as controls. The flow rate of these pumps were 0.5 μl/h. The osmotic pumps were connected to a 10-cm polyethylene catheter (ID = 0.28 mm, OD = 0.61 mm) and a 1-mm-long Teflon catheter (ID = 0.18 mm, OD = 0.3 mm; Unique Medical, Tokyo, Japan). The pumps and catheters were filled with saline. Each guinea pig was anesthesized with a mixture of ketamine (16 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.). Under hypodermic injection of 1.5 ml of lidocaine, the temporal bone was exposed via a postauricular

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doi:10.1016/j.ejphar.2004.01.019

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, GC, Tokyo, Japan). After the skin incision was closed, antibiotic ointment was applied on the skin incision.

2.3. Auditory brainstem response (ABR) examination

Auditory brainstem response thresholds of all animals were assessed under xylazine (16 mg/kg, i.p.) and ketamine (16 mg/kg, i.p.) anesthesia 3 days after the operation. Responses were recorded between subcutaneous (s.c.) stainless steel electrodes located at the vertex (positive) and antinion (negative); the lower back served as ground. The sound stimuli consisted of 2–8 kHz tone bursts (rise–fall time 2 ms, duration 4 ms). Stimuli were presented through a 10-cm-long tube connecting an earphone to the external auditory canal. The stimulus intensity was evaluated with a sound-level meter (NA-60; Rion, Tokyo, Japan) adjacent to the tip of the tube. Responses to 500 stimuli were recorded using a signal processor (Synax 1100; NEC, Tokyo, Japan). Auditory brainstem response thresholds were defined as the lowest stimulus intensity that produced a reliable peak 3 or 5 in ABR waveforms.

2.4. Pump exchange

Immediately after auditory brainstem response examination, their osmotic pumps were exchanged with other pumps filled with edaravone (1.722×10^{-2} M). This concentration was previously used in an in vivo study (Yamamoto et al., 1997). Edaravone was dissolved in NaOH, then water was added and adjusted to pH 7 with HCl. The operations of pump exchange were performed under 1.5 ml of lidocaine local anesthesia, in addition to xylazine and ketamine anesthesia. Skin incisions were made on their back and their osmotic pumps were exchanged with other pumps filled with edaravone. After the skin incision was closed, antibiotic ointment was applied to it. The catheter we utilized was designed to be used 12 h after the pump exchange administration of edaravone was started, and the osmotic pump we used was designed so that the filling was administered for 2 weeks.

2.5. Noise exposure

Twenty-four hours after the exchange, the guinea pigs were exposed to intense (130 dB SPL) noise with a center frequency of 4 kHz for 3 h under pentobarbital anesthesia (33 mg/kg, i.p.). Animals were fixed, and a speaker was set on the center of each animal's head. The distance of the speaker from the head of the animal was 15 cm. The sound intensity was monitored near each external auditory canal by a sound-level meter (NA-60; Rion).

2.6. Histological analysis

Two weeks after sound exposure, auditory brainstem response thresholds were recorded and all animals were anesthesized and killed with an overdose of pentobarbital. The temporal bones of all animals were removed. Each cochlea was opened at the apex, base, and oval window, and fixative [4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS), pH 7.3] was perfused gently for 12 h. After rinsing in PBS, the organ of Corti of each inner ear was removed. The specimens were permeabilized with 0.3% Triton X-100 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. After being rinsed in PBS, specimens were mounted using the Slow-Fade Light Antifade Kit (Molecular Probes, Eugene, OR, USA). The surface structure of specimens was observed under a fluorescence microscope (Nikon, Tokyo, Japan), and we counted the missing outer hair cells of cochlea and presented them as percentages of defects of outer hair cells.

