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Mechanism of acute lung injury due to phosgene exposition and its protection by cafeic acid phenethyl ester in the rat

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ABSTRACT

The mechanism of phosgene-induced acute lung injury (ALI) remains unclear and it is still lack of effective treatments. Previous study indicated that oxidative stress was involved in phosgene-induced ALI. Caffeic acid phenethyl ester (CAPE) has been proved to be an anti-inflammatory agent and a potent free radical scavenger. The purpose of this study was to investigate the protective effects of CAPE on phosgene-induced ALI and identify the mechanism, in which oxidative stress and inflammation were involved. The phosgene was used to induce ALI in rats. The results showed that after phosgene exposure, total protein content in BALF was not significantly changed. The increase of MDA level and SOD activity induced by phosgene was significantly reduced by CAPE administration, and the decrease of GSH level in BALF and lung were significantly reversed by CAPE. CAPE also partially blocked the translocation of NF-κB p65 to the nucleus, but it had little effect on the phosphorylation of p38 MAPK. In conclusion, CAPE showed protective effects on lung against phosgene-induced ALI, which may be related with a combination of the antioxidant and anti-inflammatory functions of CAPE.

1. Introduction

Phosgene, a classic chokey poisonous gas, is an important raw material of chemosynthesis, which is used widely in the production of the medicine, tincture, pesticide etc., and also deemed as a pollutant found in different environmental samples (Sciuto, 1998). There exists the potential of accidental, environmental, and occupational exposure. It is well-know that exposure to phosgene causes acute lung injury (ALI), which can eventually lead to death (Chen et al., 2009). The mechanism of phosgene-induced acute lung injury (ALI) remains unclear and effective treatments are deficient (Diller, 1978). Our previous studies primitively demonstrated that oxidative stress was involved in phosgene-induced ALI (Qin et al., 2004). Also, some documents suggested that ALI was related to inflammation for the activity of myeloperoxidase (MPO), existing in granular leukocytes, was increased (El Kebir et al., 2008; Yang et al., 2008), which could be inhibited by chloride gadolinium (Liu et al., 2006).

Caffeic acid phenethyl ester (CAPE), a natural flavonoid-like chemical extracted from honeybee propolis. Recent studies had shown that CAPE presented several biological properties such as anti-oxidation (Michaluart et al., 1999; Rao et al., 1992), anti-inflammation (Chen et al., 2001), anti-carcinogenic activity, antiviral, and immunomodulatory activities (Fesen et al., 1993). CAPE had been also demonstrated to protect the tissues against ischemia–reperfusion injury (I/R) (Uz et al., 2002) and oxidative stress (Ozguner et al., 2005a,b,c).

It was unclear whether CAPE could play a role of anti-oxidation and anti-inflammation against the acute lung injury induced by phosgene. If it was the case, whether the classical inflammatory signal pathway would be involved in it's function? Considering the biological action of CAPE and aforementioned questions, it is necessary to investigate the effects of CAPE on phosgene-induced acute lung injury, for the toxicity of phosgene seems to be related to oxidative injury (Kennedy et al., 1990) and inflammation, at least partially. The aim of the present study is to ascertain the protective effect of CAPE on lung against phosgene-induced acute injury and its mechanism, in which oxidative stress and inflammation is involved. Previous studies had demonstrated the essential role of mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) activation in pro-inflammatory cytokine responses to a number of microbial stimuli including LPS (Sweet and Hune, 1996), mycoplasma membrane lipoproteins (Christelle et al., 1999; Rawadi et al., 1998), and Staphylococcal peptidoglycans (Dziarski

Abbreviations: ALI, acute lung injury; CAPE, caffeic acid phenethyl ester; BALF, bronchoalveolar lavage fluid.

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et al., 1996), but little was known about the role of MAPKs and NF-κB pathways in phosgene-induced lung injury, as well as the relation between the expression of MAPK and NF-κB and oxidative stress. Therefore, the change of MAPK and NF-κB signaling in phosgene-induced rat acute lung injury was also assessed in this study.

