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The effect of caffeic acid phenethyl ester on the prevention of experimentally induced myringosclerosis

Jae-Jun Song*, **Seong Keun Kwon, Chang Gun Cho, Seok-Won Park**

Department of Otorhinolaryngology-Head and Neck Surgery, Dongguk University International Hospital, 814 Siksa-Dong, Goyang, Gyeonggi 410-773, South Korea

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Summary

Objectives: Myringosclerosis is a common sequela of ventilation tube insertion for the treatment of the otitis media with effusion. Several antioxidants have been identified to prevent myringosclerosis. The objective of this study was to investigate the effect of caffeic acid phenethyl ester (CAPE) on the prevention of experimentally induced myringosclerosis.
Methods: Thirty-five Sprague—Dawley rats were unilaterally myringotomized. The rats were divided into four groups randomly: group 1 received no treatment, group 2 received intraperitoneally administered saline and group 3 received intraperitoneally administered CAPE. The tympanic membranes were examined by otomicroscopy on the 15th day after treatment. The membranes were then harvested and evaluated histologically by light microscopy.
Results: The tympanic membranes from group 1 showed extensive myringosclerosis; those from group 2 showed a similar occurrence of myringosclerosis. However, group 3 had a reduced occurrence of myringosclerosis by otomicroscopic evaluation. Under light microscopic examination, the lamina propria of the pars tensa was found to be thicker and more sclerotic in groups 1 and 2 when compared with group 3.
Conclusions: Systemic treatment with CAPE was found to be effective in the prevention of sclerotic lesions in myringotomized rat tympanic membranes.

1. Introduction

Myringosclerosis (MS) is a common sequela of ventilation tube treatment for otitis media with effusion. MS is characterized by hyalinization and

* Corresponding author. Tel.: +82 31 961 7436; fax: +82 31 961 7427.
E-mail address: entsong@empal.com (J.-J. Song).

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calcification of the collagen layer in certain areas of the tympanic membrane and appears as white chalky patches. Histologically, there is an increase in collagen fibers as well as hyaline degeneration and extracellular calcium deposition within the lamina propria [1].

The exact etiology and pathogenesis of MS is not known. Recent studies have shown that oxygen derived free radicals and mechanical injury may be the main causative factors in the formation of MS [2]. Insertion of ventilation tubes through the tympanic membrane allows for ambient air to enter into the middle ear cavity, causing a relative hyperoxia [3]. This relative hyperoxia may cause an excess production of oxygen derived free radicals, which may initiate the process involved in the development of sclerotic plaques [4].

Therefore, we thought that MS might be reduced or prevented by administration of antioxidants and anti-inflammatory agents. Previous reports have shown that the formation of MS, after experimental myringotomy, could be reduced by the application of a variety of free radical scavengers [1,5,6].

Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of propolis with several interesting biological properties including: antioxidant, anti-inflammatory, antiviral, immunostimulatory, anti-angiogenic, anti-invasive, anti-metastatic and carcinostatic activities [7]. CAPE effectively downregulates a variety of proinflammatory cytokines and inflammatory mediators by inhibition of the transcription of the nuclear factor-κB (NF-κB) [8].

The aim of this study was to investigate the possible preventive effect of intraperitoneal (IP) CAPE on the development of MS in the tympanic membrane of myringotomized rats using otomicroscopy and histopathology.

2. Materials and methods

2.1. Animal maintenance

Thirty-five healthy adult male Sprague—Dawley rats weighing 250—300 g were obtained from a specific pathogen-free colony at Coretek Inc. (Gyeonggi, Korea) and used after 1 week of quarantine and acclimatization. Animals were housed in a room maintained at a temperature of $23 \pm 3\ ^{\circ}C$ and a relative humidity of $50 \pm 10\%$ with artificial lightning from 08:00 to 20:00 and with 13—18 air changes per hour. The Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the Guidelines for Animal Experiments of the Dongguk University International Hospital. Any animal that showed signs of external or middle ear infection before or after the surgery was excluded from this study.

2.2. Experimental design and surgical procedure

All the animals were divided into four groups, 10 rats (group 1) were not treated after myringotomy for a period of 2 weeks. Ten rats (group 2) were injected with IP saline for 2 weeks. Ten rats (group 3) were injected with IP CAPE (Sigma Chemical Co., St. Louis, MO, USA) for 2 weeks (10 mg/kg, solubilized in saline containing 20% Tween 20). The remaining five rats (group 4) served as the control group for histological and anatomical comparison.

Animals were anesthetized with IP ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (5 mg/kg) injections. The animals of groups 1—3 were randomly selected and under otomicroscopic examination (Opmi Pico, Zeiss, Germany), a standard perforation occupying almost the entire posterosuperior quadrant of the left tympanic membrane, was made with a myringotomy knife through an ear speculum.

2.3. Otomicroscopic examination

Otomicroscopic examination was performed on the 15th day, and the formation of MS was assessed prior to sacrificing the rats in groups 1—3. The extent of MS in the pars tensa of the tympanic membrane was evaluated semiquantitatively as follows: (0), no visible MS; (+), white halo around umbo; (++), white halo around umbo and white line beside the handle of the malleus and along the annulus; with confluent whitish deposits, forming a horseshoe shaped pattern [9].

2.4. Histological evaluation

On the 15th day, the rats were euthanized and decapitated after otomicroscopic examination. The tympanic membrane and surrounding bony annulus were removed together by microdissection under otomicroscopy. They were fixed overnight in 4% paraformaldehyde solution and then decalcified with ethylenediamine tetra-acetic acid. The specimens were embedded in paraffin, sectioned on 5 μm slices and stained with hematosylen-eosin and Mason-trichrome for study under the light microscope. Mason-trichrome staining was used to evaluate the arrangement of collagen fibers and the sclerotic changes in the connective tissue of the lamina propria. Stained specimens were evaluated by a blinded histologist and the thickness of the