Minireview

Arsenic, Reactive Oxygen, and Endothelial Dysfunction

David C. Ellinsworth

Bristol Heart Institute, University of Bristol, Bristol, United Kingdom

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ABSTRACT

Human exposure to drinking water contaminated with arsenic is a serious global health concern and predisposes to cardiovascular disease states, such as hypertension, atherosclerosis, and microvascular disease. The most sensitive target of arsenic toxicity in the vasculature is the endothelium, and incubation of these cells with low concentrations of arsenite, a naturally occurring and highly toxic inorganic form of arsenic, rapidly induces reactive oxygen species (ROS) formation via activation of a specific NADPH oxidase (Nox2). Arsenite also induces ROS accumulation in vascular smooth muscle cells, but this is relatively delayed because, depending on the vessel from which they originate, these cells often lack Nox2 and/or its essential regulatory cytosolic subunits. The net effect of such activity is attenuation of endothelium-dependent conduit artery dilation via superoxide anion–mediated scavenging of nitric oxide (NO) and inhibition and downregulation of endothelial NO synthase, events that are temporally matched to the accumulation of oxidants across the vessel wall. By contrast, ROS induced by the more toxic organic trivalent arsenic metabolites (monomethylarsonous and dimethylarsinous acids) may originate from sources other than Nox2. As such, the mechanisms through which vascular oxidative stress develops in vivo under continuous exposure to all three of these potent arsenicals are unknown. This review is a comprehensive analysis of the mechanisms that mediate arsenic effects associated with Nox2 activation, ROS activity, and endothelial dysfunction, and also considers future avenues of research into what is a relatively poorly understood topic with major implications for human health.

Introduction

Arsenic is a toxic metalloid present as the 20th most abundant element in the earth’s crust (Woolson et al., 1977), and is a major health concern for >200 million people worldwide (Naujokas et al., 2013). The Agency for Toxic Substances and Disease Registry ranks arsenic as number one on their Priority List of Hazardous Substances (http://www.atsdr.cdc.gov/spl/), and it is also classified as a Group I carcinogen by the International Agency for Research on Cancer (Straif et al., 2009). Human exposure to arsenic can occur through various industrial processes, as well as contaminated food and polluted air. However, the highest risk source of intoxication is contaminated drinking water. Countries that are affected by arsenic-contaminated drinking water include Bangladesh, India, China, Taiwan, Mongolia, Mexico, Argentina, Chile, and specific regions of the United States (Argos et al., 2010; Rodriguez-Lado et al., 2013). Indeed, the serious risk to 35–77 million people in Bangladesh alone has been described by the World Health Organization as “the largest poisoning of a population in history” (Argos et al., 2010), and the current estimate of ~20 million for China is likely an underestimate of the actual affected population due to the millions of wells as sources of drinking water that are still to be tested for contamination, with the expectation that such testing will take several decades to complete (Rodriguez-Lado et al., 2013). Numerous epidemiologic studies have provided evidence that chronic arsenic intoxication predisposes to a number of cardiovascular diseases, including hypertension, coronary artery disease (CAD), stroke (carotid atherosclerosis), and microvascular abnormalities (arteriosclerosis and Blackfoot disease), as well as diabetes mellitus, skin lesions, neurodegenerative disorders, and cancer (primarily of the lung, bladder, kidney, liver, and skin) (for review, see Chen, 2014).

