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The role of potassium channel in silica nanoparticle-induced inflammatory effect in human vascular endothelial cells in vitro

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HIGHLIGHTS

- Nano-SiO_2 can induce cytotoxicity and inflammation in HUVECs.
- PCBs alleviated nano-SiO_2-induced cytotoxicity and inflammatory response in HUVECs.
- The activations of potassium channel were associated with nano-SiO_2-induced inflammation and cytotoxicity in HUVECs.

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ABSTRACT

Exposure to nanoparticles became popular in industry and daily life. Nano-SiO_2 was shown to have an adverse effect to vascular endothelial cell although the mechanisms remain unclear. To test whether the nano-SiO_2's harmful effect was related to the potassium channel, human umbilical vascular endothelial cells (HUVECs) were treated with nano-SiO_2 in different dose. Cell survival rate and lactate dehydrogenase (LDH) as cytotoxic parameters, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) as inflammation indicators were determined. The electrophysiological changes and function of potassium channel were detected with patch clamp and channel blockers. It was found that nano-SiO_2 exposure decreased cell survival rate, increased LDH leakage, TNF-α and IL-6 production. The potassium channel activity was increased in the opening rate and current intensity. Furthermore, potassium channel blockers tetraethylammonium (TEA), 4-amino pyridine (4-AP), and margatoxin (MGTX) reduced the nano-SiO_2-induced cytotoxity and inflammation, i.e., increase in the cell survival rate, and decrease in the LDH leakage and production of TNF-α and IL-6. It might be concluded that the nano-SiO_2-induced inflammation and cytotoxicity at HUVECs was associated with the activation of potassium channel.

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1. Introduction

Nanoparticles were defined as particles whose aerodynamic diameter were less than 100 nm, and also called ultrafine particles (UFPs) in the occupational and environmental research field (Delfino et al., 2005). Because of physicochemical properties such as small size, large surface area as well as high reactivity (Oberdörster et al., 2005), their biological activity and health effects will depend on those not routinely considered in toxicity studies (Sayes et al., 2011; Paino et al., 2010). Nanoparticles were able to penetrate pulmonary blood barrier and enter into the systemic circulation (Terzano et al., 2010; Oberdörster et al., 2004; Nilius and Droogmans, 2001), and the vascular endothelial cells (VECs) therefore became the direct targets. The particles could induce vascular inflammation and injury, leading to some chronic cardiovascular diseases such as atherosclerosis (Gojova et al., 2009; Kreyling et al., 2002). It was already known that Nalp3 inflammasone (as a protein complex studied mostly), composed of the Nod-like receptor (NLR) protein Nalp3, Cardinal, the adaptor ASC, and caspase-1, was implicated in the production of some cytokines in response to the signals by a variety of agents, and mediated the inflammatory reactions (Dostert et al., 2008; Martinon et al., 2009). For the activation of Nalp3 inflammasome intracellular K^+ outflow, namely reduced intracellular K^+ concentration, was one of the preconditions (Dostert et al., 2008; Pétrilli et al., 2007).

However, it was not certain whether this K^+ outflow was through the potassium channel. In VECs, potassium channels are responsible for maintaining the resting membrane potential. There are four types of potassium channels in the VECs: voltage-gated (K_v); Ca^{2+}-activated (K_{Ca}); ATP-sensitized (K_{ATP}); and inward rectified (K_{ir}) (Nilius and Droogmans, 2001). The role of K_v and K_{ir} in setting or modulating the membrane potential was well recognized (Fan and Walsh, 1999; Ince et al., 1987), and K_v involved

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also in endothelial cells' function (Nilius and Droogmans, 2001). Furthermore, it was confirmed that blockade of outwardly rectifying potassium channels (K_{or}) by 4-AP or increasing extracellular K^+ concentration could inhibit phorbol myristate acetate (PMA)-induced inflammatory cytokine (TNF-α, IL-8) production in human macrophages, suggesting that K^+ and its regulation through K_{or} were the early inflammatory events following PMA stimulation (Qiu et al., 2002). By use the patch clamp, our previous-laboratory study showed that quartz was able to activate the outward delayed potassium channel, which might act as a signal to initiate the inflammatory response in macrophages (Sun et al., 2009). It seemed that potassium channels, outward potassium channels especially, might be involved in the activation process of inflammatory response or in the activation of inflammasome directly. Dysfunction of potassium channels in vascular endothelial cells (VECs) was related to the pathogenesis and pathomechanism of some cardiovascular diseases, such as hypertension, atherosclerosis, and septic shock (Baranowska et al., 2007). However, the research on the outward potassium channels in VECs is rare. How the inflammatory reaction is induced by nanoparticles is still unclear. In addition, the study early was carried out separately, i.e., by either toxicological test or physiological measurement of the channel. In this way, it is difficult to assess the relation between channel function and harmful effect.

Nano-SiO_2 is a kind of important nanoparticles, which has good stability, easy dispersibility, and melting degeneration, and is widely used in rubber, paints, biomedical and biotechnology fields (Baun et al., 2008). The aim of this study was to investigate whether nano-SiO_2 could activate the potassium channel, and thereby to influence K^+ outflow and the inflammation in VECs. More importantly, the outward potassium channel was studied by patch clamp and channel blockers at the same time, as a regulatory mechanism to explore how was it related to the inflammation response in VECs. The human umbilical vein endothelial cells (HUVECs) that were generally used as a cellular model to investigate the cardiovascular system were employed in this study.

