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Atorvastatin restores arsenic-induced vascular dysfunction in rats: Modulation of nitric oxide signaling and inflammatory mediators

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ABSTRACT

We evaluated whether atorvastatin, an extensively prescribed statin for reducing the risks of cardiovascular diseases, can reduce the risk of arsenic-induced vascular dysfunction and inflammation in rats and whether the modulation could be linked to improvement in vascular NO signaling. Rats were exposed to sodium arsenite (100 ppm) through drinking water for 90 consecutive days. Atorvastatin (10 mg/kg bw, orally) was administered once daily during the last 30 days of arsenic exposure. On the 91st day, blood was collected for measuring serum C-reactive protein. Thoracic aorta was isolated for assessing reactivity to phenylephrine, sodium nitroprusside and acetylcholine; evaluating eNOS and iNOS mRNA expression and measuring NO production, while abdominal aorta was used for ELISA of cytokines, chemokine and vascular cell adhesion molecules. Histopathology was done in aortic arches. Arsenic did not alter phenylephrine-elicited contraction. Atorvastatin inhibited E_{max} of phenylephrine, but it augmented the contractile response in aortic rings from arsenic-exposed animals. Sodium nitroprusside-induced relaxation was not altered with any treatment. However, arsenic reduced acetylcholine-induced relaxation and affected aortic eNOS at the levels of mRNA expression, protein concentration, phosphorylation and NO production. Further, it increased aortic iNOS mRNA expression, iNOS-derived NO synthesis, production of pro-inflammatory mediators (IL-1β, IL-6, MCP-1, VCAM, sICAM) and serum C-reactive protein and aortic vasculopathic lesions. Atorvastatin attenuated these arsenic-mediated functional, biochemical and structural alterations. Results show that atorvastatin has the potential to ameliorate arsenic-induced vascular dysfunction and inflammation by restoring endothelial function with improvement in NO signaling and attenuating production of pro-inflammatory mediators and cell adhesion molecules.

Introduction

Arsenic as a contaminant in water and food chain or as inhaled particulates in the atmosphere is of great concern to human and animal wellbeing. However, contaminated groundwater is believed to be the major source of arsenic exposure. Human exposure takes place by direct intake of arsenic rich water (*via* drinking or cooked foods) or indirectly through food crops grown using arsenic-contaminated water. Arsenic contamination of groundwater and its impact on human health have been reported from several countries around the world. But the situation is worst in Asia, where groundwater contamination was reported in India, Bangladesh, Nepal, Vietnam, China, Taiwan and Japan (Jiang et al., 2013). A study revealed that one in five deaths in Bangladesh could be attributed to high arsenic exposure through drinking water and the WHO described this as the largest mass poisoning of a population in history (Argos et al., 2010). The magnitude of arsenic contamination is severe in Bangladesh followed by West Bengal, India. Serious health hazards can occur due to drinking of arsenic contaminated water for a period of about 5–15 years, but the duration can be even 2–5 years for high level exposure (Roy et al., 2013). Arsenic exposure was linked to increased risks of cancer and nonmalignant diseases, including cardiovascular diseases (CVDs) like hypertension, ischemic heart disease, atherosclerosis and peripheral vascular disease (Stea et al., 2014).

Abbreviations: ACh, acetylcholine; ATV, atorvastatin; CMC, carboxymethyl cellulose; CRP, C-reactive protein; CVD, cardiovascular disease; cNOS, constitutive nitric oxide synthase; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal regulated kinase; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; IL-1β, interleukin-1 beta; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; LDL, low density lipoprotein; MCP-1, monocyte chemoattractant protein-1; MMAsIII, monomethyl arsenous acid; NO, nitric oxide; PE, L-phenylephrine; p-eNOS, phosphorylated-eNOS; sGC, soluble guanylyl cyclase; cGMP, cyclic nucleotide of guanosine monophosphate; sICAM, soluble intercellular adhesion molecule-1; SMCs, smooth muscle cells; SNP, sodium nitroprusside; TNF-α, tumor necrosis factor alpha; VCAM, vascular cell adhesion molecule; VED, vascular endothelial dysfunction.

