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Arsenic causes aortic dysfunction and systemic hypertension in rats: Augmentation of angiotensin II signaling

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ABSTRACT

The groundwater pollutant arsenic can cause various cardiovascular disorders. Angiotensin II, a potent vasoconstrictor, plays an important role in vascular dysfunction by promoting changes in endothelial function, vascular reactivity, tissue remodeling and oxidative stress. We investigated whether modulation of angiotensin II signaling and redox homeostasis could be a mechanism contributing to arsenic-induced vascular disorder. Rats were exposed to arsenic at 25, 50 and 100 ppm of sodium arsenite through drinking water consecutively for 90 days. Blood pressure was recorded weekly. On the 91st day, the rats were sacrificed for blood collection and isolation of thoracic aorta. Angiotensin converting enzyme and angiotensin II levels were assessed in plasma. Aortic reactivity to angiotensin II was assessed in organ-bath system. Western blot of AT_1 receptors and G protein ($G\alpha_{q/11}$), ELISA of signal transducers of MAP kinase pathway and reactive oxygen species (ROS) generation were assessed in aorta. Arsenic caused concentration-dependent increase in systolic, diastolic and mean arterial blood pressure from the 10th, 8th and 7th week onwards, respectively. Arsenic caused concentration-dependent enhancement of the angiotensin II-induced aortic contractile response. Arsenic also caused concentration-dependent increase in the plasma levels of angiotensin II and angiotensin converting enzyme and the expression of aortic AT_1 receptor and $G\alpha_{q/11}$ proteins. Arsenic increased aortic protein kinase C activity and the concentrations of protein tyrosine kinase, extracellular signal-regulated kinase-1/2 and vascular endothelial growth factor. Further, arsenic increased aortic mRNA expression of Nox2, Nox4 and p22phox, NADPH oxidase activity and ROS generation. The results suggest that arsenic-mediated enhancement of angiotensin II signaling could be an important mechanism in the arsenic-induced vascular disorder, where ROS could augment the angiotensin II signaling through activation of MAP kinase pathway.

1. Introduction

Arsenic is widely distributed in the environment and its exposure occurs primarily through contaminated water, food and soil. Arsenic contamination in groundwater has been reported in several countries, including India, Bangladesh, Taiwan, Chile, Argentina and the USA [1,2]. The chronic poisoning caused by high levels of arsenic in well waters led to public health emergency in Bangladesh [3,4]. While arsenic contamination in groundwater in West Bengal, India, was designated as the biggest arsenic calamity in the world [5]. Arsenic exposure was linked to both carcinogenic and non-carcinogenic diseases. Several studies have associated high-level of arsenic exposure from drinking water with elevated risk of vascular diseases, including peripheral vascular disease, hypertension, ischemic heart disease and carotid atherosclerosis [6]. Epidemiological studies reported increased incidence of hypertension in arsenic-exposed populations [7–9]. Further, evidences showed that arsenic induced hypertension in rats [10,11] and mice [12].

Abbreviations: AngII, angiotensin II; ACE, angiotensin converting enzyme; AT_1R, AT_1 receptor; CVD, cardiovascular disease; DAG, diacylglycerol; DBP, diastolic blood pressure; ED, endothelial dysfunction; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase 1/2; iNOS, inducible nitric oxide synthase; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MKHS, Modified Krebs Henseleit Solution; Nox, NADPH oxidase; PBS-T, Tween-20 phosphate buffered-saline; PKC, protein kinase C; PLC, phospholipase C; PTK, protein tyrosine kinase; RLU, relative light unit; ROS, reactive oxygen species; SA, sodium arsenite; SBP, systolic blood pressure; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

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Increased vascular resistance resulting from enhanced vasoconstriction or impaired vasodilation is a hallmark of hypertension. Angiotensin II (AngII) is a potent vasoconstrictor. It plays an important role in hypertension and cardiovascular diseases (CVDs) by promoting changes in endothelial function, vascular reactivity, tissue remodeling and oxidative stress [13]. The predominant effect of AngII in vascular tone is modulated via interaction with the G protein-coupled AT_1 receptors (AT_1Rs). AT_1R-mediated signaling was associated with vasoconstriction, cell growth, inflammation and fibrosis [14,15]. Increased expression of AT_1R in endothelial cells and/or vascular smooth muscle cells (VSMCs) was observed both in patients with ischemic heart disease and in hypertensive rats [16,17]. Further, in an *in vitro* study in mouse aortic endothelial cell line, Hossain et al. [18] reported that arsenic-induced upregulation of AT_1R expression can promote the pathogenic actions of AngII via an aberrant AT_1R signaling pathway, thus could contribute to development of endothelial dysfunction (ED) and hypertension. Besides, ability of acute arsenic exposure to increase AngII level was reported in mouse liver [19]. Hossain et al. [18] also showed that the arsenic-induced AT_1R up-regulation was due to generation of reactive oxygen species (ROS). Arsenite stimulated NADPH oxidase (Nox) of endothelial cells, VSMCs and thoracic aorta causing increased generation of ROS [10,20,21], which activated mitogen activated protein kinase (MAPK) signaling pathways and resulted in vascular endothelial growth factor (VEGF) over-expression [22]. The stress inducer arsenic (sodium arsenite) was shown to activate members of the MAPK family in different cell lines [23–25]. Moreover, Shin et al. [26] observed rapid increase in the levels of phosphorylated ERK1/2, JNK1/2 and p38 MAPK in the sodium arsenite-exposed human keratinocyte cells and suggested arsenic-mediated activation of these major MAPK pathways. Arsenite also activated these MAPKs in human osteosarcoma cells [27].