2. Materials and methods

2.1. Reagents

HUVECs were purchased from Promoter Biological Ltd (Wuhan, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were obtained from Gibco (Carlsbad, CA, USA). Tetraethylammonium (TEA), 4-aminopyridine (4-AP) and Margatoxin (MGTX) were purchased from Sigma (St. Louis, MO, USA), Tocris Cookson Ltd (Bristol, UK) and Alomone (Jerusalem, Israel), respectively. The Cell Counting Kit-8 (CCK-8), LDH kit and tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) ELISA kits were obtained from Dojindo (Tokyo, Japan), Jian Cheng Biological Engineering Institute (Nanjing, China) and R&D Systems (Minneapolis, MN, USA), respectively. The rabbit polyclonal antibody was purchased from Alomone (Jerusalem, Israel), Alexa-Fluor-647-conjugated goat anti-body against rabbit IgG was obtained from Cell Signaling Technology Inc (Boston, USA).

Table 1
The characteristics of nano-SiO_2 particles. The stock solution of nano-SiO_2 particles (100 μg/ml) were prepared in culture media (DMEM + 10% FBS) and were measured by transmission electron microscopy (TEM). And the hydrodynamic diameters of nano-SiO_2 particles were determined by dynamic light scattering (DLS) in water and in medium respectively.

Particles (nm)	TEM (in medium)	DLS-measurements	
		(in water)	(in medium)
Silica (VK-SP15)	56.8 ± 14.1	290.1	224.9

Table 2
The ζ-potentials of the nano-SiO_2 were prepared in water and in culture media with the nano-sizer (Malvern Instruments Ltd., UK).

ζ-potential (mV)	In water	In medium
Silica (VK-SP15)	−30.2	−24.6

2.2. Nanoparticles application

The nano-SiO_2 particles (VK-SP15) used in the present study was provided by Wanjing New Material Ltd., Hangzhou, China. Transmission electron microscopy (TEM, Tecnai G2 12, FEI, 300 kV, Philips, Holland) image showed that most of the nano-SiO_2 particles were linear or circular with a mean diameter of 56.8 ± 14.1 nm (Fig. 1 and Table 1). Stock solution (100 μg/ml) of nano-SiO_2 was prepared in water and culture media (DMEM + 10% FBS). The hydrodynamic diameters were determined by dynamic light scattering (DLS) (Malvern Instruments Ltd., UK). The particle size distribution had a wide range from 224.90 nm to 2111 nm due to aggregation. The mean hydrodynamic diameter was 290.1 nm (in water) and 224.9 nm (in culture medium, Table 1). The ζ-potentials were measured by nano-sizer (Malvern Instruments Ltd., UK). The particles showed relatively similar values in water (−30.2 mV) and in medium (−24.6 mV) (Table 2). The nano-SiO_2 suspension was stirred on the vortex agitator before every use.

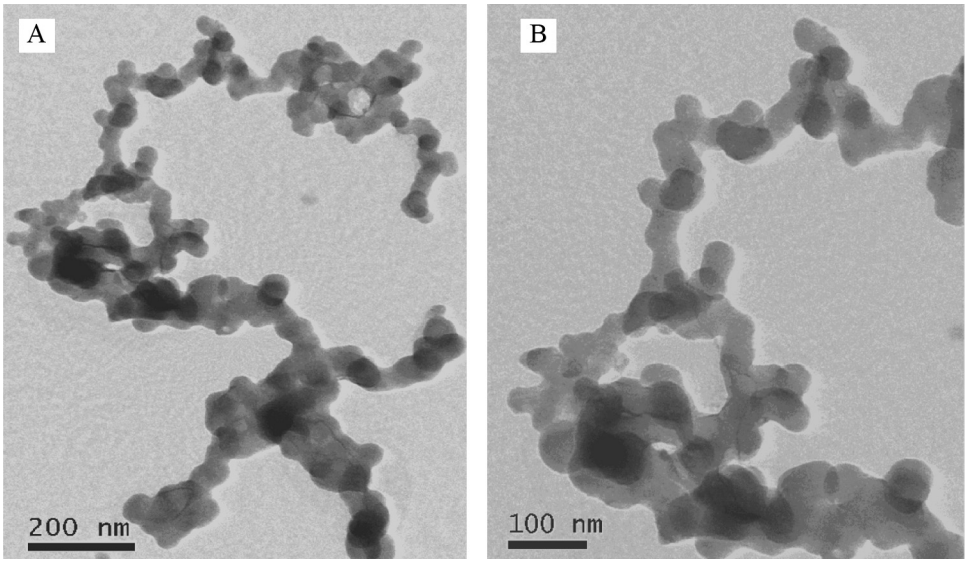

Fig. 1. The morphology of silica nanoparticles. The micrographs of nano-SiO_2 particles were measured by transmission electron microscopy (TEM). (A) The scale bar of Silica nanoparticles (VK-SP15) was 200 nm. (B) The scale bar was 100 nm.

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