

Future opportunities in preventing ototoxicity: Caffeic acid phenethyl ester may be a candidate (Review)

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Abstract. Caffeic acid phenethyl ester (CAPE) is an important active component of propolis, which is derived from honeybee hives. It has received increasing attention in a variety of medical and pharmaceutical research, due to its anti-oxidant, antiproliferative, anti-inflammatory, antiviral and antifungal activity, in addition to its antineoplastic properties. Besides the use of CAPE as an antioxidant and anti-inflammatory agent in a number of *in vivo* studies of ear disease, its beneficial effects have been reported in the treatment of cancer, arthritis, allergies, heart disease, diabetes, kidney disease, liver disease and neurological disease. CAPE influences a number of biochemical pathways, as well as several targets involved in ear diseases, in particular, in ototoxicity. The protective effects of CAPE in ototoxicity, which may be induced by a number of factors, including lipopolysaccharides, hydrogen peroxide and streptomycin, are evaluated and discussed in the present review.

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1. Introduction

Caffeic acid phenethyl ester (CAPE; Fig. 1), a well known component of the natural honeybee product, propolis, has been used for centuries in medicine, due to its anti-inflammatory, antioxidant and antineoplastic properties (1-4). CAPE is a naturally occurring phenolic compound, and is an ester derived from caffeic acid and phenethyl alcohol. It downregulates a number of pro-inflammatory cytokines and inflammatory mediators, by inhibiting the transcription of nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) (5). CAPE exhibits antimitogenic, anticarcinogenic, anti-inflammatory and immunomodulatory properties *in vitro* (6). There are a number of articles that have reviewed the positive effects of CAPE in models of neoplasm (7), prostate, lung and melanoma cancers (8), chemotherapy and radiotherapy-induced toxicity (9), and in heart disease (10). However, to the best of our knowledge, there has been no review discussing the protective role of CAPE on diseases of the ear, such as ototoxicity.

Reactive oxygen species (ROS) are associated with ototoxicity and presbycusis (11). Not all cell types found in the cochlea share the same vulnerability to ROS injury. Outer hair cells on the base of the cochlea are susceptible to ROS, while supporting cells show significantly greater survival capacity compared with hair cells, following exposure to ROS (12). Glutathione levels were found to be higher in apical outer hair cells, compared with outer hairs at the base (12). The literature demonstrates that oxidative stress, regardless of its origin, is associated with ototoxicity (13).

Since a number of oxidative pathways may affect the structures in the ear (Fig. 2), CAPE may be considered as a promising agent with which to prevent ROS-induced ototoxicity caused by certain medicines, myringosclerosis, otitis media and inflammation of the ear. Therefore, the present

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review aims to summarize and critically evaluate the evidence for the protective role of CAPE in ototoxicity.

2. Use of CAPE in antibiotic-induced ototoxicity

Aminoglycoside antibiotics, which are polycationic compounds, are widely utilized in clinical practice. However, they induce adverse ototoxic effects in 2-5% of patients (14). The common cochleotoxic or vestibulotoxic side effects thus limit the use of aminoglycoside antibiotics. A number of the oxidation products of polyunsaturated fatty acids (PUFA) in phospholipids act as mediators of apoptosis. ROS activate apoptotic or necrotic intracellular pathways, including the c-Jun N-terminal kinase (JNK) pathway (15). The inhibition of the JNK pathway has been demonstrated to help hair cells injured by aminoglycosides (15). Due to the fact that the trigger mechanism starts with ROS activation, CAPE may be a promising agent to block this cascade at two different points (Fig. 3). A number of studies have investigated underlying mechanisms of action of numerous antibiotics and cytotoxic medications, which result in ototoxicity (16-18). Once inside the negatively charged apex of the hair cell, aminoglycosides induce the generation of ROS, which are involved in molecular pathways leading to ototoxicity (19). Bakir *et al* (20) conducted a study in order to assess the antioxidant properties of CAPE in the prevention or attenuation of ototoxicity caused by long-term use of aminoglycosides in a rat model. Specifically, the authors investigated the toxic effects of intramuscular injections of streptomycin (20 mg/kg/day for 45 days). On day 45, a marked decrease in cochlear activity was observed in the streptomycin group compared with that in the control group, according to distortion product otoacoustic emissions (DPOAEs). However, no significant differences were observed between the control and CAPE groups. The distortion product-gram, DP-gram (a measurement for DPOAEs) of the streptomycin group had significantly deteriorated compared with the control and streptomycin plus CAPE-treated rats. The number of cochlear hair cells was shown to be reduced in rats treated with streptomycin. Immunohistochemical examination revealed caspase-3 immunoreactivity in rats treated with streptomycin, while this was not observed in streptomycin plus CAPE-treated rats, which indicates a protective effect of CAPE on hair cells, supporting cells, and the basilar membrane. CAPE, when applied alone, exhibited no obvious harmful effects on hair cells. Histopathological examination with hematoxylin and eosin, and immunohistochemistry, in addition to hearing test measurements, did not reveal deterioration in the cochlear hair cells of rats in the CAPE-treated group. These results demonstrate that CAPE exhibits protective effects against ROS generated by oxidative pathways in the ear (Fig. 2).

