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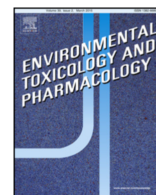

Arsenite suppresses angiogenesis of vascular endothelial cells mediated by Platelet Derived Growth Factor Receptor-beta

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ABSTRACT

The present study aimed to investigate the effects of sodium arsenite ($NaAsO_2$) on the angiogenesis of human umbilical vein endothelial cells (HUVECs) and the mechanism involved. Firstly, a Matrigel-based in vitro angiogenesis assay demonstrated that arsenite suppressed the angiogenesis of HUVECs in a dose-dependent manner. Then by using a global inhibitor for multiple growth factor receptors (E7080) and a specific inhibitor of PDGFR-beta (CP-673451), we found that E7080 completely prevented and CP-673451 significantly decreased the angiogenesis of HUVECs. This suggested that angiogenesis of HUVECs depends on the signal pathway mediated by tyrosine kinase receptors and that among them, PDGFR-beta has an important regulatory function. Finally by using porcine aortic endothelial cells which stably express human PDGFR-beta, we found that arsenite suppressed the angiogenesis mediated by PDGFR-beta. Based on these results, we conclude that arsenite suppressed the angiogenesis of the vascular endothelial cells, that this effect is mediated by PDGFR-beta, and postulate that it might contribute to the injuries of blood vessel in arsenism.

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1. Introduction

High arsenic levels of drinking water in endemic regions remain a major public health concern which affects more than a hundred million people worldwide (Rahman et al., 2009). Chronic exposure to inorganic arsenic has been shown to elicit multiple health disorders, such as skin lesions, cancers of the skin, lung, kidney, bladder, and liver, cardiovascular disease (Chang et al., 2004; Wu et al., 1989) and diabetes mellitus (Liu et al., 2015). The best known adverse effect of long-term arsenic exposure is gangrene, secondary to peripheral vascular disease (Tseng et al., 2007).

Healthy vascular endothelial cells are responsible for the maintenance of vascular integrity, repair of the injured vessel wall, and the neovascularization of damaged tissue (Mannarino and Pirro, 2008). Using human umbilical vein endothelial cells (HUVECs) as an in vitro experimental model it has been found that arsenic inhibits the proliferation of HUVECs by preventing cell cycle progression (Woo et al., 2005). Sodium arsenite enhanced the apoptosis of HUVECs by its effects on mitochondrial function (Shi et al., 2010). Lower concentrations (up to 1 μM) of sodium arsenite increase vascular tube formation (Kao et al., 2003). However, studies about the effects of arsenite on the angiogenesis of HUVECs have not been reported, nor have potential mechanisms been elucidated.

Angiogenesis is a major function of vascular endothelial cells (Hofer and Schweighofer, 2007), and, in tumor angiogenesis is largely mediated by alterations of receptor tyrosine kinase pathways (Zhang and Simons, 2014). They are a diverse group of transmembrane proteins which function by transducing growth factor signals from the external milieu to intracellular processes (Haluska and Adjei, 2001). A multi-targeted tyrosine kinase inhibitor lenvatinib (E7080) has been demonstrated to have anti-angiogenesis activities in colorectal cancer xenografts (Wiegering et al., 2014), in preclinical human thyroid cancer models (Zhang and Simons, 2014) and in patients with advanced solid tumors (Roberts et al., 2005). A selective inhibitor of PDGFR-beta kinase, CP-673,451, has also been shown to be capable of inhibiting PDGF-BB-stimulated angiogenesis in malignant tumors (Roberts et al., 2005). In the present study, we analyzed the effects of arsenite

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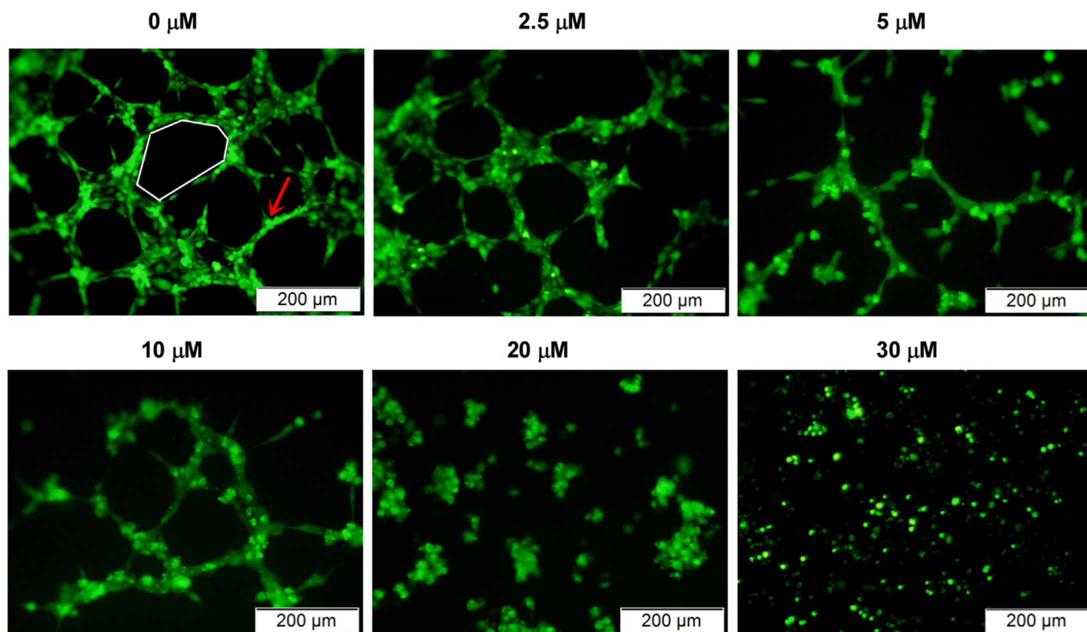