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The endothelial cells (ECs) of blood vessels regulate vascular homeostasis by balancing production of vasodilators, including nitric oxide (NO) and vasoconstrictors. Vascular endothelial dysfunction (VED) is an early process in the event of various CVDs and it could result from impairment in NO activity (Virdis et al., 2010). Inflammation plays a pivotal role in VED. Arsenic produces or exacerbates inflammatory states and oxidative stress (States et al., 2009). Arsenic can enhance or reduce NO production, depending on the type of cell, species and dose (Gurr et al., 2003). Arsenite can suppress relaxation of rat blood vessels by inhibiting endothelial NO synthase (eNOS) activity (Lee et al., 2003). In humans, long-term exposure to arsenic-contaminated drinking water reduces NO production in the ECs partially due to inhibition of eNOS activity (Kumagai and Pi, 2004). Reduced bioavailability of endothelial NO is involved in the initiation and progression of atherosclerosis (Li and Forstermann, 2009). Deficiency of eNOS can accelerate atherosclerotic lesion formation in eNOS knockout mice (Kawashima and Yokoyama, 2004). Inducible NO synthase (iNOS) is induced in diseases associated with inflammation and oxidative stress (Sun et al., 2010). iNOS is up-regulated by arsenic and may contribute to the inflammatory response, increased reactive oxygen species generation, vascular remodeling, decreased aortic blood flow, attenuation of endothelium-dependent relaxation and endothelial cell damage (Sharma and Sharma, 2013; Steed et al., 2010).

Statins are the most effective and best-tolerated drugs for treating dyslipidemia and lowering cardiovascular risk associated with the elevation of low density lipoprotein (LDL) cholesterol. They are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyzes an early, rate limiting step in cholesterol biosynthesis (Bersot, 2011). They reduce the risk of both primary and secondary coronary heart disease, myocardial infarction, stroke and peripheral artery disease by lowering the LDL cholesterol and improving lipid profile. However, the clinical benefits of statin therapy may also be attributed to mechanisms independent of their cholesterol-lowering effects. The multiple cardioprotective effects of these drugs are ascribed to their pleiotropic effects, *viz*., anti-inflammatory, antioxidative, antiproliferative and immunosuppressive properties. Further, statins can increase NO bioavailability, neovascularization of ischemic tissue, circulation of endothelial progenitor cells and fibrinolysis, provide stability to atherosclerotic plaque, prevent platelet aggregation and thrombus formation and normalizes sympathetic outflow (Liao and Laufs, 2005). Efficacy of statins in reducing the risks of cerebrovascular diseases (Amarenco and Labreuche, 2009) and perivascular diseases (Aung et al., 2007) in humans was demonstrated.

Statins are prescribed for treating hyperlipidemia or atherosclerosis and once statin therapy is initiated, it is mostly continued for life. When a drug is being taken daily for a life-long period for treating hyperlipidemia and minimizing the risk of associated CVDs, if the same drug simultaneously possesses efficacy against arsenic-induced vascular dysfunction, it might provide an ameliorative measure for chronic arsenicosis. There is no proven treatment for chronic arsenic toxicity. Safe water, nutritious food, fruits, vegetables and physical exercise are the only preventive measures to combat arsenicosis. We wanted to understand whether statins could be a potential remedial measure for arsenic-induced VEDs. Biswas et al. (2010) demonstrated that atorvastatin (ATV) and N-acetyl cysteine treatment can synergistically recover the arsenic-induced death signaling in rat erythrocytes. Given that arsenic can induce and aggravate vascular disorders and statins have beneficial effects in CVDs, we assumed that statins could mitigate the risk of arsenic-induced development of VED, which eventually leads to development of CVDs in the arsenic-exposed subjects. Therefore, we evaluated whether ATV, an extensively prescribed statin, can reduce the risk of arsenic-induced vascular inflammation and dysfunction in rat model and whether the ATV-mediated modulation could be linked to improvement in vascular NO signaling.