More recently, Sakalliglu *et al* (21) examined the effects of exogenous glucocorticoid exposure during the prenatal effect on hearing, in addition to the protection of this inner ear injury by CAPE. Dexamethasone, which is capable of causing an excessive production of ROS, was administered to pregnant Sprague-Dawley rats (one group received only dexamethasone, and the other received dexamethasone plus CAPE) prior to exposure to 110 dB of noise for 4 h. Exogenous dexamethasone administration did not alter hearing thresholds prior to the noise exposure. Following noise exposure, dexamethasone

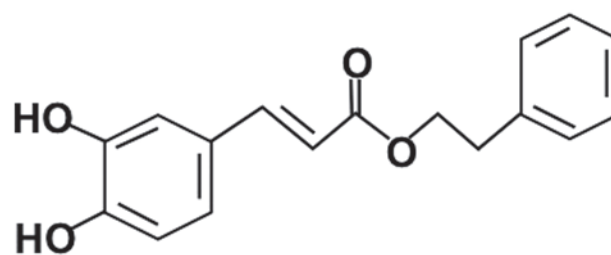


Figure 1. Chemical structure of caffeic acid phenethyl ester.

treatment elevated the hearing thresholds. Therefore, prenatal exposure to dexamethasone may cause inner ear susceptibility to noise. In this study, CAPE treatment did not prevent the damage to hearing caused by dexamethasone. This finding supports the hypothesis that the protective effects of CAPE are due to its antioxidant and anti-inflammatory properties. In conclusion, CAPE does not appear protect the inner ear against dexamethasone-induced oxidative stress.

3. Use of CAPE in otitis media

Otitis media (OM) is the leading cause of visits to physicians and consequent antibiotic prescriptions, and is a common cause of hearing impairment in children (22). Pro-inflammatory mediators have been reported to be responsible for the inflammation observed in OM. Several cytokines, including fibroblast growth factor, tumor necrosis factor α (TNF- α), platelet activating factor, interleukin (IL) 2, 6, 8 and 1 β , and interferon γ , have been isolated in middle ear effusions, and are responsible for increases in vascular permeability, chemotaxis, secretion, and consequently, middle ear inflammation (Fig. 4) (23). Additionally, lipopolysaccharide (LPS) is a pro-inflammatory mediator that is associated with middle ear inflammation. Furthermore, a number of other ROS products may directly damage the epithelium of the middle ear. For example, nitric oxide (NO) has been reported to contribute to vasodilatation, increased vascular permeability and mucoid effusions (24). Furthermore, NO produced by activated inflammatory cells, regulates cells and induces inflammation. Therefore, NO may be a secondary inflammatory mediator, produced by the middle ear epithelium in response to primary cytokines, such as IL-1 β , TNF- α (25).

Previously, *in vivo* and *in vitro* studies have shown that cytokines increase inducible nitric oxide synthase (iNOS) production in middle ear epithelial cells (26). Intracellular NO production inhibition prevents the hypersecretion of mucin that is stimulated by ROS and other inflammatory mediators (27). A number of studies have suggested that CAPE is involved in the suppression of LPS-induced inflammatory responses in the HMEEC human middle ear epithelial cell line, via the inhibition pro-inflammatory mediators (Fig. 4) (28). Thus, the effects of CAPE on TNF- α , may underlie the LPS-stimulation of HMEECs. In these studies, the bacterial endotoxin, LPS (10 μ g/ml), which is isolated from *Pseudomonas aeruginosa*, was used in order to induce an inflammatory response in HMEEC cells. CAPE was administered at doses of 0, 10, 50, 100 and 200 μ M for 1 h. LPS treatment led to an increase in TNF- α gene expression in HMEEC cells. CAPE treatment

