Original Article

TANAFFOS

Evaluation of the Effect of Caffeic Acid Phenethyl Ester (CAPE) on Pharmacological Responses of Isolated Rat Trachea in vitro

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Background: Caffeic acid phenethyl ester (CAPE) is one of the major components of honeybee propolis and its structure is similar to flavonoids. CAPE has been shown to possess anti-inflammatory, immunomodulatory, and antioxidant properties. Despite a wide range of biological activities of CAPE, detailed biochemical mechanisms of its action are poorly described. The aim of the present study was to investigate the in vitro effect of CAPE on isolated rat trachea.

Materials and Methods: A 20 mm long portion of rat tracheal spiral was submerged in 20 ml Krebs solution in an isolated organ bath at 37°C. Changes in tracheal contractility in response to the application of agonist agents were measured using an isometric transducer connected to a Harvard polygraph.

Results: Acetylcholine (ACH), histamine (HIS), and CaCl2 caused the trachea to contract in a dose-dependent manner. Incubation of trachea with 10-7 M and 10-6M of CAPE induced a significant reduction in contraction induced by ACH and HIS. The degree of drug-induced tracheal contraction or relaxation was dose-dependent.

Conclusion: The CAPE potential to relax the trachea may antagonize cholinergic and histaminergic receptors of the trachea. The findings provide new insight into the effectiveness of CAPE in the control of asthma and the possible use of propolis for its treatment. The results highlight the anti-muscarinic, anti-histaminic, anti-inflammatory, and relaxant activities of CAPE and critically show its potential therapeutic effects.

Key words: Caffeic Acid Phenethyl Ester (CAPE), Rat, Trachea, Asthma, Pharmacological response

INTRODUCTION

Asthma is a common chronic inflammatory disease of the airways. It is characterized by airway obstruction, hyperresponsiveness, and remodeling (1). The rate and prevalence of asthma episodes have climbed dramatically among developing countries. Despite the increased use of medications that suppress airway inflammation and repress contraction of smooth muscle that encircles the airways, there is only a small decrease in total asthma exacerbation (2). Corticoids are the currently used medications as anti-inflammatory agents in combination with β_2-adrenergic agonists as bronchodilators (3). These drugs cause serious side effects even in those with mild to moderate disease. Therefore, there has been an increase in demand for finding new medicines in asthma therapy. Natural products are novel products for treating asthma

and offer a remarkable pharmacological perspective for drug development with regard to their widespread acceptability and broad-spectrum activities.

Propolis is a honey-bee hive product, which has long been used due to its several beneficial medical properties (4). Caffeic acid phenethyl ester (CAPE) found in propolis (bee glue) is a derivative of a honeybee product (5). CAPE has anti-inflammatory, immunomodulatory, and antioxidant properties (6). CAPE may pass easier through the cell membrane and has greater pharmacologic effects than caffeic acid (7). The activation of nuclear factor kappa B (NF-KB) is induced by various agents producing reactive oxygen species (ROS) in human histiocytic cells and coronary artery endothelial cells. This induction was inhibited by CAPE (8, 9). The inhibition of NF-KB is crucial for the development of novel and safe anti-inflammatory agents (10). The current treatment of chronic inflammatory diseases, such as asthma is based on pharmacological interventions. Due to numerous pharmacological effects, such as anti-inflammatory, immunomodulatory, and antioxidant properties, the aim of the present study was to evaluate the effect of CAPE on pharmacological responses of isolated rat trachea *in vitro*.

MATERIALS AND METHODS

All chemical reagents were obtained from Sigma (St Louis, MO, USA). This study was approved by the animal ethics committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS). Albino rats of either sex were anesthetized by intraperitoneal administration of pentobarbital (45 mg/kg) and two sections of trachea about 20 millimeters in length were removed from each rat and cut into a spiral strip. The tracheal specimen was mounted using two steel plates and submersed in a 20ml organ bath at 37°C. The bath was filled with ml Krebs solution consisting of 118 mmol/l NaCl, KCl (4.7 mmol/l), $CaCl_2$ (2.5 mmol/l), $MgSO_4 \cdot 7H_2O$ (1.2 mmol/l), KH_2PO_4 (1.2 mmol/l), $NaHCO_3$ (25.0 mmol/l); and glucose (10.0 mmol/l). Continuous aeration with a mixture of 95% O_2 and 5% CO_2 was applied. The upper side of the tracheal strip was attached to a UF1 isometric transducer another