Fig. 1. Effects of arsenite on the angiogenesis of HUVECs.
HUVECs were exposed to different concentration of arsenite, as indicated for 24 h. Representative microscopic fields are shown. The typical branch point and tube length measured by using Image J software were marked by red arrow and white hexagon, respectively.

on the angiogenesis of HUVECs and the possible signals involved in order to explore the mechanisms through which arsenic causes injuries of blood vessels.

2. Materials and methods

2.1. Materials

Human Umbilical Vein Endothelial Cells (HUVECs, Cat. No. 8000) and endothelial cell medium (ECM, Cat. No. 1001) were purchased from the ScienCell Research Laboratories (San Diego, USA). Porcine aortic endothelial cells with stably transfected human PDGF beta-receptors were from Professor Rainer Heuchel, Karolinska Institute, Sweden. IMDM (Cat. No. 21056023) was purchased from Gibco BRL (Rockville, USA). Fetal bovine serum (FBS, Cat. No. SH30071.03) was purchased from HyClone Inc. (Logan, USA). Endothelial cell growth supplement (ECGS, Cat. No. 1052) was purchased from ScienCell Research Laboratories (San Diego, USA). In Vitro Angiogenesis Assay Kit (Cat. No. ECM625) was purchased from Millipore (Billerica, USA). Calcein-AM (Cat. No. sc-203865) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, USA). Sodium arsenite ($NaAsO_2$, CAS No. 7784-46-5) was purchased from Sigma-Aldrich (St. Louis, USA). E7080 (Cat. No. S1164) and CP-673451 (Cat. No. S1536) were purchased from Selleck Chemical (Huston, USA).

2.2. Cell culture and treatments

The HUVECs were grown in ECM medium containing 5% FBS and 1% endothelial cell growth supplement (ECGS). PDGFR-beta/PAE cells were grown in IMDM containing 10% FBS. Both cell types were incubated at 37 °C in 5% CO_2 and a humidified atmosphere. HUVECs were used for all experiments at passages 2–6. For arsenic treatment, the cells were plated in dishes of a 6 cm diameter at a density of 0.5×10^5 cells per dish. After incubating them for 24 h, the medium was exchanged with fresh medium containing various concentrations of arsenite or vehicle and incubated for different time, as indicated in Figs. 1–5 .

2.3. In vitro angiogenesis assay

The angiogenesis of the cells was evaluated by a Matrigel in vitro angiogenesis assay technique. The assay was performed with a detailed procedure as described previously (Mou et al., 2016). Briefly, 100 μl stock solution of Matrigel was added to each well in 48-well plates and kept at 37 °C for 30 min in order to form the Matrigel. Cell suspensions containing 3×10^4 cells in 100 μl of ECM were seeded on the Matrigel of each well, and incubated for 6 h. Then Calcein-AM (0.1 mM) was directly added to each well for 20 min at 37 °C to stain the cells which were imaged under a phase contrast microscope with an excitation wavelength of 490 nm and an emission wavelength of 515 nm. For quantification, the values for the pattern recognition, branch point and total capillary tube length were determined following the manufacturer's guidelines (ECM625; Millipore). Image J software was used in the first instance prior to double-checking by an independent assessor. 5 random microscopic (×100) fields per well were included and the data are expressed as Mean ± SD of 5 samples.

2.4. Statistical analysis

All calculations and statistical analyses were performed by using GraphPad Prism 5.0 software (San Diego, USA). T test was used to analyze the significance of any differences between two groups. The statistical significance was defined as $p < 0.05$.

3. Results

3.1. Arsenite suppressed angiogenesis of HUVECs in a dose-dependent manner

To evaluate the effects of arsenite on the angiogenesis of HUVECs, an in vitro angiogenesis assay was performed for the cells exposed to varying concentration of arsenite for differing periods of time. Fig. 1 shows representative microscopic appearances. Cells not exposed to arsenic displayed morphologic features of angiogenesis, specifically, cells aligned themselves; there was formation

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