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Main Article

Protective effect of edaravone in inner-ear barotrauma in guinea pigs

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Abstract

Objective: The purpose of this study was to determine the protective effect of edaravone, a free radical scavenger, on inner-ear barotrauma (IEB) in guinea pigs, based on a hypothesis implicating free radicals in the development of IEB.

Materials and methods: One hundred and twenty-five guinea pigs were divided into a control group and a pretreatment group. After auditory brainstem response (ABR) testing, the pretreatment group received 9.0 mg/kg intraperitoneal edaravone. Animals were exposed to pressure loading and then to further ABR testing.

Results: The incidence of IEB was 62.7 per cent in the control group and 42.9 per cent in the pretreatment group ($p < 0.01$). The distributions of threshold elevation in the control group were 37.3 per cent (for 10 dB or less), 21.3 per cent (for 20–30 dB), 18.0 per cent (for 40–60 dB) and 23.4 per cent (for 70 dB or more), and those in the pretreatment group were 57.1 per cent, 19.1 per cent, 14.3 per cent and 9.5 per cent, for the same respective decibel levels ($p < 0.01$).

Conclusions: These results suggest that protective treatment with edaravone can significantly reduce both the incidence of IEB and the severity of the resultant ABR threshold elevation.

Key words: Barotrauma; Inner Ear; Free Radical Scavengers; 3-methyl-1-phenyl-2-pyrazolin-5-one; Edaravone

Introduction

Inner-ear barotrauma (IEB), which is related to pressure changes in the middle and inner ear, can produce permanent and disabling injury to the cochleovestibular system. Many authors have attempted to determine its aetiology; however, no clear mechanism has been established. Four hypotheses have been advocated: (1) haemorrhage within the inner ear;[1] (2) rupture of Reissner's membrane;[2] (3) round or oval window fistula;[3] and (4) a mixed injury consisting of one, two, or three of these entities.[4] All of these hypotheses appear relevant; however, they are focused on morphological change, not a metabolic mechanism, which may include the effect of free radicals.

Recently, free radicals have been proved to be implicated in several inner-ear disorders, such as cisplatin ototoxicity,[5] noise-induced hearing loss,[6] gentamicin-induced oto-vestibular toxicity,[7] Ménière's disease,[8] cochlear ischaemia[9] and lipopolysaccharide-induced labyrinthitis.[10] Furthermore, several free radical scavengers have been proved to give effective protection.[9,11,12] However, no author has investigated the role of free radicals in IEB.

We therefore hypothesized that free radicals may play a role in IEB. To investigate this hypothesis, we assessed the protective effect of edaravone (MCI-186), a novel free radical scavenger, on IEB in guinea pigs.

Materials and methods

Experiments were performed in 125 healthy guinea pigs weighing between 300 and 350 g, with normal Preyer reflexes. The animals were divided into a control group of 90 animals and a pretreatment group of 35 animals. The experiments were carried out under the guidelines for animal experiments of the Japanese National Defense Medical College and the law and notification requirements of the government of Japan.

Methods

All steps were performed under general anaesthesia using intraperitoneal injections of ketamine (55 mg/kg) and medetomidine (1.0 mg/kg). Before the pressure loading, auditory brainstem response (ABR) testing was performed using a signal recorder

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(Synax 1200, NEC, Tokyo, Japan). The auditory stimulus was a click at 4 kHz. One hundred sweeps were recorded and averaged per stimulus. Auditory brainstem response threshold was defined from 90 to 20 dB in 10 dB steps, as determined by the presence of wave form one. Guinea pigs with a normal ABR threshold (i.e. less than 40 dB) were entered into the experiment. We thus had available 150 ears in the control group and 63 ears in the pretreatment group.

Drug administration

The animals in the pretreatment group received edaravone (MCI-186, Mitsubishi Pharma Corporation, Tokyo, Japan) at a dose of 9.0 mg/kg intraperitoneally 15 minutes before pressure-loading. The edaravone was dissolved in 1 N NaOH and the pH was adjusted to 7.0 with 1 N HCl just before administration. The animals in the control group received no pretreatment.

Pressure-loading

One week after ABR testing, all animals were exposed to pressure change sufficient to induce IEB. To limit the movement of the tympanic membrane, which makes IEB more likely, ear plugs were inserted into the external auditory canal of the animals, under general anaesthesia. The guinea pigs were then placed within the animal chamber (Haniuda P-5100, Haniuda Tekko, Tokyo, Japan) (Figure 1).

The animal chamber can produce pressure change within the cage. When the inflow bulb is opened, compressed air flows into the chamber, producing a pressure increase. When the outflow bulb is opened, the air in the chamber is released, producing a pressure decrease. In this study, the pressure was increased from one absolute pressure (ATA) to two ATA in three seconds. Then, the pressure was released from two ATA to one ATA in two seconds (Figure 2).

