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Protective effect of calcineurin inhibitors on acoustic injury of the cochlea

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Abstract

This study examined the effect of immunosuppressants, cyclosporin A, FK506 and rapamycin on functional recovery of the cochlea after acoustic overexposure, in guinea pigs and mice. Thirty guinea pigs were exposed to a 2 kHz pure tone at 120 dB SPL for 10 min. The compound action potential threshold shift induced by acoustic overexposure was examined. Twenty-five mice were exposed to a 4 kHz pure tone at 128 dB SPL for 4 h. Auditory brainstem response was used to examine the hearing threshold shift. In both the guinea pig and mouse experiments, cyclosporin A and FK506, intraperitonally given just before acoustic overexposure, significantly decreased the hearing threshold shift one or two weeks after acoustic overexposure. However, neither rapamycin nor the FK506 and rapamycin combined treatment groups showed improvement of the threshold shift. The present findings suggest that these two calcineurin inhibitors have a protective effect against acoustic injury of the cochlea, whereas the non-calcineurin inhibitor, rapamycin, not only has no effect against acoustic injury, but rather blocked the effect of FK506. This indicated a possible role of calcineurin against acoustic injury.

Keywords: Acoustic injury; Cochlea; Calcineurin; Cyclosporine A; FK506; Rapamycin

1. Introduction

Cyclosporin A (CsA), FK506 and rapamycin have been widely used in clinical practice as immunosuppressant drugs. The receptors for CsA and FK506 are classified as immunophilins and are cyclophilins and FK506 binding proteins (FKBP), respectively. CsA and FK506 inhibit calcineurin (CaN) by binding the immunophilins and mediating the immunosuppressive actions. Rapamycin and FK506 bind to the same immunophilin (FKBP12) with similar affinities and rapamycin does not inhibit CaN (Dumont et al., 1990; Fruman et al., 1992), thus resulting in rapamycin having an antagonistic effect on FK506 when the two drugs are used together.

Recent studies have demonstrated that CaN is widely distributed in various mammalian tissues and that the drug/immunophilin complex has neuroprotective actions in the brain (Sharkey and Butcher, 1994; Uchino et al., 1995; Lautermilch and Spitzer, 2000). Administration of CsA or FK506 significantly decreased apoptosis of neurons induced by ischemic brain injury or neurodegenerative diseases.

In the present study, we examined whether CsA and FK506 can protect against hearing deficits induced by acoustic overexposure.

2. Materials and methods

2.1. Animals

Female Hartley guinea pigs (250–300 g) and female ddY mice (8 weeks of age) were used. The animals were anesthetized by intraperitoneal injection of pentobarbital sodium (Dainihon Parmaceuticals Inc., Osaka,

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Japan). The care and use of animals were approved by the animal experimental committee of the University of Tsukuba.

2.2. Recording of compound action potential (CAP)

Hearing thresholds of guinea pigs were evaluated by the compound action potential (CAP). Methods for recording CAP have been described in detail elsewhere (Tabuchi et al., 2003), and a brief summary is presented here. After the auditory bulla was opened, CAP was recorded with a fine silver wire at the bony edge of the round window. Tone bursts of 2–16 kHz pure tones (rise/fall time: 1 ms and duration: 10 ms) were used in an open field system to elicit CAP. One hundred responses were filtered and averaged with a signal processor (Synax 1200, NEC, Tokyo, Japan). The sound pressure level necessary to evoke 10 μV of CAP was defined as the threshold.

2.3. Auditory brainstem response (ABR) testing

Hearing thresholds of mice were assessed by auditory brainstem response (ABR). The needle electrodes were placed subcutaneously at the vertex, in the right retroauricular region and in the presacral region of the mice. Tone bursts of 4–16 kHz (rise/fall time: 1 ms and duration: 10 ms) were used for ABR testing. The evoked reponses was summed and filtered with a bandpass of 200 Hz to 3 kHz with a SYNAX 1200 system (NEC, Tokyo, Japan). The thresholds were determined visually in 5 dB steps.

2.4. Hair cell loss induced by acoustic overexposure

At the end of the study period, the guinea pigs and mice were sacrificed under deep anesthesia. Cochleas were fixed with 4% paraformaldehyde for 8 h. After fixation, the organs of Corti were dissected for surface preparation. Nuclei of hair cells were stained with 5 μg/ml of propidium iodine (Molecular Probes Inc., OR, USA) for 30 min at room temperature.

All the specimens were thoroughly inspected under a laser confocal microscope (TCS 4D, Leica Microsystems, Wetzlar, Germany). The number of missing hair cells (absence of staining with propidium iodine) was counted from the apex to the base. Hair cell loss was evaluated in each animal and findings were compared between the drug-treated and control groups.

2.5. Experimental protocol

2.5.1. Experiment I

Thirty guinea pigs were exposed to a 2 kHz pure tone at 120 dB SPL for 10 min through a hollow ear bur. CsA or FK506 was dissolved in physiological saline solution and given to the animals just before acoustic overexposure. The animals were divided into the following three groups:

(1) *FK506-treated group ($n = 15$)*. The animals were treated with intraperitoneal administration of 0.1 mg/kg ($n = 5$), 1 mg/kg ($n = 5$) or 5 mg/kg of FK506 ($n = 5$).
(2) *CsA-treated group ($n = 10$)*. Ten mg/kg ($n = 5$) or 50 mg/kg ($n = 5$) of CsA was intraperitonally given to the animals.
(3) *Control group ($n = 5$)*. Physiological saline solution only was given to the animals.

CAP threshold shifts from the pre-exposure level were compared between the FK506- or CsA-treated animals and control animals immediately and one week after acoustic overexposure.

2.5.2. Experiment II

Twenty-five ddY mice were exposed to a 4 kHz pure tone of 128 dB SPL for 4 h in an open field system under awake conditions. CsA, FK506, rapamycin or both FK506 and rapamycin were given intraperitonally just before acoustic overexposure. The animals were divided into the following five groups:

(1) *FK506-treated group ($n = 5$)*. The animals were treated with 5 mg/kg of FK506.
(2) *CsA-treated group ($n = 5$)*. The animals were treated with 50 mg/kg of CsA.
(3) *Rapamycin-treated group ($n = 5$)*. The animals were treated with 10 mg/kg of rapamycin.
(4) *FK506 and rapamycin-treated group ($n = 5$)*. The animals were treated with both 5 mg/kg of FK506 and 10 mg/kg of rapamycin.
(5) *Control group ($n = 5$)*. The animals were treated with physiological saline solution.

ABR threshold shifts were determined immediately, one and two weeks after acoustic overexposure.

2.6. Statistics

All results are presented as mean ± SEM. The CAP and ABR thresholds were assessed with two-way ANOVA and Scheffe test. Loss of hair cells were evaluated with one-way ANOVA. p values less than 0.05 were considered significant.

3. Result

3.1. Electrophysiological studies

Fig. 1 demonstrates the threshold shift of CAP immediately (a) and (b) and one week (c) and (d) after