In contaminated water, arsenic is present in the inorganic forms of pentavalent arsenate (AsV) and trivalent arsenite (AsIII) (Vahter, 2002). Once ingested, AsV is rapidly reduced (primarily in the blood) to the more reactive and toxic AsIII. This is then methylated (primarily in the liver) to monomethylarsonic
acid, with subsequent reduction/methylation cycles producing monomethylarsonous (MMA\(^{\text{III}}\)), dimethylarsinic, and dimethylarsinous (DMA\(^{\text{III}}\)) acids (Vahter, 2002). With the exception of As\(^{\text{V}}\) reduction to As\(^{\text{III}}\), which is catalyzed by purine nucleoside phosphorylase, these steps are catalyzed by glutathione \(S\)-transferases \(\omega1\) and \(\omega2\) (using glutathione as the reducing agent) and arsenic methyltransferase (using \(S\)-adenosymethionine as the methyl donor) (De Chaudhuri et al., 2008) (Fig. 1). Although the methylated species are the primary products excreted in urine, their trivalent forms (i.e., MMA\(^{\text{III}}\) and DMA\(^{\text{III}}\)) are intrinsically more toxic than As\(^{\text{III}}\), with the pentavalent species themselves being generally nontoxic at experimental levels relevant to human environmental exposure (Aposhian et al., 2003). Notably, arsenic trioxide (As\(_2\)O\(_3\)) is used clinically as a highly effective treatment of acute promyelocytic leukemia and is converted to As\(^{\text{III}}\) and, subsequently, to methylated species in vivo after administration (Chen et al., 2013).

In the vascular system, and primarily in endothelial cells (ECs), exposure to arsenic induces overproduction of reactive oxygen species (ROS), which modulate signaling events that are central to the ability of this cell monolayer to regulate the tone of the underlying vascular smooth muscle cells (VSMCs), an event widely designated as “endothelial dysfunction” (Bilszta et al., 2006; Verma et al., 2010; Edwards et al., 2013). Notably, in individuals exposed chronically to arsenic in drinking water there is a positive correlation between blood levels of arsenic and plasma markers of ROS accumulation (Wu et al., 2001). Endothelial dysfunction is widely accepted as an initiating precursor to the development of chronic vascular abnormalities (Vanhoutte, 2009), and so elucidation of the mechanisms that underlie arsenic-induced disturbances in the regulation of vascular tone could therefore provide insight into the associated etiology of hypertension and vascular disease.

### Reactive Oxygen and the Endothelium

In both health and disease, ROS play an important role in numerous signaling processes in most cell types. Vascular cells are capable of producing ROS via a number of different sources, including several NADPH oxidase (Nox) isoforms (see below), nitric oxide (NO) synthases (NOS), the mitochondrial electron transport chain, cytochrome P450 epoxygenases, xanthine oxidase, cyclooxygenases (cyclooxygenase-1 and -2), and lipooxygenases (Weaver et al., 2012). With the exception of the Nox family, these sources reduce molecular \(O_2\) to produce superoxide anions (\(O_2^{-}\)) as a byproduct of their metabolic activity, whereas Nox isoforms synthesize ROS [variably \(O_2^{-}\) or hydrogen peroxide (\(H_2O_2\); see below)] as their sole product. Depending on the origin of synthesis and/or activity, \(O_2^{-}\) is further reduced by one of potentially three superoxide dismutases (SODs) (cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, or extracellular SOD) to produce \(H_2O_2\) (Zinkевич and Gutterman, 2011), a weaker, but more stable oxidant that is known to play an important role in vascular tone regulation in a number of artery types (for examples, see Matoba et al., 2003; Hatoum et al., 2005; Phillips et al., 2007; Larsen et al., 2009; Liu et al., 2011b).

Signaling via Nox-derived ROS is integral to a number of aspects of vascular physiology, such as differentiation, angiogenesis, cell survival, growth, and contraction, but acute and chronic increases in the activity and expression of Nox isoforms are implicated in the development of hypertension, atherosclerosis, ischemia-reperfusion injury, and endothelial dysfunction (Lassegue et al., 2012). The Nox family consists of seven catalytic subunits, designated as Nox1–5 and the dual oxidases Duox1 and Duox2, of which Nox1, 2, 4, and 5 are expressed in arteries (Lassegue et al., 2012). With the exception of Nox5, these catalytic subunits are bound to \(p22^{\text{phox}}\) as a membrane-associated heterodimeric complex (designated as cytochrome b558, for Nox2-p22\(^{\text{phox}}\)). With the exception of Nox4, which is constitutively active, the translocation of regulatory cytosolic subunits, variably including \(p47^{\text{phox}}, \text{Nox}o1, p67^{\text{phox}}, \text{Nox}a1, p40^{\text{phox}}, \) and the small GTPase Rac1, to the plasmalemma and their association with the Nox-p22\(^{\text{phox}}\) heterodimer is an essential step to initiate oxidase activity (Lassegue et al., 2012).

