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Resveratrol preserves cardiac function, but does not prevent endothelial dysfunction or pulmonary inflammation after environmental tobacco smoke exposure

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ABSTRACT

The mechanisms by which environmental tobacco smoke (ETS) causes adverse cardiovascular effects remain unclear. Resveratrol is a natural polyphenol from red wine which may be beneficial to the cardiovascular system. Therefore, the ability of daily oral resveratrol (5 mg/kg) to prevent adverse effects of a 14-day ETS exposure (1 h/day) on endothelial function (flow-mediated dilation), left ventricular function (echocardiography) and blood pressure (oscillometry) was assessed in juvenile male pigs ($n = 4$ pigs/group). After a 14-day exposure to ETS, flow-mediated dilation was impaired while plasma nitrotyrosine was increased compared to sham-exposed pigs indicating impaired endothelial function. In ETS-exposed pigs, plasma C-reactive protein levels, lung cytochrome P4501A1 activity, bronchoalveolar lavage fluid total white blood cell count and leukocyte elastase activity were all significantly increased compared to sham-exposed pigs. Resveratrol treatment failed to prevent most ETS-mediated effects examined, but did increase left ventricular end-diastolic volume and ejection fraction in the presence of ETS exposure. In summary, ETS exposure impaired endothelial function and increased oxidative stress which was associated with pulmonary and systemic inflammation, but resveratrol failed to protect against these changes. More importantly, resveratrol exerted a positive effect on left ventricular function which may help explain the French paradox.

1. Introduction

The adverse health effects of cigarette smoke involve almost every organ system in smokers (Alberg, 2008). However, cardiovascular and respiratory effects are most prevalent including diseases such as atherosclerosis, coronary artery disease, asthma, and chronic obstructive pulmonary disease (COPD) (Bhalla et al., 2009). Passive exposure to tobacco smoke, also called environmental tobacco smoke (ETS) exposure, poses an increased risk for many of these same diseases. In fact, smoking and ETS exposure is considered the number one preventable risk factor contributing to cardiovascular disease.

Endothelial cells play a key role in the regulation of vascular tone and impairment of normal endothelial function is thought to be an early step in the development of all cardiovascular diseases, including in smokers (Rahman and Laher, 2007; Peluffo et al., 2009). Flow-mediated dilation (FMD) is an ultrasound technique which measures the endothelium-dependent ability of conduit arteries to relax in response to a shear stress and hypoxia stimulus. FMD has been shown to be impaired in both smokers and non-smokers passively exposed to ETS (Celermajer et al., 1996; Karatzi et al., 2007). In smokers, FMD impairment has been shown to be due to an oxidative stress-mediated decrease in nitric oxide (NO) bioavailability (Celermajer et al., 1996; Karatzi et al., 2007; Peluffo et al., 2009). However, it is not clear if the mechanism of endothelial dysfunction is similar after ETS exposure. Also, it is not known whether agents that combat oxidative stress will rescue endothelial function and FMD responses after ETS exposure.

Cigarette smoke-induced oxidative stress is a major pathway contributing to the development of cardiovascular disease (Rahman and Laher, 2007). Of the more than 4000 compounds found in cigarette smoke, numerous classes of toxic chemicals have been implicated in causing this increased oxidative stress including reactive metabolites of polycyclic aromatic hydrocarbons (PAHs)

Abbreviations: AHR, aryl hydrocarbon receptor; BAL, bronchoalveolar lavage; CO, carbon monoxide; CO-Hb, carboxyhemoglobin; COPD, chronic obstructive pulmonary disease; CRP, C-reactive peptide; CYP1A1, cytochrome P450 1A1; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; ETS, environmental tobacco smoke; FMD, flow-mediated dilation; Met-Hb, met-hemoglobin; NO, nitric oxide; NOx, nitrate/nitrite; Oxy-Hb, oxygen-hemoglobin; PAH, polycyclic aromatic hydrocarbon.

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via cytochrome P450 1A1 (CYP1A1) activity (Ding et al., 2008), carbon monoxide (CO) (Thom et al., 1997) and nicotine (Rahman and Laher, 2007). In addition to lung inflammation, epidemiologic studies have also shown that cigarette smokers have higher levels of C-reactive peptide (CRP) in their serum compared to non-smokers (Antoniades et al., 2004), indicating activation of acute systemic inflammation pathways. Individuals with high CRP serum levels exhibit increased oxidative stress (van der Vaart et al., 2004) and are at increased risk of developing cardiovascular diseases (May and Wang, 2007). Therefore, not only is increased lung inflammation linked to lung dysfunction (Chan-Yeung and Dimich-Ward, 2003; Salvi and Barnes, 2009) and eventual development of emphysema (Sharafkhaneh et al., 2008), but may also serve as a source of systemic inflammatory mediators that increases systemic oxidative stress and impairs endothelial function.

