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Alpha B-crystallin prevents the arrhythmogenic effects of particulate matter isolated from ambient air by attenuating oxidative stress

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ABSTRACT

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is activated by particulate matter (PM) isolated from ambient air and linked to prolonged repolarization and cardiac arrhythmia. We evaluated whether alpha B-crystallin (CryAB), a heat shock protein, could prevent the arrhythmogenic effects of PM by preventing CaMKII activation. CryAB was delivered into cardiac cells using a TAT-protein transduction domain (TAT-CryAB). ECGs were measured before and after tracheal exposure of diesel exhaust particles (DEP) and each intervention in adult Sprague–Dawley rats. After endotracheal exposure of DEP (200 μg/mL for 30 minutes, $n=11$), QT intervals were prolonged from 115 ± 14 ms to 144 ± 20 ms ($p=0.03$), and premature ventricular contractions were observed more frequently (0% vs. 44%) than control ($n=5$) and TAT-Cry ($n=5$). However, DEP-induced arrhythmia was not observed in TAT-CryAB (1 mg/kg) pretreated rats ($n=5$). In optical mapping of Langendorff-perfused rat heats, compared with baseline, DEP infusion of 12.5 μg/mL ($n=12$) increased apicobasal action potential duration (APD) differences from 2 ± 6 ms to 36 ± 15 ms ($p<0.001$), APD restitution slope from 0.26 ± 0.07 to 1.19 ± 0.11 ($p<0.001$) and ventricular tachycardia (VT) from 0% to 75% ($p<0.001$). DEP infusion easily induced spatially discordant alternans. However, the effects of DEP were prevented by TAT-CryAB (1 mg/kg, $n=9$). In rat myocytes, while DEP increased reactive oxygen species (ROS) generation and phosphated CaMKII, TAT-CryAB prevented these effects. In conclusion, CryAB, a small heat shock protein, might prevent the arrhythmogenic effects of PM by attenuating ROS generation and CaMKII activation.

Introduction

Particulate air pollution is an environmental health risk factor that is associated with increased cardiovascular morbidity and mortality. Epidemiologic studies have suggested that elevated ambient particulate matter (PM) concentrations are strongly associated with increases in hospital admissions, atherosclerosis, episodes of acute ischemia, increased blood pressure and decreased heart rate (Brook et al., 2004; Peters et al., 2001; Pope et al., 2004). A recent epidemiologic study has also demonstrated that the risk of mortality associated with life-threatening arrhythmias increases in relation to 7-day mean levels of black smoke and PM_{10} (particles measuring 10 μm or less) (Hoek et al., 2001). In addition, studies in patients with implanted cardioverter-defibrillators have shown significant associations between air pollution and ventricular arrhythmias (Dockery et al., 2005). A more recent study by Ljungman et al. (2008) further reported that the incidence of ventricular arrhythmias increased with elevated levels of PM_{10} in as quickly as 2 hours.

Both the size and composition of PM are relevant to resultant cardiopulmonary toxicity. Fine particles (diameter <2.5 μm) and ultrafine particles (diameter <0.1 μm) remain airborne for long periods of time (Xiong and Friedlander, 2001), penetrate deeply into the respiratory tract and can carry large amounts of toxic compounds, such as hydrocarbons and metals on their surfaces. Ambient ultrafine particles are the most abundant particles in urban environments. A large portion of ambient ultrafine particles are composed of diesel exhaust particles (DEP) (Tobias et al., 2001). The common constituents of PM include

Abbreviations: APD, action potential duration; CAMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CL, cycle length; CryAB, alpha B-crystallin; DEP, diesel exhaust particles; EAD, early afterdepolarization; LV, left ventricle; PM, particulate matter; PVC, premature ventricular contractions; ROS, reactive oxygen species; SCA, spatially concordant alternans; SDA, spatially discordant alternans; VF, ventricular fibrillation; VT, ventricular tachycardia.

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nitrates, sulfates, elemental and organic carbon, organic compounds (e.g., polycyclic aromatic hydrocarbons), biological compounds (e.g., endotoxin, cell fragments), and a variety of metals (e.g., iron, copper, nickel, zinc, and vanadium) (Tobias et al., 2001). Despite evidences for the arrhythmogenic action of PM, the underlying arrhythmogenic mechanisms of PM are still poorly understood.

Oxidative stress increases following exposure to elevated levels of ambient particles (Cozzi et al., 2006). Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is activated by enhanced intracellular Ca^{2+} from β-adrenergic receptor stimulation (Zhang et al., 2005). However, recently, it was revealed that oxidation of paired regulatory domain methionine residues sustains CaMKII activity in the absence of Ca^{2+}/CaM (Erickson et al., 2008). CaMKII activation also prolongs action potential duration (APD) and induces afterdepolarization in cardiomyocytes by impairing I_{Na} inactivation and enhancing I_{CaL} (Xie et al., 2009). In the previous study, we found that PM can cause arrhythmia via oxidative stress and CaMKII activation (Kim et al., 2012).

Heat shock proteins (HSP) play an essential role in numerous diseases. Their function is necessary for the homeostasis of the living cell, when cells are faced with stressful environments including oxidative stress (Benjamin and McMillan, 1998). Alpha B-crystallins (CryAB) can be induced by heat shock and are members of the small HSP 20 family. CryAB is abundantly expressed in the ocular lens, heart and skeletal muscle (Kappe et al., 2003). CryAB is the most abundant small HSP in cardiomyocytes. Several studies suggested that the CryAB has the antioxidative effects (Golenhofen et al., 2006; Rajasekaran et al., 2007). We hypothesized that CryAB could decrease oxidative stress and CaMKII activation caused by PM. Second, CryAB could prevent the arrhythmogenic effects of PM. Finally, CryAB could also attenuate the repolarization gradient and triggered activity. To prove this hypothesis, we evaluated the arrhythmic events of DEP after pretreatment with CryAB using in vivo model and in vitro model. We also evaluated levels of DEP induced oxidative stress and CaMKII activity after pretreatment with CryAB. CryAB was delivered into cardiac cells using a TAT-protein transduction domain (TAT-CryAB).