In general, Nox1 is expressed at relatively low levels in ECs [notable exceptions are certain cultured ECs, including those obtained from bovine aorta and rat basilar artery, in which it is highly expressed (Ago et al., 2005)], whereas Nox2 and Nox4 are much more common in the endothelium, with Nox4 generally being the most abundant (Lassegue et al., 2012). Specialized roles for Nox2 and Nox4 are believed to rest on their specific sub-EC distributions (plasmalemma and endoplasmic reticulum membranes, respectively; see table 1 in Lassegue et al., 2012) as well as the ability of Nox4 to produce \(H_2O_2\) directly without intermediary \(O_2^{-}\) (Montezano et al., 2011; Lassegue et al., 2012).

A number of risk factors for cardiovascular disease are associated with increased vascular ROS generation that exceeds endogenous antioxidant capacity (designated as “oxidative stress”) (States et al., 2009; Tousoulis et al., 2011). Indeed, \(O_2^{-}\) reacts rapidly with endothelium-derived NO to form the toxic reactive nitrogen species peroxynitrite (ONOO\(^{-}\)), thereby reducing the bioavailability of NO. Furthermore, ONOO\(^{-}\) exacerbates oxidative stress and impaired NO by oxidizing the essential endothelial NOS (eNOS) cofactor (\(6\)R)-5,6,7,8-tetrahydrobiopterin, leading to the uncoupling of eNOS and an increase in \(O_2^{-}\) generation by the oxygenase component of the enzyme (Forstermann and Munzel, 2006).

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**Fig. 1.** Mechanisms of arsenic metabolism. As\(^{\text{V}}\) ingested through drinking water is reduced in the blood to trivalent As\(^{\text{III}}\) by purine nucleoside phosphorylase, which is then methylated in the liver by arsenic methyltransferase to pentavalent monomethylarsonic acid (MMA\(^{\text{V}}\)). Subsequent reduction [herein catalyzed by glutathione (GSH) \(S\)-transferases \(\omega1\) and \(\omega2\)] and methylation cycles lead to production of MMA\(^{\text{III}}\), dimethylarsinic (DMA\(^{\text{V}}\)), and DMA\(^{\text{III}}\) acids.
Arsenic and NO: Role of Oxidative Stress

A number of studies of isolated vascular cells and tissues have provided clear evidence that AsIII stimulates the production of ROS and promotes oxidative stress. In ECs, the accumulation of both O₂− and H₂O₂ is detectable within minutes of exposure to low, noncytotoxic, and environmentally relevant concentrations of AsIII (2.5–5 μmol/l) (Barchowsky et al., 1999; Straub et al., 2008) (Fig. 2). A similarly rapid induction of ROS has also been observed in human bladder epithelial cells exposed to 1 or 10 μmol/l AsIII (Ebin et al., 2006) and in human keratinocytes exposed to 3 μmol/l AsIII (Cooper et al., 2009).