2. Methods

2.1. Materials and animals

Twenty-four adult male SD rats (8 weeks old, mean weight 213 ± 3.6 g) obtained from Laboratory Animal Center of the Fourth Military University were used in the experiment. The animals were kept in an environment with controlled temperature (24–26 °C), constant humidity (55–60%) and controlled photoperiod (12 h light/dark cycle) for 1 week before the start of experiment. CAPE was purchased from Sigma (St. Louis, MO, USA). Rats were randomly divided into control group, phosgene-exposed group (phosgene group) and phosgene plus CAPE-treated group (phosgene + CAPE group). Control group was exposed to normal room air; phosgene and phosgene + CAPE group were exposed to 400 ppm for 1 min in a whole-body dynamic exposure chamber, which was chosen following our previous study to control lethality of SD rat under 10%. The air of phosgene was poured into the chamber (40 cm in height × 60 cm in diameter), and mixed with filtered room air with a turbine and outpoured via the other hole. Portascens II gas detector (American Analytical Technology, Inc.) was employed to detect phosgene concentration. When desired concentration was gained, rats were set in the drawer and pushed into the chamber. Time was counted when the cover of drawer was closed. For phosgene + CAPE group, after exposure to phosgene, CAPE (50 μmol/kg in 0.1 ml of vehicle solution-10% ethanol in normal saline) (Ozer et al., 2005) was immediately administered (i.p.) to rats. Instead, rats in control group and phosgene treated group were given simultaneously equivalent volumes of saline. Six hours after exposure to phosgene, all rats were anesthetized (i.p.) with 2% pentobarbital (our previous experiments demonstrated that, at this time point, ALI induced by phosgene occurred and some damage criterions such as the total protein content and the lung wet/dry weight ratio increased to maximum and maintained for 6–9 h). Four milliliter iced saline was injected into the lungs to wash pulmonary alveoli for three times and bronchoalveolar lavage fluid (BALF) was collected. BALF was centrifuged at 600 g for 5 min and the supernatant was stored at −80 °C until analysis. The right lung was rapidly removed and placed in liquid nitrogen, then stored at −80 °C until analysis. One hundred and fifty milligrams of right lung tissue was homogenized in a motor-driven tissue homogenizer with phosphate buffer (pH 7.4). Unbroken cells, cell debris and nuclei were deposited at 2000 g for 10 min and the supernatant was stored at −80 °C until determination.

2.2. Bronchoalveolar lavage (BAL)

BAL was performed with the whole lung. Four milliliter aliquots of 37 °C, sterile, pyrogen-free, 0.9% saline were flushed through the tracheotomy tube. Four milliliter iced saline was injected into the lungs to wash pulmonary alveoli for three times and bronchoalveolar lavage fluid (BALF) was collected. The total number of cells was counted using a standard haemocytometer. BALF was then centrifuged at 600 × *g* for 5 min. The supernatant was collected and stored at −80 °C. Protein content was measured by the method of Lowry et al. (1951).

2.3. Wet-to-dry lung weight ratio

The fresh harvested lung was separated, weighed, then dried at 75 °C for 72 h, and finally weighed again. The W:D ratio was calculated by dividing the wet weight by the dry weight.

2.4. Myeloperoxidase (MPO) activity

MPO activity in lungs was assessed according to previous method (Kumar et al., 2002). Lungs were lysed in Triton X-100 0.05% and were kept frozen at −70 °C until use. The substrate cocktail contained 5 ml of 0.1 M citrate buffer (pH 5.5), 32 μl of Triton X-100 20%, 50 μl of 82.4 mM O-dianisidine or OPD in dimethyl sulphoxide (20.15 μg/ml in DMSO) and 20 μl of 26.4 mM H_2O_2. To 0.5 ml of cell lysate, 0.7 ml of 0.1 M citrate buffer (pH 5.5) was added. Then 4.8 ml of the above assay mixture was again added. The mixture was kept at room temperature for 1 h and the OD450 was measured.

2.5. Malondialdehyde determination

The MDA levels in lung tissue and BALF were determined according to the modified method described by Yagi (1992). 200 μl sample of lung tissue homogenate or BALF was gently mixed with 4.0 ml of 1/12 N H_2SO_4 and 0.5 ml of 10% phosphotungstic acid, then centrifuged at 1600 × *g* for 10 min. The supernatant was discarded and the sediment was mixed again with 2.0 ml of 1/12 N H_2SO_4 and 0.3 ml of 10% phosphotungstic acid, then centrifuged at 1600 × *g* for 10 min. The supernatant was discarded again. The pellet was resuspended with 1.0 ml distilled water, and equal volume of 0.67% thiobarbituric acid (TBA, Merck), then was boiled for 60 min. After cooling, the reacts were extracted by n-butyl alcohol for 5 min and the upper layer was suctioned after centrifugation at 1000 × *g*. The reaction of MDA with TBA at 100 °C forms a pink fluorescence pigment. The absorbance of extractions was read at 515/553 nm. Arbitrary values obtained were compared with a series of standard solutions 1,1,3,3-tetraetoxypropane (Sigma). The results were standardized with lung tissue protein concentration and expressed as milligram per milliliter in BALF and milligram per gram protein in lung tissue.

2.6. Superoxide dismutase activity determination

Total (Cu–Zn and Mn–SOD) SOD activity was determined according to the modified method of Kono (1978). The principle of the method was based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. The sample was mixed with 50 mM phosphate buffer, 50 mM sodium carbonate buffer (pH 10.2, 0.1 mM ethylenediaminetetracetic acid EDTA), 24 μM NBT and 0.03% (v/v) TritonX-100. The reaction was initiated by 1 mM hydroxylamine in the 37 °C water bath, and terminated by adding TCA. Absorbance measurements were carried out with spectrophotometer at 560 nm. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was standardized with lung tissue protein concentration, and also expressed as units per milliliter in BALF and units per milligram protein in lung tissue.

2.7. Reduced glutathione determination

Glutathione (GSH) content was determined as we described previously (Wang et al., 2010). One hundred milligrams of lung was mixed with 1.5 ml sodium phosphate–ethylenediaminetetraacetic acid (EDTA) buffer and 0.4 ml 25% sodium phosphate to precipitate proteins, homogenized for 30 s using a motor-driven tissue homogenizer, and centrifuged at 6500 × *g* for 15 min at 25 °C.