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Short communication

Protective effect of edaravone against streptomycin-induced vestibulotoxicity in the guinea pig

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Abstract

This study investigated alleviation of streptomycin-induced vestibulotoxicity by edaravone in guinea pigs. Edaravone, a free radical scavenger, has potent free radical quenching action and is used in clinical practice to treat cerebral infarction. Streptomycin was administered to the inner ear by osmotic pump for 24 h, and edaravone ($n=8$) or saline ($n=6$) was intraperitoneally injected once a day for 7 days. We observed horizontal vestibulo-ocular reflex as a marker of postoperative vestibular function. Animals injected with saline showed statistically smaller gains than those injected with edaravone. These results suggest that edaravone suppresses streptomycin-induced vestibulotoxicity.

Keywords: Edaravone; Free radical scavenger; Horizontal vestibulo-ocular reflex; Osmotic pump; Streptomycin; (Guinea pig)

1. Introduction

Many drugs are known to be toxic to the inner ear. Aminoglycoside antibiotics enjoy widespread clinical use as highly effective antimicrobial agents. However, a serious limitation to the use of these drugs is their side effect of ototoxicity (loss of hearing or balance). Streptomycin causes preferential damage to the vestibular system with little auditory involvement (Song et al., 1998; Sugasawa et al., 2001; Wanamaker et al., 1999). Free radical scavengers have been shown to protect against aminoglycoside-induced ototoxicity (Nakagawa et al., 1999; Sha and Schacht, 2000; Song et al., 1998; Takumida and Anniko, 2002). Studies show that edaravone is a free radical scavenger and has potent free radical quenching action (Watanabe et al., 1998, 1994; Yamamoto et al., 1997). In the present study, we investigated the protective action of edaravone against vestibulotoxicity from streptomycin.

2. Materials and methods

2.1. Animals

Experiments were performed on 14 male Hartley guinea pigs (380–565 g) with normal Preyer's reflexes and tympanic membranes. The experimental protocol was approved by the Committee for Ethics in Animal Experiments of the Yamaguchi University School of Medicine. Experiments were carried out under the Guidelines for Animal Experiments of the Yamaguchi University School of Medicine and the Law and Notification of the Government of Japan.

2.2. Evaluation of vestibular function

Before osmotic pump implantation, we calculated horizontal vestibulo-ocular reflex gains using our method (Horiike et al., 2002). For the purpose of immobilizing the guinea pig, a cage designed to hold the animal still during experiments was mounted on top of a turntable apparatus (Daiichi Medical, Tokyo, Japan). The cage was of two parts, one for the head and the other for the body, and the two parts were joined. The two-part construction

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was intended to remove as much influence of the animal's body movements during sinusoidal rotation as possible. The animal's head was fixed firmly with both auricles held between sponge-covered plates that held both acoustic meati horizontally such that the midpoint of a straight line joining the lateral semicircular canals was located on the rotation axis of the turntable. We set up the infrared CCD camera (Nagashima Medical, Tokyo, Japan) perpendicular to the sagittal plane of the guinea pig's head and in a plane parallel to the rotational plane of the turntable apparatus. By opening an aperture on the left side of the head cage, eye movements of guinea pigs were videotaped (Hi8 format, Sony, Tokyo, Japan) in the dark with the infrared CCD camera. We stored the video images on a computer (Power Mac G4, Apple Computer, CA, USA). Each image was converted to an image file with QuickTime 4.0 optional (Apple Computer). For automatic analysis of guinea pig eye movement, we created a macro for use with the National Institutes of Health (NIH) Image analysis software (http://rsb.info.nih.gov/nih-image/). Our macro is available at http://www.cc.yamaguchi-u.ac.jp/~ent/gankyu3d/ikeda.html. After capturing eye movement on the computer with this macro, we removed unnecessary portions of the images, and set the threshold to provide for clear outlines of the pupil. The X–Y center of the pupil was analyzed, and the horizontal and vertical components of eye movements were calculated. We calculated slow-phase velocities, found the maximum slow-phase velocity, and calculated the horizontal vestibulo-ocular reflex gain by dividing the maximum slow-phase velocity by the peak angular velocity.