Methods

This study protocol was approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine and Cardiovascular Research Institute, and conformed to the guideline for the care and use of laboratory animals published by the United States National Institutes of Health. We used commercially available DEP (SRM1650b, National Institute of Standards Technology, USA).

Construction of TAT-CryAB

The CryAB fragment was amplified by PCR using the human cDNA library as a template and PCR primers 5′-CACCTAGAATTCATGGACATCG CCATCCAC-3′ (upstream primer, the underlining indicates the *Eco*RI site) and 5′-AAGAAACTCGAGCTATTTCTTGGGGGCTGC-3′ (downstream primer, the underlining indicates the *Xho*I site). The CryAB fragment was inserted into the EcoRI and XhoI sites of the pHis/TAT vector (Kwon et al., 2007) for TAT-CryAB fusion protein expression. Plasmid constructs were confirmed by both restriction enzyme mapping and DNA sequence analyses.

Expression and purification of TAT-CryAB protein

Escherichia coli BL21 (DE3) transformed with recombinant plasmids was grown in LB broth at 37 °C. Protein expression was induced by the addition of 1 mM IPTG for 4 hours. The bacterial pellet was harvested by centrifugation and resuspended in buffer Z (8 M urea, 100 mM NaCl, and 20 mM HEPES, pH 8.0). The clarified lysate was loaded onto a Ni-NTA affinity column (Qiagen, Hilden, Germany). His-tagged proteins were eluted with a linear gradient from 100 mM to 1 M imidazole in buffer. The proteins were loaded onto a PD-10 desalting column to facilitate rapid exchange to the phosphate buffered saline (PBS). The recombinant proteins were further purified using endotoxin-removing gel (Pierce, Rockford, IL).

In vitro and in vivo protein delivery

The rat heart-derived myoblast cell line H9c2 was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and was cultured in DMEM/F-12 supplemented with 10% FBS. For the transduction of the TAT-CryAB protein, cells were grown to confluence in six-well plates. The culture medium was replaced with fresh medium containing 10% FBS and was then treated with the TAT-CryAB protein. The intracellular transduction of the TAT-CryAB protein was confirmed by immunoblot analysis using the anti-His-tag antibody. TAT-CryAB protein (1 mg/kg) was also intraperitoneally injected into Sprague–Dawley (SD) rats. After 2 hours, the heart was isolated and transduced TAT-CryAB was detected by immunoblot analysis using anti-His-tag antibody.

In vivo exposure of DEP

Adult male SD rats (250–300 g) were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After endotracheal intubation and mechanical ventilation (room air, rate 60 cycles/min, tidal volume 1 mL per 100 g of body weight, Harvard Apparatus Rodent Ventilator, model 683), ECG lead II was recorded continuously. In 11 rats (DEP group), after baseline recording, DEP dissolved in 0.1 ml PBS was given via endotracheal intubation at the concentrations of 100, 200 and 400 μg/mL. The lung burden of DEP per rat was 10, 20 and 40 μg at the DEP concentrations of 100, 200 and 400 μg/mL, respectively. For the control ($n=5$) and TAT-CryAB group ($n=5$), the same amounts of PBS or TAT-CryB (1 mg/kg) were given via endotracheal intubation, respectively. In the DEP and TAT-CryAB group ($n=5$), DEP were given after intraperitoneal injection of TAT-CryAB (1 mg/kg). In the DEP and TAT-GFP group ($n=5$), DEP were given after intraperitoneal injection of TAT without CryAB. At each concentration, ECG was measured at 5 minutes after the instillation of DEP, and continuously recorded for 30 minutes. The ECGs were manually evaluated by a cardiologist. QT intervals were measured from Q to the end of T in 10 beats and averaged (online supplementary Fig. 1).

Optical mapping and experimental protocol

Thirty adult male SD rats (250–300 g) were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). The chests were opened via median sternotomy and the hearts were rapidly excised and immersed in cold Tyrode's solution (composition in mmol/L: 125 NaCl, 4.5 KCl, 0.25 $MgCl_2$, 24 $NaHCO_3$, 1.8 NaH_2PO_4, 1.8 $CaCl_2$, and 5.5 glucose). The ascending aorta was immediately cannulated and perfused with 37 °C Tyrode's solution equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. The contractility of the heart was inhibited by 10–17 μmol/L of blebbistatin (Fedorov et al., 2007). The hearts were stained with di-4-ANEPPS (Invitrogen, California, USA) and excited with quasi-monochromatic light (520 ± 30 nm) from two green LED lamps. Emitting light was collected by an image-intensified charge-coupled device camera (Dalsa Inc., Waterloo, Canada) with a 610-nm long pass filter.

Optical recordings were performed during steady-state and programmed stimulation. Programmed stimulation was performed with bipolar electrodes at the lateral side of the left ventricle (LV). Action potential duration (APD_{90}) was measured at the base and apex of the LV. Apicobasal APD difference was defined as the difference of APD between the left ventricular base and apex. After the initial electrophysiological study, we attempted to induce ventricular tachycardia (VT) or ventricular fibrillation (VF) using the standard