By contrast, in human aortic VSMCs exposed to 1–10 μmol/l AsIII, ROS production takes up to 60 minutes to initiate and oxidative stress develops over a period of hours (Lynn et al., 2000). Even at extremely high concentrations (100 μmol/l; i.e., ~10–20-fold higher than the generally accepted maximum level relevant to human environmental/clinical exposure) incubation with AsIII for 30 minutes fails to increase dihydroethidium (DHE) fluorescence (a specific O₂− indicator) in either the media or adventitia of rabbit aortic and iliac artery segments, although under similar conditions the metalloid induces an expected robust increase in fluorescence in aortic valve endothelium (Edwards et al., 2013). Furthermore, in reactivity studies of these vessels incubation with AsIII for either 30 or 90 minutes does not affect endothelium-dependent, NO-mediated relaxations to the G protein–coupled receptor agonist acetylcholine, or to cyclopiazonic acid, an agent that promotes store-operated extracellular Ca²⁺ influx by inhibiting the endothelial sarcoplasmic reticulum Ca²⁺-ATPase (Edwards et al., 2013). It could be argued that the ability of AsIII at concentrations as low as 0.5 μmol/l to rapidly induce endothelial nitrotyrosine formation (indicative of ONOO− accumulation) (Bunderson et al., 2002; Straub et al., 2008) implies that O₂− accumulates sufficiently to impair NO bioavailability. However, the aforementioned findings in rabbit arteries nevertheless suggest that endothelium-selective increases in ROS formation that follow acute exposure to AsIII are insufficient to affect the increased flux of NO associated with vessel relaxation (Edwards et al., 2013) (Fig. 2). This idea is consistent with the demonstration that exposure of rats to a single high intravenous dose (6 mg/kg) of AsIII for 4 hours does not affect acetylcholine-induced relaxation of ex vivo preparations of aorta (mediated entirely by NO in this vessel), but does potentiate the development of tone induced by phenylephrine such that, in contrast to control preparations, tone is not further enhanced when NO’s activity is inhibited, implying that only basal levels of NO are affected (Bilszta et al., 2006).

The generality of these findings relate to observations that 1) acetylcholine-induced relaxation is maintained in aortic tissue isolated from transgenic mice with endothelium-selective overexpression of Nox2 (Bendall et al., 2007), and 2) the well known ability of angiotensin II to suppress NO-mediated relaxation is associated with rapid and global increases in vascular NO activity (nonselectively via Nox1 or Nox2, whichever is present) in the intimal, medial, and adventitial layers (Landmesser et al., 2002; Rey et al., 2002; Lassegue and Griendling, 2010). As such, suppression of endothelium-dependent relaxation induced by chronic AsIII exposure is more likely to be mediated by oxidant accumulation in multiple layers of the vessel wall (see below and Fig. 2).

Consistent with this proposal, more prolonged exposure of rats to AsIII (1.5 mg/kg per day for 2 weeks) attenuates ex vivo aortic relaxations to acetylcholine in association with elevated serum markers of oxidative stress (Kaur et al., 2010). Indeed, in this particular experimental model of arsenic toxicity, serum oxidant markers and impaired vessel relaxation (see Fig. 2) are restored by the peroxisome proliferator-activated receptor γ ligands fenofibrate and rosiglitazone and also by atorvastatin (Jindal et al., 2008; Kaur et al., 2010; Verma et al., 2010), agents that are associated variously with increased (6R)-5,6,7,8-tetrahydrobiopterin synthesis (through the upregulation of GTP-cyclohydrolase-I), reduced serum levels of asymmetric dimethylarginine (an endogenous NOS inhibitor), increased eNOS phosphorylation, and suppressed vascular p22phox expression, nitrotyrosine formation, and O₂− content (Okayasu et al., 2008; Potenza et al., 2009; Antoniades et al., 2011; Liu et al., 2011a). Time- and concentration-dependent effects are also apparent after exposure of rats to environmentally relevant low-to-moderate AsIII levels in drinking water [20 μg/l; with 10 μg/l deemed as the safe level by the World Health Organization (http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/), where acetylcholine-induced aortic relaxation is maintained after 2 months but reduced after 7 months’ exposure (Cifuentes et al., 2009)].