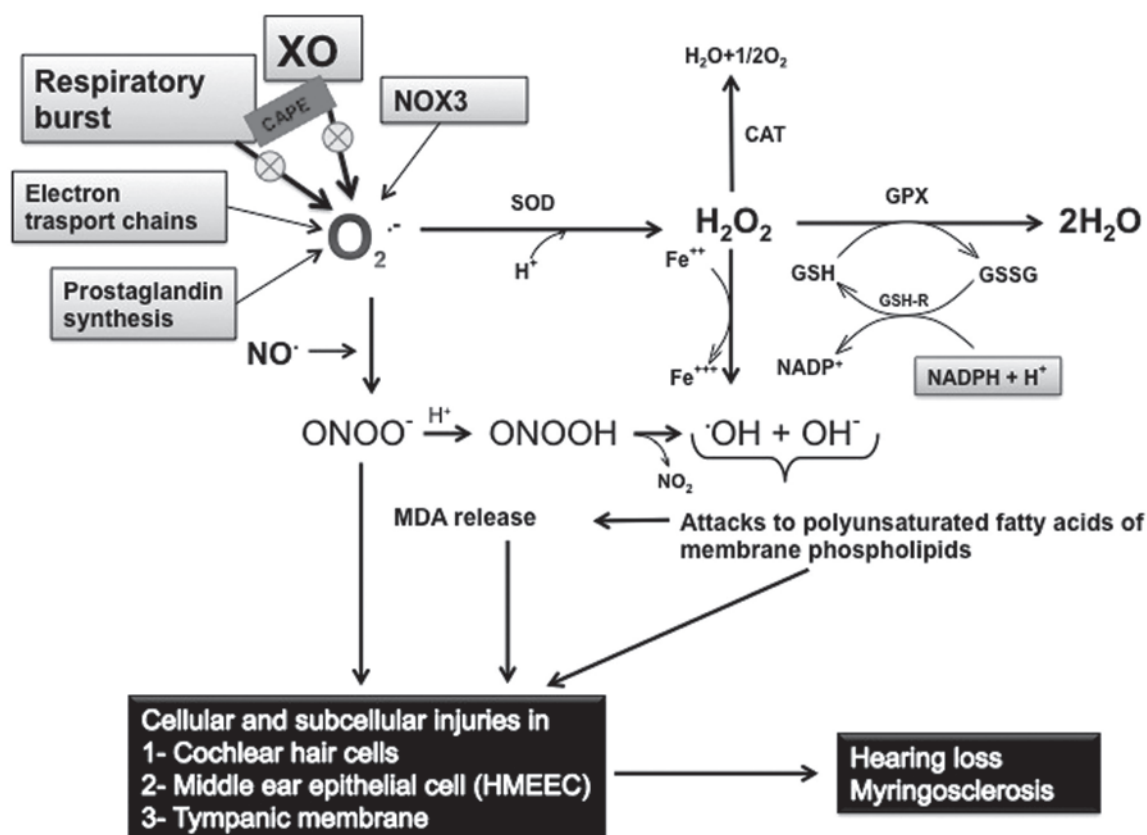


Figure 2. Proposed mechanism for the induction of oxidative stress, and its effects on hearing pathways and cells, as well as the use of CAPE for mitigating the damaging effects of ROS. CAT, catalase; Fe^{++} , ferrous iron; GPx, glutathione peroxidase; GSH, reduced glutathione; GSH-R, glutathione reductase; GSSG, oxidized glutathione; H^+ , hydrogen ion/proton; H_2O , water; H_2O_2 , hydrogen peroxide; MDA, malondialdehyde; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; $NADPH + H^+$, reduced nicotinamide adenine dinucleotide phosphate; NO^{\cdot} , nitric oxide radical; NO_2 , nitrite; NOX3, NADPH oxidase 3; O_2 , molecular oxygen; $O_2^{\cdot-}$, superoxide anion radical; OH^{\cdot} , hydroxyl ion; OH^- , hydroxyl radical; $ONOO^-$, peroxynitrite; PUFA, polyunsaturated fatty acid; SOD, superoxide dismutase; tNOS, total nitric oxide synthases; XO, xanthine oxidase; ROS, reactive oxygen species; CAPE, caffeic acid phenethyl ester.