Materials and methods

Drugs/kits/chemicals. Sodium-*m*-arsenite (94%), acetylcholine (ACh), L-phenylephrine (PE) and sodium nitroprusside (SNP) were procured from Sigma-Aldrich, St. Louis, MO, USA. Atorvastatin (ATV) was purchased from Vivan Life Sciences, Mumbai (India). The EIA kits for assessing the levels of pro-inflammatory mediators, cell adhesion molecules, C-reactive protein (CRP) and eNOS were procured from Blue Gene Biotech, Inc., Shanghai, China. The Pathscan®136 #ser1177 phospho-eNOS sandwich ELISA kit was purchased from Cell Signaling Technology, Inc., USA. Chemicals for quantitative Real Time PCR *viz*., RNAlater®, Ribozol® were procured from Qiagen (Germany) and Amresco (USA), respectively, while Revertaid® Firststrand cDNA synthesis kit, Thermo Scientific Maxima SYBR Green/Fluorescein qPCR Master Mix (2X) from Thermo Fisher Scientific (USA). Carboxymethyl cellulose (CMC) sodium salt was purchased from G.S. Chemical Testing Lab and Allied Industries, Bombay. All other chemicals were of analytical or molecular grade.

Dose/concentration selection. The dose of ATV was selected from our earlier work, where effects of ATV on sepsis-induced VED were examined in rat thoracic aorta and pulmonary arteries (Subramani et al., 2009). There ATV at 10 mg/kg bw orally restored the impaired vascular endothelium-dependent relaxations mediated by NO and endothelium-derived hyperpolarizing factors. So we used ATV @ 10 mg/kg bw. Literature reveals that substantial progress in understanding the cardiovascular effects of arsenic has resulted from the studies conducted on specific genetic mouse models (*viz*., apolipoprotein E and low-density lipoprotein receptor double knockout or apolipoprotein E knockout mice) that make the mouse highly susceptible to the cardiovascular disorders (Simeonova et al., 2003; States et al., 2009). In these models, lower concentrations of arsenic through drinking water were shown to exacerbate vascular disorders. However, to simulate the natural arsenic exposure-mediated vascular disorders in a rat model – either normal rat (50 and 100 ppm of sodium arsenite; Sharma and Sharma, 2013; Yang et al., 2007) or even spontaneous hypertensive rat (100 ppm of arsenic; Cheng et al., 2011) – higher concentrations of arsenic were used. In a study on male Wistar rats, exposure to sodium arsenite at 50 ppm through drinking water for 200 successive days induced hypertension from the 80th day onwards, indicating development of vascular dysfunction (Yang et al., 2007). Since we exposed the male Wistar rats to arsenic for a subchronic duration of 90 days, we used 100 ppm of sodium arsenite to induce vascular dysfunction in order to examine the ameliorative effects of ATV. Further, this concentration is also relevant from an environmental perspective. The range of arsenic concentrations found in natural waters around the world vary from <0.0005 to >5 ppm (Rahaman et al., 2013). But there is a report that in West Bengal, India, people were exposed to arsenic-contaminated water in the range of 0.05–14.2 ppm (Guha Mazumder and Dasgupta, 2011). In the current study, the 100 ppm sodium arsenite used is equivalent to 57.7 ppm of elemental arsenic, which is about 4 times the 14.2 ppm environmental contamination level. Thus, considering the heterogeneity of drinking water resources and interspecies variation, the findings of the current study would be useful for risk assessment.

Animals and experimental design. The study was conducted on adult male Wistar rats (200–230 g) procured from the Laboratory Animals Resource Section of the Institute. They were housed in polypropylene cages with chopped wheat straw as the bedding material and given standard rat pellet feed (Amrut Feeds, Pranav Agro Industries Ltd, New Delhi, India) and provided water *ad libitum*. Before the commencement of the experiment, they were kept in laboratory conditions for 7 days for acclimatization. They were maintained under standard management conditions and handled as per the Institute Animal Ethics Guidelines.