Activation of upstream regulators by AngII, such as tyrosine kinases, MAPKs, phospholipase C (PLC) and Nox, leads to activation of various signaling pathways that modulate long-term functions of VSMCs [15,28]. AngII activates MAPKs, particularly extracellular signal-regulated kinase 1/2 (ERK1/2), in VSMCs by a protein kinase C (PKC) and tyrosine kinase-dependent pathways [29,30]. Studies in spontaneous hypertensive rats demonstrated that angiotensin converting enzyme (ACE) inhibitors and AT_1R blockers reduced ERK activity, implicating a role for AT_1Rs in enhanced ERK activation in hypertension [31]. VEGF serves as a downstream target for ERK signaling [32,33]. VEGF is a probable essential mediator in AngII-induced vascular inflammation and remodeling. It acts as a pro-inflammatory and pro-arteriosclerotic factor in AngII-induced hypertension [34]. It was demonstrated that the AngII-induced VEGF gene expression and production occurs through an AT_1R-ERK1/2-dependent mechanism [32].

Arsenic was shown to cause concentration-dependent increase in protein tyrosine phosphorylation and subsequent activation of PKC and MAPK in human aortic endothelial cells [35]. Arsenite induced diacylglycerol (DAG) and stimulated PKC activity as an upstream signaling event of MAPK pathway mediating increased VEGF expression and level in porcine smooth muscles cells [36]. Wang et al. [37] reported that VEGF up-regulation in murine saphenous vein endothelial cells was mediated through ERK1/2 and p38MAPK-dependent signaling pathways in response to arsenic.

Given that arsenic has the potential to cause vascular disorder and can upregulate AT_1R expression and ROS generation in an endothelial cell line [18]; it will be of particular interest to examine, in an *in vivo* model, the mechanistic association between arsenic-induced cardiovascular disorders and alteration in angiotensin action. We evaluated in a rat model whether arsenic-induced vascular dysfunction could relate to modulation in AngII signaling. Further, since AngII-induced signal transduction could involve activation of Nox, we examined if the modulation could be associated with alteration in vascular redox homeostasis.

2. Materials and methods

2.1. Chemicals/kits

Sodium (meta)arsenite ($NaAsO_2$) was purchased from Sigma–Aldrich, St. Louis, USA. Primary and secondary antibodies for western blot of AT_1R and $G\alpha_{q/11}$ proteins as well as ELISA kits for measuring AngII level and PKC activity were purchased from Enzo Life Sciences, USA. ELISA kits for protein tyrosine kinase (PTK), ERK1/2 and VEGF were obtained from Blue Gene Biotech Inc., Shanghai, China, while for ACE from Uscn Life Sciences Inc., USA. Chemicals for quantitative Real-Time PCR (qRT-PCR) viz., RNAlater®, Revertaid® Firststrand cDNA synthesis kit were procured from Thermo Fisher Scientific (USA), while SsoFastTM EvaGreen® supermix (2×) from Bio-Rad, USA. All other chemicals used were of analytical or molecular grade.

2.2. Selection of arsenic concentration

Literature shows that to simulate the cardiovascular effects of natural arsenic exposure through drinking water in the rat model – either normal rat (50 and 100 ppm of sodium arsenite [10,11,38,39] or spontaneous hypertensive rat (100 ppm of arsenic [40]) – different concentrations of arsenic were used. In another recent study, Afolabi et al. [41] exposed rats to sodium arsenite (SA) at 50, 100 and 150 ppm for 12 weeks to investigate the association between inorganic arsenic exposure through drinking water and CVDs. Based on these reports, to characterize the dose-response relationship of arsenic-induced vasculotoxic effects, we used 3 concentrations in the current study, where along with 50 and 100 ppm we took a lower concentration of 25 ppm of SA. Based on the weekly water consumption data, the respective arsenic intake (mean ± S.E.) during the course of 13 weeks of SA exposure through drinking water at 25, 50 and 100 ppm concentrations was 1.34 ± 0.08, 2.6 ± 0.14 and 5.10 ± 0.31 mg/kg b.wt./-day. We have already reported the effects on water consumption, food intake and body weights of these rats [42]. The 25 and 50 ppm concentrations of SA did not alter these physical attributes. But, the 100 ppm concentration decreased all these attributes from the 11th week onwards.

2.3. Animals and experimental design

The study was conducted in the apparently healthy adult male Wistar rats (200–230 g) procured from the Laboratory Animals Resource Section of the Institute. All animals were housed in polypropylene cages with chopped wheat straw as the bedding material. They were maintained under standard management conditions and handled as per the Institute Animal Ethics Guidelines. They were given standard pellet feed (Amrut Feeds, Pranav Agro Industries Ltd, New Delhi, India) and provided water *ad libitum* throughout the experiment. Before the experiment, all the animals were kept in the laboratory conditions for a period of 7 days for acclimatization. Rats were divided randomly into 4 groups consisting of 20 each. Animals of Group I received only drinking water, while of groups II, III and IV received 25, 50 and 100 ppm of SA, respectively, through drinking water for 90 consecutive days.

2.3.1. Measurement of blood pressure

Systolic (SBP), diastolic (DBP) and mean arterial (MAP) blood pressure were measured weekly in conscious rats by