2.3. Pump implantation

Under pentobarbital anesthesia (28 mg/kg, i.p.), 1.5 ml of lidocaine HCl was injected into the right postauricular region of each guinea pig, and the mastoid bulla was opened by a postauricular incision to allow visualization of the round window under a surgical microscope. A tiny hole was made adjacent to the round window with a perforating burr. A catheter filled with streptomycin (30%, dissolved in saline; Meiji Seika Kaisha, Tokyo, Japan) and connected to an osmotic pump (Model 2002, Alza, Palo Alto, CA, USA) was then inserted. The pump was placed under the skin on the back. The pump ran continuously for 24 h, injecting 0.5 μl/h; next, 3.6 μg of streptomycin was injected over 24 h into the inner ear. After the wound was washed with saline, a small amount of piperacillin sodium was introduced. After wound closure, piperacillin sodium at a dose of 40 mg/kg was injected i.m., and oxytetracycline HCl ointment was applied to the wound. During the operation and for 24 h following the operation, each animal was kept warm with an electric blanket. In our previous study, vestibular function after the implantation of an osmotic pump and the infusion of saline was within the preoperative range (Shimogori and Yamashita, 2000; Shimogori et al., 1999), and auditory function was in the same way (Sugahara et al., 2001).

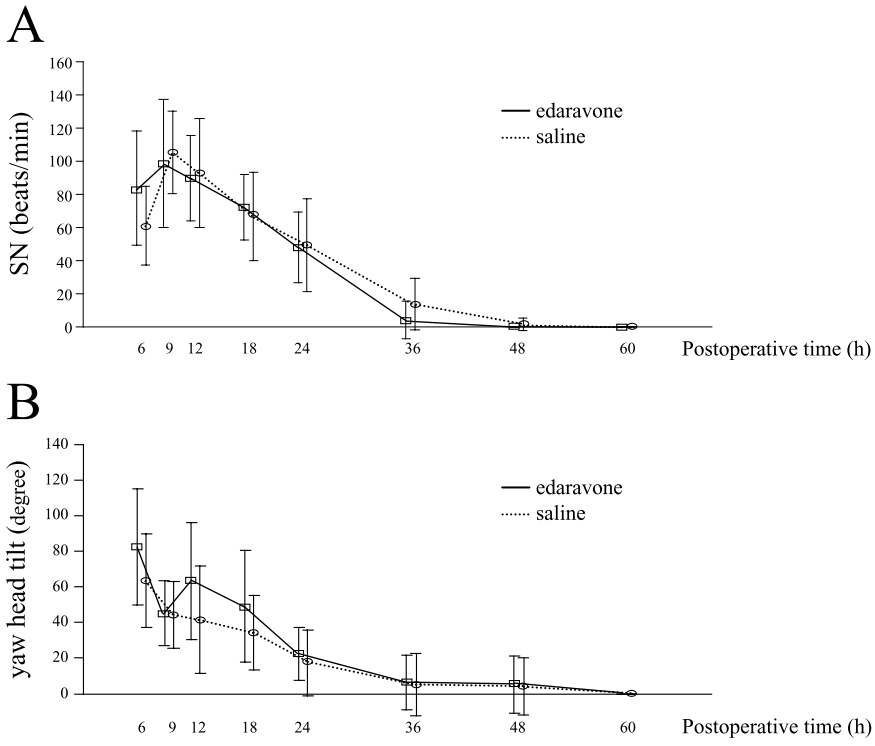

Fig. 1. (A) Spontaneous nystagmus (SN) after the operation. (B) Yaw head tilt after the operation. Error bar, ± 1 S.D.

2.4. Drug administration

These animals were divided into two groups. Eight animals of the fourteen received edaravone (Mitsubishi Pharma, Tokyo, Japan) at a dose of 3 mg/kg i.p. once a day for 7 days after the operation (edaravone group). Edaravone was dissolved in 1 N NaOH, and the pH was adjusted to 7.0 with 1 N HCl. The remaining six animals received the same amount of saline i.p. in the same manner (control group).

2.5. Statistical analysis

At 6, 9, 12, 18, 24, 36, 48 and 60 h after the operation, we measured spontaneous nystagmus beats per min and yaw head tilt (Curthoys et al., 1988). We measured the gains with sinusoidal rotation at 0.1 Hz and a peak angular velocity of 60°/s before the operation and at 3 and 7 days postoperatively. We calculated the gain ratio by dividing the postoperative gain by the preoperative gain. The Mann–Whitney *U*-test was used to assess differences between the two groups with significance set at $P<0.05$. All data are the means ± S.D. for the two groups.

3. Results

3.1. Spontaneous nystagmus and yaw head tilt

No statistical difference in postoperative spontaneous nystagmus or yaw head tilt was found between the two groups. In both groups, the peak of spontaneous nystagmus was observed at 9 h after the operation, and spontaneous nystagmus disappeared within 60 h after the operation, additionally, yaw head tilt also disappeared within 60 h after the operation (Fig. 1A,B).