2.7. Statistical analysis

Data of auditory brainstem response thresholds and the percentage of hair cells were analyzed with StatView version 4.5 J for Macintosh (Abacus Concepts, Berkley, CA, USA). The auditory brainstem response thresholds of all animals were calculated at preexposure and postexposure. The differences between the treated ears and the untreated ears were compared with Mann–Whitney U test used to determine significant values. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Auditory brainstem response thresholds

The auditory brainstem response thresholds before sound exposure and after sound exposure are shown in Fig. 1. There is no difference between the thresholds of treated ears and that of control ears before sound exposure. After exposure, auditory brainstem response thresholds of all animals increased. The 8-kHz threshold shifts in treated ears were significantly less than those of the control ears. This indicates that the edaravone infused into the cochleae suppressed threshold shifts after sound exposure.

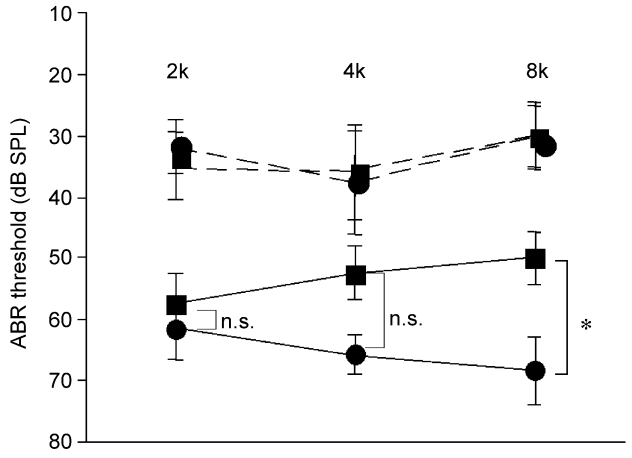

Fig. 1. Auditory brainstem response thresholds of edaravone-treated ears (closed squares) and control ears (closed circles). Broken lines indicate the auditory brainstem response thresholds before sound exposure, and straight lines indicate thresholds at 2 weeks after sound exposure. Error bar ± 1 S.E.M. (*$P < 0.05$).

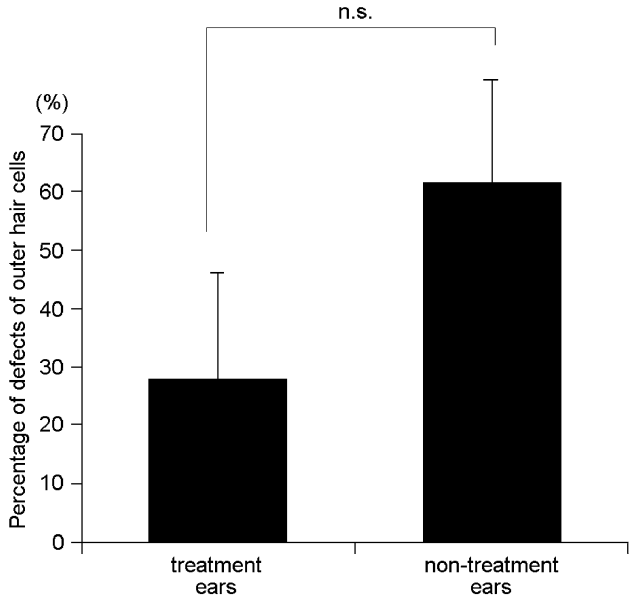

Fig. 3. Percentages of defects of outer hair cells 2 weeks after sound exposure. In edaravone-treated ears, the proportion of defects of outer hair cells is less than that in control ears. Error bar ± 1 S.E.M.

3.2. Deficiency of outer hair cells

Fig. 2 shows the surface structure of the organ of Corti (cochlear second turn). There are three rows of outer hair cells and a single row of inner hair cells. All animals showed defective outer hair cells on the cochlear second turn after sound exposure. There were fewer defects in the outer hair cells of the treated ears than of the control ears. Inner hair cells of both groups were relatively well conserved. We counted the missing outer hair cells of the cochlear second turn, and presented them as percentages of defects of outer hair cells (Fig. 3). The proportion of defective outer hair cells in treated ears was less than that in the control group.