AsIII-induced disturbances in endothelial function may not, however, be simply due to reductions in the bioavailability of preformed NO and may also involve chronic effects on eNOS activity and protein expression. For example, in isolated segments of rat aorta incubated for 14 hours with high

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**Fig. 2.** Effects of acute and chronic AsIII exposure on endothelium-dependent, NO-mediated vasodilation. Acute exposure to AsIII reduces basal NO but does not affect NO-dependent vasodilation, whereas chronic exposure impairs NO-mediated vasodilation in association with EC and VSMC O₂− accumulation, as well as inhibition of eNOS through modulation of phosphorylation status (increased Thr⁶⁴⁷ phosphorylation and decreased Ser¹⁸⁷ phosphorylation, for bovine eNOS). BH₄, tetrahydrobiopterin; PP-1, protein phosphatase-1.
concentrations of As\textsuperscript{III} (25–50 μmol/l) the attenuation of acetylcholine-induced relaxation is associated with the reduced conversion of L-arginine to L-citrulline (suggestive of reduced NO production by eNOS) (Lee et al., 2003). However, these authors demonstrate that As\textsuperscript{III}-induced attenuation of eNOS activity is independent of oxidative stress (Lee et al., 2003), which contrasts with findings in bovine aortic ECs that a similar high concentration of As\textsuperscript{III} (30 μmol/l) suppresses eNOS activity via ROS-mediated inhibition of protein phosphatase-1, resulting in derestricted phosphorylation of eNOS-Thr\textsuperscript{497} (Seo et al., 2014) (Fig. 2). The reason for these disparate findings may rest on the duration of As\textsuperscript{III} exposure, i.e., 14 hours (Lee et al., 2003) versus 4 hours (Seo et al., 2014). Notably, more prolonged (>24 hours) exposure of bovine aortic ECs to 30 μmol/l As\textsuperscript{III} promotes endothelial dysfunction via coordinated increased Thr\textsuperscript{497} (equivalent to Thr\textsuperscript{495} in human eNOS) phosphorylation, reduced Ser\textsuperscript{1179} (Ser\textsuperscript{1177} in human eNOS) phosphorylation (Fig. 2), and downregulation of eNOS (Seo et al., 2014).

However, although exposure of rats chronically to drinking water containing extremely high concentrations (100 mg/l) of As\textsuperscript{III} modulates aortic eNOS phosphorylation and expression (Kesanav et al., 2014; Sarath et al., 2014), it is currently unclear whether environmentally relevant levels of As\textsuperscript{III} evoke similar effects in vivo. Indeed, in human aortic ECs treated with 100 μg/l As\textsubscript{2}O\textsubscript{3} (equating to ∼0.5 μmol/l) reductions in NO bioavailability (observed after 1-hour incubation) are followed by decreased activity and down-regulation of eNOS (after 72-hour exposure), but although the authors of this study investigated the significance of these findings in rats in vivo, neither serum NO metabolites nor aortic eNOS content were affected (Chou et al., 2007). It should also be noted that low level MMA\textsuperscript{III} (100–500 nmol/ml) potentiates vasoconstrictor responses to agonists via an endothelium-independent mechanism mediated by Ca\textsuperscript{2+} sensitization and increased myosin light chain phosphorylation (Lim et al., 2011).

**Arsenic and Nox2**

The possibility that As\textsuperscript{III} induces EC oxidative stress via a mechanism involving a Nox-based oxidase was first alluded to in studies of cultured porcine aortic ECs (Barchowsky et al., 1999; Smith et al., 2001). Treatment of porcine aortic ECs with 5 or 10 μmol/l As\textsuperscript{III} rapidly induces translocation of Rac1 to membrane fractions and triggers membrane-associated oxidase activity (Fig. 3), whereas direct treatment of isolated membranes or cells deficient in p67\textsuperscript{phox} (an indispensable cytosolic subunit of Nox2) or Rac1 is without effect (Smith et al., 2001).