side of the strip was fixed to a steel tissue holder. A resting tension of 500mg was applied to the tissue and allowed for equilibration in the bath solution for 30 min prior to the main tests. Preliminary tests showed that a tracheal strip immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. Stepwise increases in the number of drugs were employed to study the contraction or relaxation responses of tracheal strips. All drugs were administered by adding a defined volume of stock solution to the organ bath to attain molar concentration. A cumulative dose-response curve was attained using acetylcholine (ACH) and histamine (HIS). The addition of CAPE into the tissue bath could not cause any contraction or change in the baseline tissue tension. However, incubation of trachea with 10^{-7} M and 10^{-6}M of CAPE induced a significant reduction in contractions induced by ACH and HIS, which was dose-dependent. To assess the possible roles of extracellular Ca^{2+} on the CAPE effect, the preparation was allowed to stabilize in the normal Krebs solution, then replaced with Ca-free and high-K (60mM) Krebs solution. Dose-response curve for $CaCl_2$ (10^{-6}M to 10^{-3}M) was obtained in the absence of CAPE (control group), or after a 20 min incubation with 10^{-7} M and 10^{-6}M of CAPE, the procedure was repeated. Finally, the contractile effect induced by $CaCl_2$ was compared in the absence (control group) and the presence of CAPE.
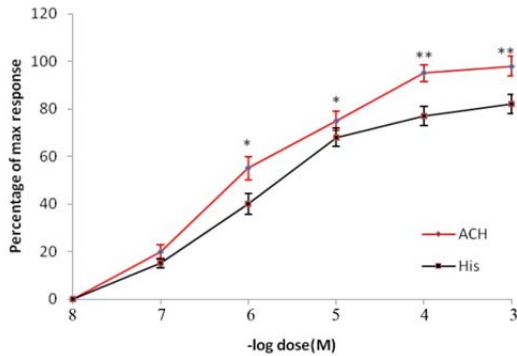
To evaluate the involvement of relaxing factors in the CAPE-induced bronchodilator activity, the effect of N omega-nitro-L-arginine methyl ester (L-NAME (0.1 mM); a non-selective inhibitor of nitric oxide synthase), as a *nitric oxide synthase* (*NOS*) inhibitor, was examined. Tracheal relaxant effects of CAPE on ACH contractile activity significantly reduced after the treatment of tracheal tissue with L-NAME prior to CAPE incubation.

RESULTS

Effects of ACH and HIS

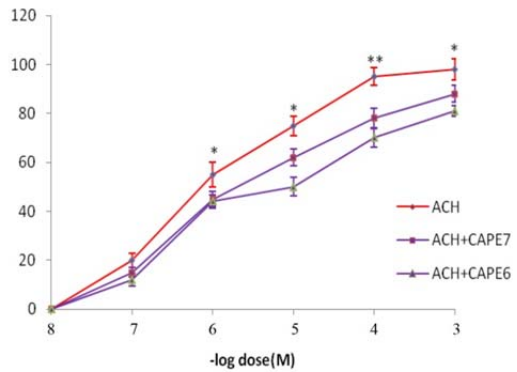As shown in Figure 1, ACH and HIS could cause the contraction of the tracheal strip in a dose-dependent

manner. The potency of ACH in this preparation seems to be higher than HIS because the ED_{50} for ACH is 7.6×10^{-7}M and for HIS is 6.3×10^{-6}M.

Figure 1. Comparative dose-response curves of ACH and HIS on rat tracheal spiral strips. Symbols and vertical bars represent means and SEM. Value significantly different from HIS are presented *(p<0.05) and ** (p<0.01).

Effect of CAPE on ACH response

Figure 2 displays the inhibitory effect of CAPE on the contractile efficacy of ACH. Incubation of the tissue with CAPE could shift the dose-response curve of ACH to the right and increase the ED_{50} of ACH from 9×10^{-7}M to 3×10^{-6}M and 9×10^{-6}M in the presence of CAPE (10^{-7}M and 10^{-6}M), respectively.
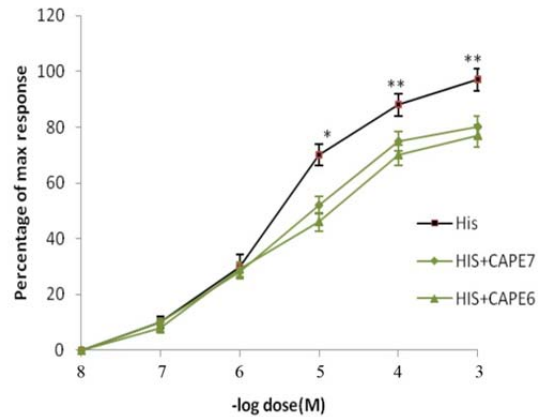

Figure 2. Dose-effect curves of ACH on tracheal spiral strips of rat in the absence and in the presence of CAPE (10-7 and 10-6 M). Symbols and vertical bars represent means and SEM. Value significantly different from CAPE are presented *(p<0.05) and **(p<0.01).