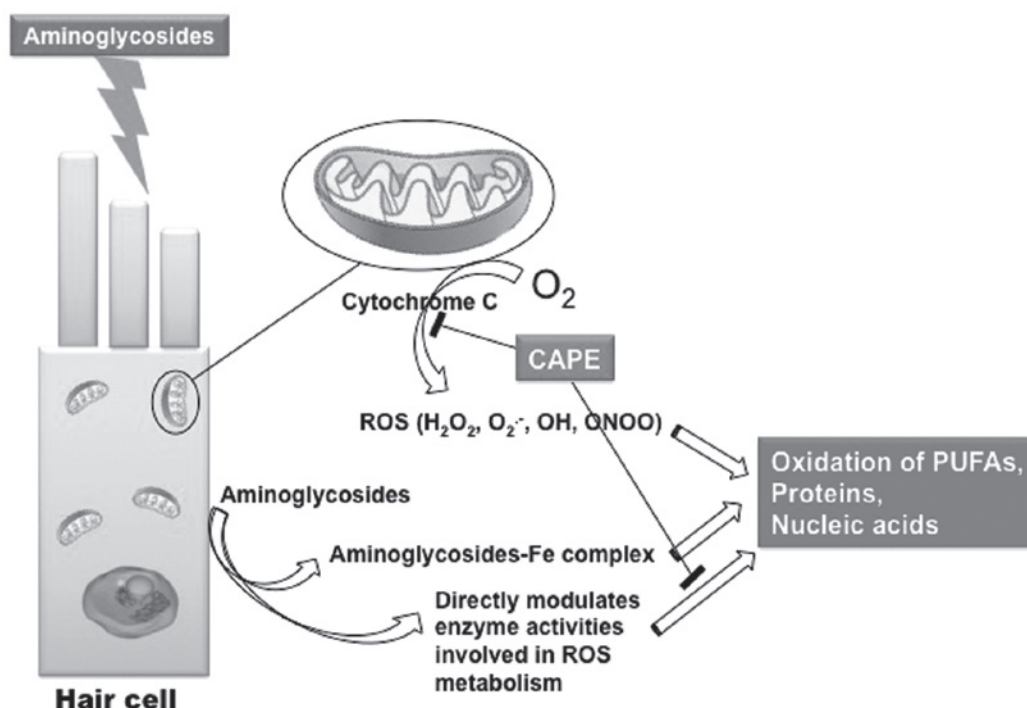


Figure 3. Schematic representation of the ototoxic effects of aminoglycosides. A number of the oxidation products of PUFA in phospholipids, which are located particularly in membranous structures, act as mediators of apoptosis. H_2O_2 , hydrogen peroxide; O_2 , molecular oxygen; $O_2^{\cdot-}$, superoxide radical; OH^{\cdot} , hydroxyl radical; $ONOO^-$, peroxynitrite; ROS, reactive oxygen species; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; CAPE, caffeic acid phenethyl ester; JNK, c-Jun N-terminal kinase.

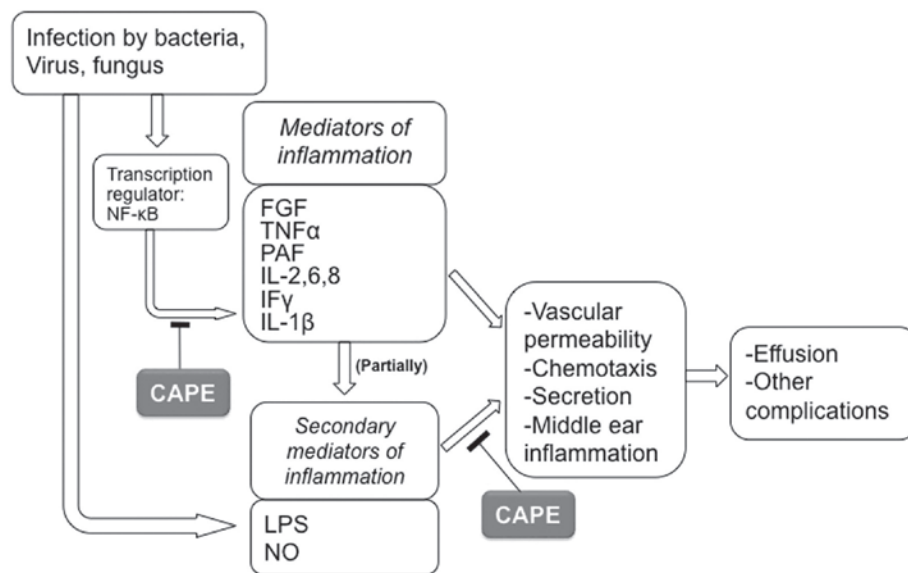


Figure 4. Proposed mechanism by which CAPE diminishes or blocks otitis media via different pathways. The transcription regulator, NF-κB has been shown to be involved in regulating the expression of large numbers of genes, such as cytokines, chemokines and other mediators involved in inflammatory responses. CAPE is a potent and specific inhibitor of NF-κB. FGF, fibroblast growth factor; PAF, platelet activating factor; LPS, lipopolysaccharide; NO, nitric oxide; CAPE, caffeic acid phenethyl ester; IL, interleukin; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor α.

suppressed LPS-induced TNF-α expression, IL-8 production and NF-κB activity in HMEEC cells, according to the results of quantitative polymerase chain reaction (qPCR), enzyme-linked immunosorbent assay (ELISA) and western blot analyses.