The "French paradox" was coined almost twenty years ago and originated with an epidemiologic study that reported low mortality among the French population from coronary heart disease compared to North Americans, despite much higher smoking rates (Renaud and de Lorgeril, 1992). This difference was attributed to the higher French consumption of red wine (Renaud and de Lorgeril, 1992). Resveratrol is a polyphenolic compound found in the skin and seeds of red grapes with reports of antioxidant (Kawada et al., 1998), anti-inflammatory (Donnelly et al., 2004), and anti-carcinogenic properties (El-Attar and Virji, 1999). Since then there have been many reported benefits of resveratrol including attenuation of cigarette smoke-induced oxidative stress, decreased proinflammatory phenotypic alterations within endothelial cells (Csiszar et al., 2008) and prevention of acute endothelial dysfunction in healthy smokers (Karatzi et al., 2007). However, there are also increasing numbers of studies from independent researchers (Turrens et al., 1997), pharmaceutical companies (Beher et al., 2009; Pacholec et al., 2010) and a comprehensive review of clinical studies that have firmly questioned resveratrol's effectiveness (Karatzi et al., 2009). Therefore, the major goal of this study was to confirm whether resveratrol would counteract ETS-mediated cardiovascular effects and support the French paradox.

To address these questions, a 2×2 factorial study design using daily 1-h sham or ETS exposure alone or in combination with daily oral resveratrol for 14 days was examined in juvenile pigs. Effects of ETS and resveratrol alone or in combination on cardiovascular function were assessed and related to changes in blood gases, blood markers of nitric oxide production (serum nitrate/nitrite), oxidative stress (plasma nitrotyrosine), inflammation (serum C-reactive peptide, bronchoalveolar lavage cytology, neutrophil elastase) and PAH exposure (CYP1A1 activity). In addition, histological analyses were performed on the abdominal aorta, brachial, and coronary arteries as well as histopathological evaluation of lung, liver and heart.

2. Materials and methods

2.1. Animals and experimental design

All protocols were in accordance with the Canadian Council on Animal Care guidelines and were approved by the Animal Care and Use Council at the University of Saskatchewan. Castrated male pigs (10–12 kg) were obtained from Prairie Swine Center (Saskatoon, SK) and randomized into 4 groups i.e. sham, resveratrol, ETS and resveratrol + ETS (4 pigs/group). The pigs were group-housed with each treatment group kept in separate pens under a 12 h dark/12 h light cycle. Pigs were fed normal pig starter chow (Federated Co-Operatives Ltd., Saskatoon, Canada) and water *ad libitum* except during exposure, blood collection or cardiovascular assessment.

2.2. Environmental tobacco smoke (ETS) and resveratrol exposure

A single cigarette manual smoking machine from CH Technologies Westwood, USA) was used to generate ETS (mainstream plus side stream smoke). The machine was adjusted to a rate of 3 puffs/min with 57 ml/puff of 2 s duration. The ETS, mixed with unfiltered indoor air, was then pumped into a 500 gallon polyethylene plastic water tank modified to include inflow and outflow ports and a sealed, removable plexiglass door in which whole body exposures were conducted in unsedated, unrestrained pigs (ETS plus resveratrol and ETS without resveratrol groups exposed at the same time). Pumps controlling inflow were set at 6 L/min. A total of 12 cigarettes (Canadian Classics, Rothmans, Benson & Hedges, Canada) were burned over 1 h every day for 14 days. Sham exposed pigs (+/− resveratrol groups at the same time) were placed in the same cleaned chamber for 1 h/day, but with an unlit cigarette attached to the smoking machine. The total particulate concentration in the chamber was assessed for ETS- and sham-exposed pigs using a SKC constant airflow pump (Universal 224-PCXR, Eighty Four, PA) fitted with pre-weighed mixed cellulose-ester filter (0.08 μm, SKC Inc., Eighty Four, PA). Total particulates were sampled continuously for 1 h at 2 L/min. Carbon monoxide levels were also monitored in using a T40 Rattler CO monitor (Industrial scientific, Corp., Oakdale, USA) placed inside the chamber. The air levels of O_2/CO_2 within the chamber were measured using a Criticare Poet IQ multiparameter gas monitor (Criticare Systems, Inc., Waukesha, USA). Resveratrol-treated pigs were force-fed resveratrol (5 mg/kg; Megaresveratrol, Danbury, CT) in gelatin capsules for 14 days.