Effect of CAPE on HIS response

The inhibitory effect of CAPE on the contractile efficacy of HIS was observed in this experiment. Incubation of the tissue with CAPE could reduce the response of HIS and increase the ED_{50} of HIS $6. \times 10^{-6}$M to 9×10^{-6}M and 2×10^{-5}M
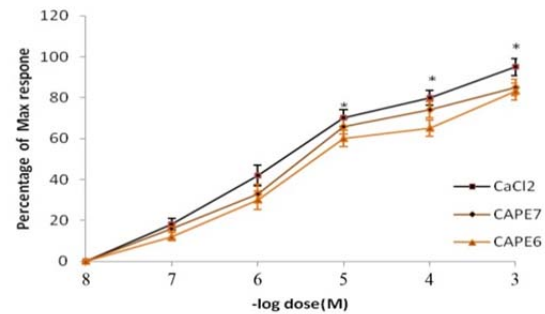
in the presence of CAPE (10^{-7}M and 10^{-6}M), respectively (Figure 3).

Figure 3. Dose-effect curves of HIS on tracheal spiral strips of rat in the absence and in the presence of CAPE (10-7 and 10-6 M). Symbols and vertical bars represent means and SEM. Value significantly different from CAPE are presented *(p<0.05) and ** (p<0.01).
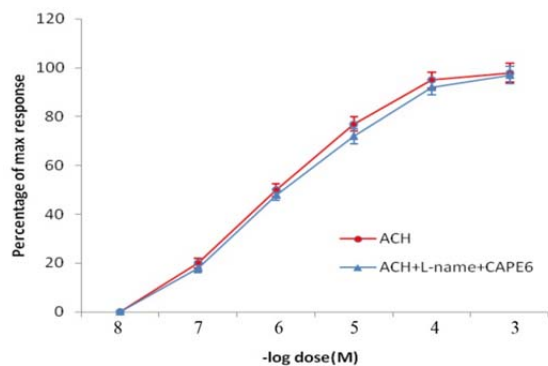
Effect of CAPE on CaCl2 response

Incubation of the tissue with CAPE in Ca^{2+}-free Krebs could reduce the contractile response of $CaCl_2$ and increase the ED_{50} of $CaCl_2$ from 2×10^{-6}M to 4×10^{-6}M and 6×10^{-6}M in the presence of CAPE (10^{-7}M and 10^{-6}M), respectively (Figure 4).

Figure 4. Dose-effect curves of CaCl2 on rat tracheal spiral strips in the absence and in the presence of CAPE (10-7 and 10-6 M). Symbols and vertical bars represent means and SEM. Value significantly different from CAPE are presented *(p<0.05)

Effect of L-NAME on relaxant effect of CAPE

Figure 6 shows the inhibitory effect of L-NAME on the relaxant efficacy of CAPE. Incubation of the tissue with L-NAME prior to CAPE could abolish the relaxant activity of CAPE on ACH-induced contraction of tracheal smooth muscle (Figure 5).

Figure 5. Dose-effect curves of ACH on rat tracheal spiral strips in the presence of L-NAME (10-4M) + CAPE (10-6 M). Symbols and vertical bars represent means and SEM. No significant differences are seen between to curves.

DISCUSSION

Asthma is one of the most important diseases of the respiratory system. It is a long-term inflammatory disease of the airways of the lungs, which is characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm (8). Asthma is thought to be caused by a combination of environmental factors and genetic (11). Many environmental factors are associated with the formation and exacerbation of asthma, including allergens, air pollution, and other environmental chemicals. Other potential triggers include medications, such as aspirin and beta-blockers (12).

There is no cure for asthma and the currently used therapy includes inhaled corticosteroids and short- and long-acting beta-agonists. Anti-leukotriene agents can be also used in addition to classic drugs (13, 14). However, numerous studies are currently trying to develop more appropriate drugs with higher efficacy and lower adverse effects; however, the use of agents with natural sources has attained great attention. Natural products provide a remarkable pharmacological perspective for drug development with regard to their widespread acceptability and broad-spectrum activities. Positive effects of CAPE have been investigated in a variety of diseases, such as pulmonary fibrosis (15, 16), heart disease (17, 18), and rheumatoid arthritis (19). Its effectiveness has not yet been studied in all aspects of asthma. Therefore, it is valuable to search for effective medicines for treating asthma using natural products.