3.2. The gain ratio

In the edaravone group, gains on the intact side preoperatively, 3 days postoperatively and 7 days postoperatively were 0.383 ± 0.081 (mean ± S.D.), 0.252 ± 0.09 and 0.241 ± 0.067, respectively, and those on the lesioned side were 0.378 ± 0.08, 0.204 ± 0.085 and 0.216 ± 0.053, respectively. In the control group, gains on the intact side were 0.377 ± 0.073, 0.177 ± 0.06 and 0.211 ± 0.048, respectively, and those on the lesioned side were 0.443 ± 0.066, 0.09 ± 0.045 and 0.137 ± 0.059, respectively. At 3 and 7 days after the operation, statistical differences in the gain ratio on the lesioned side were found between the edaravone and control groups (day 3, 0.575 ± 0.333 vs. 0.202 ± 0.086, $P<0.01$; day 7, 0.589 ± 0.149 vs. 0.308 ± 0.124, $P<0.05$) (Fig. 2).

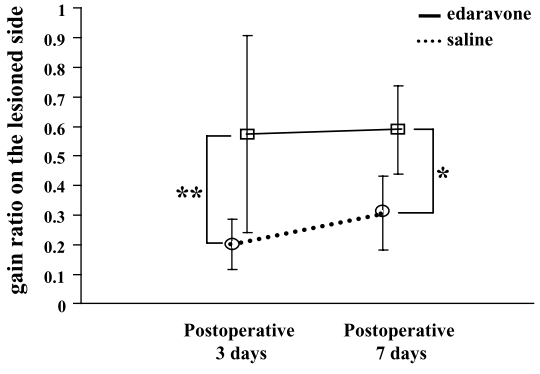

Fig. 2. The gain ratios on the lesioned side at 3 and 7 days after the operation. Error bar, ± 1 S.D., **$P<0.01$, *$P<0.05$.

4. Discussion

The gain ratios on the lesioned side in the control group in this study were equivalent to those in the hemilabyrinthectomized guinea pigs in the study by Vibert et al. (1993). Additionally, the S.D. of the gain ratio on the lesioned side at 3 days in the control group was small, indicating that our method may produce stable vestibular disorder (Fig. 2). The S.D. of the gain ratios on the lesioned side at 7 days after the operation were almost equal between the groups, which might be thought that central vestibular compensation led to the correction of vestibular imbalance in all animals (Smith and Curthoys, 1989).

Evidence is accumulating that generation of reactive oxygen species is an important factor in inner ear damage (Nakagawa et al., 1999; Sha and Schacht, 2000; Song et al., 1998; Tabuchi et al., 2001; Takumida and Anniko, 2002). The hydroxyl radical is one of reactive oxygen species and an extremely powerful oxidant that is indiscriminately reactive with almost all biological substances (Tabuchi et al., 2001). It is therefore suspected that the tissue damage induced by reactive oxygen species is mainly due to hydroxyl radicals. It has been reported that edaravone is capable of directly trapping a variety of free radical species and reacts with hydroxyl or peroxyl radicals to form some oxidized compounds (Watanabe et al., 1998, 1994; Yamamoto et al., 1997). As stated earlier, inner ear damage caused by aminoglycoside is ameliorated by the presence of free radical scavengers (Sha and Schacht, 2000; Sinswat et al., 2000; Song et al., 1998; Takumida and Anniko, 2002). However, we cannot find any report about the effects of edaravone on the inner ear in vivo and in vitro. In this study, the gain ratios of the edaravone group on the lesioned side, which were significantly larger than those of the control group at 3 and 7 days after the operation (Fig. 2). From this evidence, it appears that the free radical scavenging action of edaravone may contribute to its protective effect against streptomycin-induced vestibulotoxicity in the guinea pig.

Edaravone showed no effect on reducing spontaneous nystagmus or yaw head tilt. Concerning this, we cannot find any previous report showing the correlation between spon-

taneous nystagmus, yaw head tilt and horizontal vestibulo-ocular reflex after vestibular disorder. However, we expect that the gain may be a good indicator to evaluate vestibular function, rather than spontaneous nystagmus or yaw head tilt that is static symptom. Horizontal vestibulo-ocular reflex is induced by rotation stimulation, indicating that this reflex is dynamic and more physiological phenomenon after vestibular disorder (Curthoys et al., 1988).

We conclude that edaravone may improve streptomycin-induced balance loss. We expect further studies to evaluate the efficacy of edaravone against cochleotoxicity and vestibulotoxicity caused by many drugs, including aminoglycoside antibiotics, and edaravone be useful in vestibular disease therapy.

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