The effects of pycnogenol on antioxidant enzymes in a mouse model of ozone exposure

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Background/Aims: Ozone is an environmentally reactive oxidant, and pycnogenol is a mixture of flavonoid compounds extracted from pine tree bark that have antioxidant activity. We investigated the effects of pycnogenol on reactive nitrogen species, antioxidant responses, and airway responsiveness in BALB/c mice exposed to ozone.

Methods: Antioxidant levels were determined using high performance liquid chromatography with electrochemical detection. Nitric oxide (NO) metabolites in bronchoalveolar lavage (BAL) fluid from BALB/c mice in filtered air and 2 ppm ozone with pycnogenol pretreatment before ozone exposure (n = 6) were quantified colorimetrically using the Griess reaction.

Results: Uric acid and ascorbic acid concentrations were significantly higher in BAL fluid following pretreatment with pycnogenol, whereas γ-tocopherol concentrations were higher in the ozone exposed group but were similar in the ozone and pycnogenol pretreatment groups. Retinol and γ-tocopherol concentrations tended to increase in the ozone exposure group but were similar in the ozone and pycnogenol pretreatment groups following ozone exposure. Malonylaldehyde concentrations increased in the ozone exposure group but were similar in the ozone and pycnogenol plus ozone groups. The nitrite and total NO metabolite concentrations in BAL fluid, which parallel the in vivo generation of NO in the airways, were significantly greater in the ozone exposed group than the group exposed to filtered air, but decreased with pycnogenol pretreatment.

Conclusions: Pycnogenol may increase levels of antioxidant enzymes and decrease levels of nitrogen species, suggesting that antioxidants minimize the effects of acute ozone exposure via a protective mechanism.

Keywords: Antioxidants; Nitric oxide; Reactive nitrogen species; Ozone

INTRODUCTION

The lung interfaces with the external environment and is frequently exposed to airborne pollutants such as ozone and particulates, and is prone to oxidant-mediated cellular damage [1-7]. The production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) associated with oxidative stress are important factors in lung disease [2]. Ozone, a component of photochemical air pollution, is formed from volatile hydrocarbons, halogenated organics, and oxides of nitrogen in the presence of sunlight [2-7]. Ozone can react directly with unsaturated fatty acids and cell membranes to produce lipid ozonation products, which are small, diffusible, and relatively stable [8]. Ozone also leads to the oxidative modification of surfactant...
proteins, such as surfactant protein-A, which causes the lung to be more susceptible to lipid peroxidation and inflammation, and results in a reduction in phagocytosis [6]. Exposure of human airway epithelial cells to lipid ozonation products in vitro leads to the activation of eicosanoid metabolism, phospholipases A2, C, and D, and the induction of inflammatory mediators such as interleukin (IL)-6, IL-8, and prostaglandin E2 [9].

The dietary supplement pycnogenol is a water-soluble mixture of flavonoid compounds extracted from French maritime pine bark. It is utilized throughout the world as a phytochemical remedy for various diseases ranging from chronic inflammation to circulatory dysfunction. The flavonoids in pycnogenol have antioxidant properties [12,13] and may also act as modulators of metabolic enzymes [14-16] and other cellular functions [12,16,17]. Pycnogenol is a very potent antioxidant for scavenging ROS and RNS [16], has anti-inflammatory effects [18], may have efficient antioxidant activity [19-21], and shows some modulatory effects on the immune system [22].

Acute ozone exposure decreases pulmonary function, increases airway responsiveness, and induces airway inflammation [1-7,23-26]. There may be a common ozone adaptation mechanism that involves the regulation of ascorbic acid in the fluid that lines the lungs [27]. Antioxidant transport contributes to the maintenance of normal airway tone and reactivity under conditions of oxidative stress [28]. In the present study, we evaluated the effects of pycnogenol on ROS, RNS, and the antioxidant responses in BALB/c mice following acute ozone exposure.

**METHODS**

**Animals and ozone exposure**

Five- to 6-week-old female BALB/c mice, obtained from Daehan Laboratories (Daejeon, Korea), were maintained on ovalbumin-free diets. The mice were individually housed in rack-mounted stainless steel cages with free access to food and water. The mice housed in whole body exposure chambers were exposed to normal ozone concentrations of 0 (filtered room air) and 2 ppm for 3 hours (n = 6/group). Ozone was generated with Sander Model 50 ozonizers (Sander, Eltze, Germany). The concentration of ozone within the chambers was monitored throughout the exposure with ambient-air ozone motors (Model 49C, Thermo Environmental Instruments Inc., Franklin, MA, USA).

Air sampling probes were placed in the breathing zone of the mice. The mean chamber ozone concentration (± SEM) during the 3-hour exposure period was 1.89 ± 0.06 ppm. The temperature and humidity were maintained at constant levels within the chamber. Pycnogenol was purchased from Horphag Research Ltd. (Guernsey, UK). The mice housed in whole body exposure chambers were treated with pycnogenol (100 mg/kg/day) orally for 5 days before ozone exposure. The study protocol was approved by the local research ethics committee of the Soonchunhyang University Bucheon Hospital research board.

**Determination of airway responsiveness**

An increase in the enhanced pause (Penh) was measured by barometric plethysmography using whole body plethysmography (Buxco, Troy, NY, USA) as an index of airway obstruction, immediately after ozone exposure while the animals were awake and breathing spontaneously [29]. Before taking readings, the box was calibrated by rapid injection of 150-µL air into the main chamber. The pressure differences between the main chamber of the whole body plethysmography-containing animal, and a reference chamber (box pressure signal) were measured. This box pressure signal is caused by changes in volume and resultant pressure in the main chamber during the respiratory cycle of the animal. A pneumotachograph with defined resistance in the wall of the main chamber acted as a low pass filter and allowed thermal compensation. The time constant of the box was determined to be approximately 0.02 seconds. Mice were placed in the main chamber, and baseline readings were taken over 3 minutes and averaged.

**Bronchoalveolar lavage (BAL) fluid preparation and analysis**

BAL was performed immediately after the last measurement of airway responsiveness. The mice were anesthetized intraperitoneally with 50 mg/kg pentobarbital sodium and were sacrificed by exsanguination.