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Endosulfan induces cell dysfunction through cycle arrest resulting from DNA damage and DNA damage response signaling pathways

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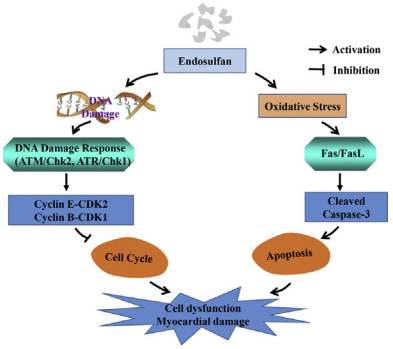
HIGHLIGHTS

- Endosulfan induces DNA damage and activates DNA damage response pathway in human umbilical vein endothelial cells.
- Endosulfan induces endothelial dysfunction in human umbilical vein endothelial cells.
- Endosulfan promoted the cell apoptosis through death receptor pathway resulting from oxidative stress in Wistar rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Our previous study showed that endosulfan increases the risk of cardiovascular disease. To identify toxic mechanism of endosulfan, we conducted an animal study for which 32 male Wistar rats were randomly and equally divided into four groups: Control group (corn oil only) and three treatment groups (1, 5 and 10 mg $kg^{-1} \cdot d^{-1}$). The results showed that exposure to endosulfan resulted in injury of cardiac tissue with impaired mitochondria integrity and elevated 8-OHdG expression in myocardial cells. Moreover, endosulfan increased the expressions of Fas, FasL, Caspase-8, Cleaved Caspase-8, Caspase-3 and Cleaved Caspase-3 in cardiac tissue. In vitro, human umbilical vein endothelial cells (HUVECs) were treated with different concentrations of endosulfan (1, 6 and 12 μg mL^{-1}) for 24 h. An inhibitor for Ataxia Telangiectasia Mutated Protein (ATM) (Ku-55933, 10 μM) was added in 12 μg mL^{-1} group for 2 h before exposure to endosulfan. Results showed that endosulfan induced DNA damage and activated DNA damage response signaling pathway (ATM/Chk2 and ATR/Chk1) and consequent cell cycle checkpoint. Furthermore, endosulfan promoted the cell apoptosis through death receptor pathway resulting from oxidative stress. The results provide a new insight for mechanism of endosulfan-induced cardiovascular toxicity which will be helpful in future prevention of cardiovascular diseases induced by endosulfan.

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1. Introduction

Endosulfan, a kind of agricultural insecticide, was defined as one of persistent organic pollutants (POPs) by the Stockholm Convention in 2011 (Desalegn et al., 2011). However, due to bioaccumulation and migration, endosulfan residues have also been detected in various fruits, vegetables, nuts, grains and fish (Canlet et al., 2013). Data showed that endosulfan has a half-time residual period of 60–800 days in soil giving rise to it as being frequently identified compound in environment (Jia et al., 2010). Hence, it poses a serious threat to agriculture eco-system and human health. Additionally, endosulfan has been proved to have adverse effects on different organ systems including nervous, endocrine, reproductive, developmental and cardiovascular systems (Chan et al., 2006; Ozmen, 2013; Rastogi et al., 2014; Silva et al., 2015).

Cardiovascular diseases (CVD) induce 17.3 million deaths every year all over the world (Moran et al., 2014). A study showed that persistent organic pollutants (POPs) exists largely in individuals with CVD compared to healthy people suggesting a possible association of these compounds with CVD (Ljunggren et al., 2014). Degeneration of myocardium and granular myofibrils with pyknotic nuclei were observed in the heart of rats exposure to endosulfan (Jalili et al., 2007). Furthermore, excessive exposure to endosulfan led to abnormal heart rate and blood pressure according to a case report further evidenced the relevance of endosulfan to CVD (Moon and Lee, 2013). Furthermore, endosulfan can result in CVD via oxidative stress. Previous research indicates that endosulfan leads to a significant increase in the levels of lipid peroxidation and malondialdehyde (MDA), while reduces the antioxidant levels such as superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT) in the cardiac tissue of Wistar rats (Alva et al., 2012; Kalender et al., 2004).

There is evidence that elevated level of DNA damage was found in heart failure patients (Mondal et al., 2013). CVD represent the leading cause of mortality in humans. Endothelial dysfunction has been recognized in CVD as a pathogenetic primary booster of various cardiovascular events that accelerates vascular injury resulting from vascular wall damage and atherosclerotic plaque formation (Cimellaro et al., 2016). It has been known that oxidative stress can lead to DNA damage which plays a vital role in the progression of CVD (Marin-Garcia, 2016). Genomic instability due to DNA lesion occurs when cells incur DNA damage persistently. In response to genotoxic stress eukaryotic cells have evolved the DNA damage response (DDR), a network of signal transduction pathways that can detect and repair DNA damage to maintain genomic integrity (Chen et al., 2016; Palou et al., 2016).