After pressure exposure, the ear plugs were removed immediately. Auditory brainstem response testing was then performed again in the same manner to evaluate threshold elevation.

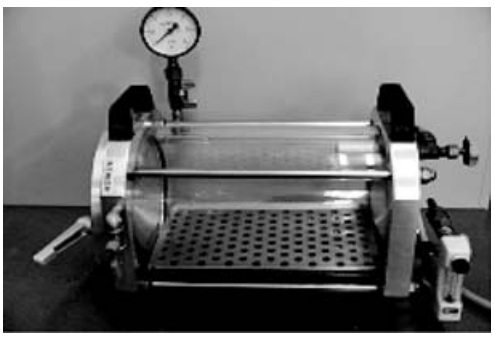

FIG. 1

The animal chamber, within which atmospheric pressure can be changed, producing an inner-ear barotrauma model in guinea pigs.

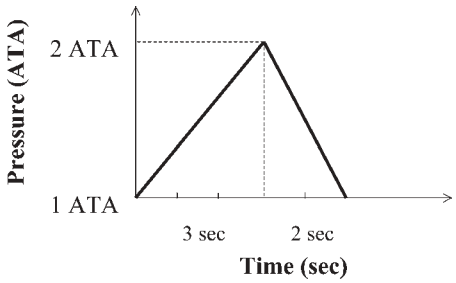

FIG. 2

The pressure change produced in the chamber to induce inner-ear barotrauma.

When wave form one was not found, even at maximum amplitude (90 dB), the threshold was deemed unresponsive.

Finally, we evaluated the incidence of IEB and the distribution of ABR threshold elevation due to IEB, within the two groups. The presence of IEB was determined by threshold elevation of more than 10 dB. Threshold elevation was divided into 10 dB or less (i.e. none), 20–30 dB (mild), 40–60 dB (moderate) and 70 dB or more (severe).

The statistical significance of the differences between the two groups was assessed by Fischer's exact probability test for the incidence of IEB and Mann–Whitney's U test for the distribution of threshold elevation due to IEB, using Stat View 5.0 software (SAS Institute, Cary, NC, USA). A p value of less than 0.01 was considered significant.

Results

Incidence of inner-ear barotrauma

The incidence of IEB in the control group was 62.7 per cent (94/150 ears). In contrast, that of the pretreatment group was 42.9 per cent (27/63 ears), statistically significantly lower than that of the control group ($p < 0.01$, Fischer's exact probability test).

Distribution of threshold elevation due to inner-ear barotrauma

The distribution of ABR threshold elevation due to IEB in the control group was 37.3 per cent (10 dB or less), 21.3 per cent (20–30 dB), 18.0 per cent (40–60 dB) and 23.4 per cent (70 dB or more), and that in the pretreatment group was 57.1 per cent, 19.1 per cent, 14.3 per cent and 9.5 per cent, respective to the same decibel ranges. The distribution of threshold elevation due to IEB in the pretreatment group was milder than that in the control group. There was a statistically significant difference in the distribution between the two groups ($p < 0.01$, Mann–Whitney's U test; see Figure 3).

Thus, the protective treatment could be seen to significantly reduce the severity of the ABR threshold elevation caused by IEB.

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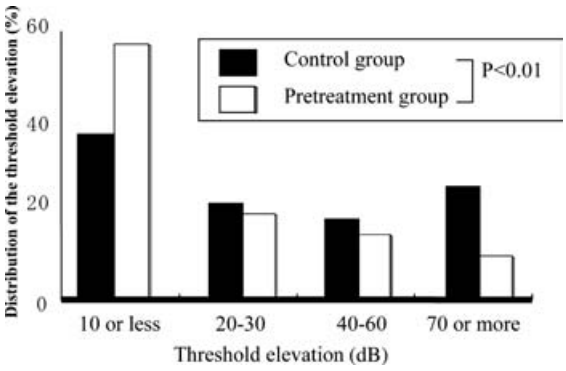

FIG. 3

Distribution of the auditory brainstem reponse threshold elevation due to inner-ear barotrauma in the two groups.

Discussion

This is the first study to investigate the protective effect of a free radical scavenger on IEB. The steady growth in the popularity of sports diving, which carries a major risk of IEB, has led to a parallel increase in the incidence of ear injuries, including IEB.[13] It is estimated that 0.5 per cent of divers will suffer IEB during their diving careers.[14] The recreational diver population is estimated at about 1 000 000 in Japan[15] and 6 000 000 in the United States.[13] Thus, many thousands of divers may be at risk of IEB.