2.3. Flow-mediated dilation (FMD), echocardiography, blood pressure, and blood gas evaluation

Immediately after each sham or ETS exposure, pigs were sedated by receiving an intramuscular injection of azaperone (2.2 mg/kg, Stresnil™ Merial Inc., Canada). The left brachial artery was visualized using a SonoSite 180 Plus ultrasound unit with a 5.0 MHz linear array transducer (SonoSite Canada Inc., Markham, Ont., Canada). The probe was placed on the medial aspect of the distal third of the left radius, approximately 10 cm from the axilla. The blood pressure cuff was applied directly distal to the ultrasound area. The brachial artery was visualized at baseline (unoccluded). The blood pressure cuff was then inflated to ~30 mmHg above systolic pressure for 4 min. Following cuff release the brachial artery was visualized for 150 s. All ultrasound views were recorded using a digital video camera for the duration of the ultrasound time and uploaded to a computer. Single digital images of the brachial artery were created when the artery showed the smallest diameter during each pulse wave at baseline and 90 s after cuff release using Adobe Premiere Elements (Adobe Inc., San Jose, CA). The perimeter (P) of the brachial artery at each time point was traced and measured using Image-Pro Plus (Media Cybernetics Inc., Bethesda, MD) and converted to a diameter using the formula: diameter = P/π. The diameter of the brachial artery at baseline and 90 s were used to calculate flow-mediated dilation (FMD). All measurements of digital ultrasound images were performed blinded. FMD was calculated using the formula:

$$\%\text{FMD} = 100\% \times [(90\text{ s post-release diameter} - \text{baseline diameter})/(\text{baseline diameter})]$$

The left ventricular end-systolic volume (ESV) and end-diastolic volume (EDV) were measured using a HP SONOS 100CF ultrasound machine. A 5.0 MHz cardiac transducer was used in the right parasternal long-axis view to visualize the left ventricular outflow tract in B-mode during systole (smallest ventricular volume during a cardiac cycle) and diastole (largest volume). Ventricular volume was measured from the 2-dimensional area and the chamber length using the following equation:

$$\text{Volume} = 5/6\ \text{Area} \times \text{Length}$$

For each end point two measurements were taken and then averaged. The ejection fraction (EF) was calculated using the formula:

$$\text{EF} = [(\text{EDV} - \text{ESV})/(\text{EDV})] \times 100\%$$

After vascular and cardiac ultrasound, a Memo Diagnostic High Definition Oscillometer (S + B medVET, Markham, ON) was placed on the distal end of the right hind limb to measure systolic pressure, diastolic pressure, mean arterial pressure (MAP) and pulse pressure. The mean of at least 5 consecutive blood pressure readings from each pig was used from a given session. Finally, blood gases were measured in venous blood collected from the jugular vein into blood gas syringes (Radiometer PICO 50; Radiometer; Copenhagen, Denmark). Oxygenated hemoglobin (oxyHb), carboxy-hemoglobin (CO-Hb), methemoglobin (MetHb) and total hemoglobin (TotalHb) levels were measured using a Rapidlab 865 blood gas analyzer (Bayer Diagnostics, East Walpole, MA, USA). Ultrasound, blood pressure and blood gas measurements were taken at baseline (*i.e.* Day 0 or prior to starting the exposures), day 1, day 7, and day 14, of the experiment.

2.4. Plasma nitrate/nitrite, cotinine, nitrotyrosine, C-reactive peptide (CRP), and ethoxyresorufin-o-deethylase (EROD) activity

Blood samples were collected from all pigs at baseline and on the last day of experiments into EDTA or serum vacutainers for plasma and serum, respectively, and stored on ice until spun at 4000*g* for 10 min. Plasma and serum were aliquoted, then stored at −80 °C until use in assays. Plasma nitrate/nitrite (NO*x*) levels were assessed using a commercially available enzyme-based kit (Nitric Oxide Quantitation Kit, Active Motif North America, Carlsbad, CA). Plasma cotinine (Bio-Quant, Inc., San Diego, CA), plasma nitrotyrosine (Cell Sciences, Canton, USA) and serum C-reactive peptide (CRP; Geneway Biotech, CA) levels were measured using