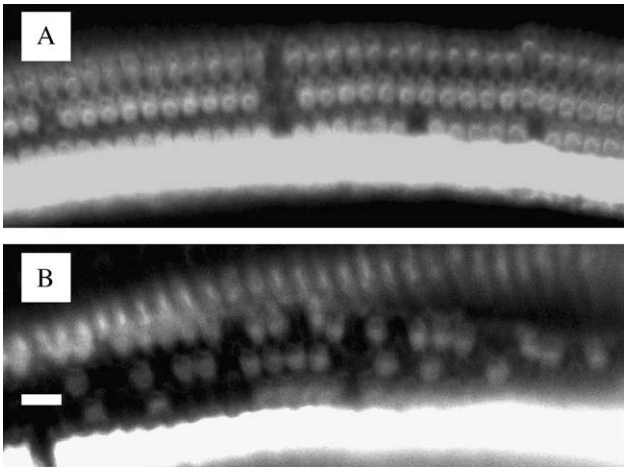

Fig. 2. Surface structure of the organ of Corti 2 weeks after sound exposure. Fluorescence microscopy reveals that the defects of the outer hair cells of edaravone-treated ears (A) appear less than those of control ears (B). Bar = 20 μm.

4. Discussion

In an earlier study, we used an osmotic pump to deliver intracochlear infusion, and we assessed the effects of several infused drugs (Shimogori et al., 1998; Shimogori and Yamashita, 2000). Sugahara et al. (2001) reported that the implantation of an osmotic pump and intracochlear infusion of saline had little influence on auditory brainstem response threshold shift. In this study, we measured auditory brainstem response threshold 3 days after operation, and confirmed that there was no postoperative threshold shift in any of the animals.

In this study, we investigated the efficacy of a free radical scavenger, edaravone, administered directly into the cochlea. It is known that histological or functional damage of the cochlea results from reactive oxygen species (Kaygusuz et al., 2001; Kopke et al., 2000; Yamasoba et al., 1999), but the pathway of injury is not sufficiently understood. We investigated the functional and histological protection against acoustic trauma offered by edaravone. Some pathways of reactive oxygen species production that have been suggested are lipid oxidation, protein oxidation, cytoskeletal protein damage, and DNA damage (Kopke et al., 1999; Pirvola et al., 2000; Yamasoba et al., 1999; Ylikoski et al., 2002). We assumed the pathway of lipid oxidation at the cell membrane phospholipids to be the target for edaravone. We hypothesized that edaravone inhibits lipoxygenase

activity. The decrease of lipoxygenase activity inhibits arachidonic cascades, resulting in reduction of reactive oxygen species. Some studies report that edaravone reduces endothelial cell injury by reactive oxygen species (Ochi et al., 1992; Watanabe et al., 1988). These studies used an increasing agent (15-hydroperoxyeicosatetraenoic acid, 15-HPETE) of reactive oxygen species that is a product from arachidonic acid (Weaver et al., 2001), and they said that edaravone protected the endothelial cells of 15-HPETE-induced injury. Therefore, we think that when edaravone inhibits lipoxygenase, 15-HPETE is decreased and reactive oxygen species injury is diminished.

This present study showed that the ABR threshold shifts of treated ears were significantly smaller than those of control ears at high frequencies 14 days after sound exposure, but histological examination did not reveal that the cochlear outer hair cells of treated ears were protected significantly from acoustic overstimulation. This evidence confirmed that the intracochlear infusion of edaravone by osmotic pump protected cochleae from acoustic trauma functionally and histologically. The auditory brainstem response thresholds in our animal model increased significantly in both groups 14 days after acoustic trauma, and all animals showed defective outer hair cells on the cochlear second turn after sound exposure because we designed the noise we used to damage cochlear hair cells at the basal end of the second turn (Yamasoba et al., 1999). Sugahara et al. (2001) reported the temporary threshold shifts model in guinea pigs exposed to intense sound of 120 dB SPL for 5 h. In this study, we exposed our animals to a sound of 130 dB SPL for 3 h using the same system to cause permanent threshold shifts. Our study revealed that a free radical scavenger, edaravone, inhibited the worst of the permanent threshold shifts and defects of outer hair cells in guinea pig cochleae, confirming that edaravone protects cochleae from reactive oxygen species injury.

Acknowledgements

This work was supported by a grant from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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