A recent study illustrated that endosulfan induces DNA damage and perturbations in DDR thereby promoting genomic instability in reproductive system (Sebastian & Raghavan, 2016). Our previous study has demonstrated that endosulfan can lead to DNA damage and cell cycle arrest in endothelial cells (Jialiu Wei et al., 2016). However, whether DDR is involved in the process of toxic action in endothelial cells and how it works are still poorly understood. Hence, the current research was designed to further clarify the role of endosulfan on the pathogenesis of cardiovascular diseases. To this end, we investigated the effect of endosulfan on cardiac tissue in Wistar rats, and further explored the toxic mechanism by measuring DNA damage and role of Ataxia Telangiectasia Mutated Protein (ATM)/Ataxia telangiectasia and Rad3 related (ATR)-cell cycle checkpoint kinase-1 (Chk1)/Chk2 signaling pathways in human umbilical vein endothelial cells (HUVECs).

2. Materials and methods

2.1. Animals and experimental design

32 specific pathogen-free (SPF) healthy male Wistar rats were obtained from Beijing Vital River Laboratory Animal Technology Limited Corporation (Animal production license number: SCXK2012-0001) (Beijing, People's Republic of China). The rats with a mean weight of 300–350 g were raised in a standard polysulfone (PSU) box (47 cm × 30 cm × 15 cm) in a ventilated animal care facility. The standard laboratory conditions (12:12 light/dark cycle) for rats was maintained at a temperature of 22 ± 2 °C with relative humidity of 50 ± 5%. The pads for rats were replaced twice per week. The foods of rats were purchased from Beijing Keao Xieli Feedstuff and drinking water ad libitum. All the animal experiments were performed in accordance with the Health Guide of Capital Medical University for the Care and Use of Laboratory Animals, and the protocol was approved by the Committee on the Ethics of Animal Experiments of the Capital Medical University, Beijing, China.

The rats were randomly divided into four groups after one-week adaptation to laboratory conditions: Control group (corn oil only), Group II, Group III and Group IV (receiving 1, 5 and 10 mg kg^{-1} endosulfan per day, respectively). The endosulfan (analytical standard, purity: 96%) consists of two stereoisomers: 70% α- and 30% β-endosulfan approximately, and it was favored by Jiangsu Kuaida Agrochemical Co., Ltd. (Jiangsu, China). The corn oil was purchased from COFCO Food Sales & Distribution Co., Ltd. (Beijing, China). To achieve a proper volume of oral gavage, endosulfan was dissolved in corn oil and treated with a volume of 2 $mg \cdot kg^{-1} \cdot d^{-1}$ via oral gavage. After 21-day exposure, all the rats were sacrificed with an intraperitoneal injection of 7% chloral hydrate. The heart tissue was collected for the further experiments.

2.2. Histopathological study of hearts

The hearts were fixed in formalin (4%) and then embedded in paraffin. The samples were stained with hematoxylin and eosin (H&E) after being sectioned at a thickness of 5 μm (Leica RM2245, Germany). Histopathological changes were examined under an optical microscope (Olympus X71-F22PH, Japan).

2.3. Heart ultrastructure assessment

Fresh heart tissue was excised and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer overnight at 4 °C. Then the tissue was post-fixed in 1% osmium tetroxide for 2 h, followed by being dehydrated with an ethyl alcohol series to 100%. The samples were embedded with epoxy resin to produce a tissue block. Ultrathin sections were cut using an ultramicrotome (Ultracut UCT, Leica, Germany), stained with lead citrate and uranyl acetate, and observed by a transmission electron microscope (Barazani et al., 2014) (JEM2100, JEOL, Japan).

2.4. Immunohistochemistry measurement for 8-OHdG

To investigate the presence of DNA damage, 8-OHdG immunohistochemistry staining was performed on paraffin sections (4-μm thickness). Tissue sections were deparaffinized and rehydrated thoroughly. After being washed with PBS three times for 10 min each, sections were blocked with 1% bovine serum albumin at room temperature for 1 h. Then the sections were incubated with rat anti- 8-OHdG monoclonal antibody (China) for 24 h. After that, the sections were washed in PBS and incubated with the secondary antibody conjugated by HRP. Finally, the sections treated with diaminobenzidine substrate (DAB) for 3 min were counterstained with Hematoxylin Harris. Images were obtained using an optical microscope (Olympus X71-F22PH, Japan). Positive staining of 8-OHdG was analyzed by average integrated optical density (IOD) per stained area (μm^2) (IOD/area) using Image-pro Plus software (Media Cybernetics, United States).

2.5. Cell culture and experimental design in vitro

HUVECs were purchased from Shanghai Institutes for Biological Sciences, China. The cells were incubated in a humid atmosphere (5% CO_2, 37 °C), cultured in a complete medium consists of DMEM (HyClone,