In the present study, we investigated the effectiveness of CAPE on bronchial contractile activity, which is a major factor in bronchospasm and asthma. The cholinergic system affects the lungs was through the release of ACH and activation of muscarinic receptors, which causes bronchospasm and asthma (20). Such activity of ACH was diminished by CAPE in isolated tracheal preparation as indicated in Figure 3. CAPE caused its effect in a dose-dependent manner with the doses of 10^{-7} and 10^{-6} M and increasing the EC_{50} of ACH (Figure 2). Therefore, CAPE may at least in part inhibit muscarinic receptors in lung tissue.

HIS is one of the oldest mediators causing bronchospasm and asthma. It is usually released in response to allergens and mast cell stimulants (21). Therefore, its role in asthma should be considered in all studies dealing with asthma. In the present study, we compared the contractile activity of the trachea in response to ACH and HIS. HIS showed less potency than ACH to elicit the contraction of the isolated trachea as exhibited in Figure 2. HIS has a higher EC_{50} than ACH. In order to elucidate whether CAPE is able to interact with HIS response, trachea tissue was incubated with 10^{-7} and 10^{-6} M of CAPE. CAPE could significantly reduce the HIS response in a dose-dependent manner and increase the EC_{50} of HIS (Fig. 3). Such finding is consistent with a previous study, which showed the inhibitory activity of CAPE in HIS--induced itching in mice (22). The results showed the therapeutic potential of CAPE and its anti-histaminic effects on the lung. Because ACH and HIS play a crucial role in the onset of lung diseases, the inhibition of CAPE seems appropriate for the development of novel and safe drugs for lung diseases.

Various spasmolytic constituents mediate their relaxant effects through calcium (Ca2+) antagonists (23). We also found that CAPE remarkably blocked $CaCl_2$-induced contraction, in Ca^{2+}-free medium containing 60 mM KCl, supporting the idea that CAPE possesses a Ca^{2+} entry blocking activity (Figure 4). These results indicate that CAPE may be able to obstruct Ca^{2+} channels. This finding is consistent with earlier studies, which showed that CAPE

is able to inhibit Ca²⁺ released from intracellular stores and block extracellular Ca²⁺ entry, which suggests that CAPE cab act as a CA antagonist (24). Our further experiment to elucidate the mechanism involved in CAPE effect showed that L-NAME can abolish the relaxing effect of CAPE on ACH response in trachea. This means that the relaxing effect of CAPE may partly be due to the release of nitric oxide (NO). NO has an important physiologic role in lung and acts as a neurotransmitter for the non-adrenergic and non-cholinergic nerves. NO is produced through an enzyme called NOS and modulates the airway tone via a bronchodilator action (25). Additionally, it is widely known that both inflammation and bronchospasm play an essential role in asthma (26). The regular use of CAPE in honey can help control asthma attacks because it acts as a bronchodilator. It is likely that bronchodilation is mediated through an inhibitory effect on Ca²⁺ movement across cell membrane (27). Also, the CAPE reduces oxidative stress through the levels of trace elements, such as zinc and copper. These elements are important for the control and treatment of asthma (28). Therefore, we propose that propolis contains CAPE that can be used as an adjuvant treatment to treat patients with mild to moderate asthma. The results showed that asthma attacks due to HIS and ACH may be significantly decreased and lung function is improved in patients receiving CAPE. The diverse effects of CAPE might be attributed to its ability to pass through the cell membranes more easily than caffeic acid. These results show that honey has antiasthma activities and confirm its use in traditional medicine. Indeed, clinical effectiveness of propolis in the treatment of asthma should be more investigated in animal studies and human clinical trials.

CONCLUSION

This study showed the protective effect of CAPE on drug-induced contractility, allergic airway inflammation, and hyperresponsiveness in asthma. Thus, it will be of great significance if the CAPE has bronchodilator effects as well as anti-inflammatory function. Many studies have proved that CAPE has anti-inflammatory effects, which can be associated with its therapeutic effect on asthma (29). Accordingly, the beneficial effects of CAPE can be used in the management of asthma. Our results indicated that CAPE may be utilized as adjuvant therapy for bronchial asthma patients.

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