The involvement of free radicals in inner-ear disorders is usually investigated indirectly, using one of three methods: (1) altering the activity of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase;[16] (2) increasing the concentration or level of expression of reactive oxygen compounds, such as 2,3-dihydroxybenzoic acid,[17] cerium perhydroxide reaction product,[18] 8-hydroxy-2′-deoxyguanosine[19] and malondialdehyde, as an index of lipid peroxidation;[20] and (3) utilizing the protective effect of free radical scavengers (antioxidants), such as diethyldithiocarbamate,[11] 4-methylthiobenzoic acid,[11] ebselen,[11] lipoic acid,[11] copper-zinc superoxide dimustase,[21] allopurinol,[21] Vitamin E[22] and edaravone.[23] To assess our hypothesis, the third method was selected for the current, preliminary study, and, of the available free radical scavengers, edaravone was selected.

Edaravone has been endorsed for clinical use as a free radical scavenger by the Japanese Ministry of Health, Labour and Welfare, the first such drug to be approved as a free radical scavenger for clinical use, anywhere in the world. Pharmacologically, edaravone inhibits not only hydroxyl radicals but also free radical-mediated lipid peroxidative damage.[24] Edaravone has been demonstrated to have protective effects in cases of cerebral oedema and tissue injury after ischaemia–reperfusion in rat models[25] and also in patients with acute cerebral ischaemia.[26] Thus, edaravone is clinically used for acute cerebral infarction; however, applications in other fields, such as mitochondrial injury in hepatic[27] and myocardial[28] ischaemia/reperfusion models and inflammatory reactions,[29] are becoming widespread at the experimental level. These effects are predominantly thought to be a result of the protective action of edaravone against lipid peroxidation. In otolaryngological problems, the effects of edaravone on streptomycin-induced vestibulotoxicity[23,30] and ischaemia-induced facial palsy[31] have already been reported.

In this study, we investigated the incidence of IEB and the distribution of ABR threshold elevation due to IEB to determine the protective effect of edaravone in cases of IEB. The incidence of IEB was 62.7 per cent in the control group and 42.9 per cent in the pretreatment group; there was a statistically significant difference between the two groups ($p < 0.01$). From these results, we suggest that the protective use of edaravone could significantly reduce the incidence of IEB. The distribution of threshold elevation due to IEB in the control group was 37.3 per cent (10 dB or less), 21.3 per cent (20–30 dB), 18.0 per cent (40–60 dB) and 23.4 per cent (70 dB or more), and that in the pretreatment group was 57.1 per cent, 19.1 per cent, 14.3 per cent and 9.5 per cent, respective to the same decibel ranges; there was a statistically significant difference in distribution between the two groups ($p < 0.01$). From these results, we suggest that the protective use of edaravone could also significantly reduce the severity of IEB. The protective effect of edaravone may also support our initial hypothesis.

Why is edaravone, a free radical scavenger, effective against IEB? In cisplatin ototoxicity, which involves free radicals, the mechanism is thought to proceed in three steps:[11] (1) cisplatin itself generates free radicals in the cochlea;[32] (2) free radicals induce cell membrane lipid peroxidation, which produces toxic aldehydes such as 4-hydroxynonenal;[33] and (3) these toxic compounds induce apoptosis and, finally, cell death.[34] Although it is impossible to adapt this mechanism to that of IEB, a mechanism of IEB action which involved free radicals might share common steps because of the pharmacological effects of edaravone, such as the inhibition of hydroxyl radicals and free radical-mediated lipid peroxidative damage[24] and the suppression of delayed neural death.[35] However, further study is required to elucidate the mechanism of IEB's effect on free radicals.

Conclusion

We suggest that protective treatment with edaravone can reduce the incidence of IEB and the severity of ABR threshold elevation due to IEB. We anticipate further study to evaluate our hypothesis and to investigate certain points in more detail, including the optimal dose, the protective time window and any adverse effects, in order to establish the best protective treatment to prevent IEB in humans.

We hope that this paper will contribute to the development of a new strategy for combatting IEB, one which takes into account the involvement of free radicals.

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- **The purpose of this study was to determine the protective effect of edaravone, a free radical scavenger, on inner-ear barotrauma (IEB) in guinea pigs, following the hypothesis that free radicals are implicated in IEB**
- **One hundred and twenty-five guinea pigs were divided into a control group and a pretreatment group. The incidence of IEB was 62.7 per cent in the control group and 42.9 per cent in the pretreatment group ($p < 0.01$)**
- **These results suggest that the protective effect of edaravone can significantly reduce the incidence of inner-ear barotrauma (IEB). Further studies are required to assess possible therapeutic use in humans**

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