Previous reports have demonstrated that CAPE is an inhibitor of NF-κB, which is a regulatory molecule that modifies the expression of genes associated with cell proliferation, inflammatory responses and cell adhesion. Downregulation of several pro-inflammatory cytokines is caused by the inhibition of NF-κB (29). Ordinarily, NF-κB is inactive in the cytoplasm, as it is bound to the IκB-α protein, which prevents NF-κB activity. When inflammation occurs, IκB-α is phosphorylated and degraded, which results in the translocation of NF-κB into the nucleus, where it activates the expression of a number of genes associated with inflammatory responses. Therefore, the inhibition of IκB-α phosphorylation may prevent NF-κB translocation (30). LPS treatment induces the degradation of IκB-α. CAPE treatment results in the inhibition of LPS-induced IκB-α degradation in a dose-dependent manner, which prevents inflammation. Overall, the literature provides an insight into the molecular pathways underlying the anti-inflammatory effects of CAPE in association with OM and other inflammatory conditions.

4. Use of CAPE in hydrogen peroxide (H₂O₂)-induced oxidative stress in HMEEC cells

Recently, Song *et al* examined the inhibitory effects of CAPE on H₂O₂-induced oxidative stress in HMEEC cells (31). H₂O₂ was added at different concentrations for up to 6 h. The H₂O₂-induced inflammatory response was subsequently analyzed by quantifying TNF-α and COX-2 mRNA expression, using reverse transcription-quantitative PCR. CAPE was applied at different concentrations for 30 and 60 min, following which, ROS production, including superoxide

dismutase (SOD) expression, was determined. H₂O₂ caused an increase in SOD protein expression. However, treatment of the cells with CAPE inhibited the expression of the SOD protein. The authors observed that CAPE treatment decreased H₂O₂-induced ROS production in HMEEC cells. Additionally, CAPE treatment inhibited COX-2 protein and TNF-α mRNA expression, which were upregulated following treatment with H₂O₂ alone. Therefore, CAPE may inhibit H₂O₂-induced oxidative injury and reduce the expression of inflammatory mediators, which are involved in the pathophysiology of OM.

5. Effect of CAPE in myringosclerosis (MS)

MS is described as hyalinization and calcification in the collagen layer of the tympanic membrane. Histopathological examination indicates an increase in collagen fibers, as well as in extracellular calcium deposition and hyaline degeneration in the lamina propria (32). Although the exact mechanisms underlying the development of MS are unknown, ROS and related oxidative stress are among the possible causes of this condition (33). Following placement of tubes into the tympanic membranes, hyperoxia occurs, which results in the excessive production of ROS (34). The preventive effects of CAPE on the development of MS in tympanic membranes of myringotomized rats have been investigated, using otomicroscopy and histopathology (35). Following myringotomy, rats were treated with CAPE for two weeks. Otomicroscopic evaluation was performed and extensive myringosclerotic plaques were observed. However, the plaques observed in myringotomized rats treated with CAPE were less extensive, compared with those treated with saline solution. Similarly, histopathological evaluation revealed extensive sclerotic membranes in the tympanic membranes of non-treated blank control as well as in saline-treated groups. Sclerotic deposits and fibroblast infiltration/production were observed in the lamina propria.

Thickening of the tympanic membranes was also observed. The tympanic membranes of the myringotomized rats that had been subjected to CAPE treatment were thinner compared with those in the group without CAPE treatment. Furthermore, the degree of fibroblastic activity in the lamina propria was lower in CAPE-treated myringotomized rats compared with those that were not treated with CAPE. In conclusion, the preventive effects of CAPE on the development of MS in myringotomized rats appears to be associated with a number of mechanisms, including the scavenging activity of free radicals.

Conclusions

This review discusses only a small number of *in vivo* studies on ear disease, and the use of CAPE exceeds the scope of this article. In addition to its use in a number of *in vivo* studies of ear pathology, as an antioxidant and anti-inflammatory agent, beneficial effects of CAPE have been reported in a number of types of cancer, arthritis, allergy, heart disease, diabetes, kidney disease, liver disease and neurological disease. CAPE modulates a number of biochemical pathways and several targets involved in ear diseases, such as ototoxicity. CAPE treatment has demonstrated promising effects in oxidative stress-mediated and inflammation-mediated diseases (Fig. 2). CAPE may be a useful treatment for patients with ear disease. Further investigation of the effects of different doses of CAPE, as well as appropriate administration regimens and the bioavailability of this agent in humans is required.

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