In murine liver, sinusoidal ECs (LSECs) As\textsuperscript{III} (2.5 or 5 μmol/l for 30 minutes)-induced DHE fluorescence is prevented by gp91ds-tat (Straub et al., 2008) [also known as Nox2ds-tat (Lassègue et al., 2012)], a synthetic docking sequence peptide that prevents p47\textsuperscript{phox} from binding to Nox2 (Rey et al., 2001). As\textsuperscript{III}-induced pathophysiological remodeling of LSECs (defenestration/capillarization and platelet/endothelial cell adhesion molecule-1 expression) is also attenuated variably by Nox2ds-tat, genetic deletion of p47\textsuperscript{phox} and selective pharmacological inhibition of Rac1 (Straub et al., 2008). Furthermore, studies of human microvascular ECs and LSECs have revealed that As\textsuperscript{III} activates a pertussis toxin-sensitive G protein (i.e., Gi)–coupled pathway to induce Rac1 translocation (Straub et al., 2009) (Fig. 3). Indeed, As\textsuperscript{III}-induced angiogenic gene expression in human microvascular ECs is prevented by pharmacological blockade or siRNA knockdown of sphingosine-1-phosphate type 1 receptors (S1PR1) (Straub et al., 2009), and blockade of S1PR\textsubscript{1,8} prevents both S1P- and As\textsuperscript{III}-induced ROS formation in LSECs (Straub et al., 2009). Pertussis toxin-sensitive As\textsuperscript{III}-induced differentiation of human mesenchymal stem cells, however, is unaffected by blockade of S1PR\textsubscript{1,8}s, but is prevented in a cumulative fashion by selective blockers of endothelin-1 type A and B receptors (Klei et al., 2013). Speculatively, As\textsuperscript{III}-induced Nox2 activation and oxidative stress in these ECs may therefore reflect a general shift toward Gi-coupled cell signaling.

By contrast, exposure of murine saphenous vein ECs to 10 μmol/l As\textsuperscript{III} does not directly induce Rac1 translocation, but instead activates another related small Rho GTPase, Cdc42 (Fig. 3), which, in turn, promotes actin filament reorganization, serine phosphorylation of p47\textsuperscript{phox}, and translocation of p67\textsuperscript{phox}, events that are temporally matched to the rapid (within 3 minutes) accumulation of O\textsuperscript{2−}, in these cells (Qian et al., 2005). The reasons underlying such differences in GTPase induction are not yet clear, but are not specific to the concentration of As\textsuperscript{III} applied (Smith et al., 2001; Qian et al., 2005) and are therefore more likely a reflection of cell-specific response to the metalloid.

In VSMCs As\textsuperscript{III} induces ROS accumulation in a manner that is prevented by knockdown of p22\textsuperscript{phox}, but observations that the elevation in ROS is potentiated by supplementation with NADH, but not NADPH, allude to the activation of an NADH oxidase but not Nox (Lynn et al., 2000). Indeed, these particular VSMCs [derived from human aorta (Lynn et al., 2000)], in contrast to those derived from human resistance arteries, do not express Nox2 (Touyz et al., 2002), and the relative delay (up to 60 minutes) in ROS formation induced by As\textsuperscript{III} appears to be more temporally matched to the upregulation of p22\textsuperscript{phox} (Lynn et al., 2000) (Fig. 3). Notably, Nox2 is barely detectable in VSMCs derived from rat aorta (Lassègue et al., 2001), a vessel that is frequently used as a model system for acute and chronic arsenic toxicity in vitro and in vivo (see Fig. 3).
Arsenic and NO: Role of Oxidative Stress). Furthermore, VSMCs do not consistently express \(p47^{phox}\) or \(p67^{phox}\) (Touyz et al., 2002; Cifuentes and Pagano, 2003; Schiffrin and Touyz, 2003), and so the catalytic cytochrome b558 and regulatory subunits that characterize the Nox2 complex are not universally present in VSMCs.

Despite compelling evidence that As\(^{III}\) acutely activates Nox2, studies of human hepatocytes have shown that exposure to As\(^{III}\) at concentrations (under 5 \(\mu\)mol/l) known to activate this oxidase in ECs (Straub et al., 2008) induces ROS formation in a manner that is prevented by either the flavoprotein inhibitor diphenylene iodonium or the mitochondrial electron transfer inhibitor rotenone (Li et al., 2014). Indeed, colocalization studies using DHE and MitoTracker appear to confirm mitochondria as the principal effector source of ROS under these conditions (Li et al., 2014). However, observations that As\(^{III}\) fails to induce ROS release from isolated mitochondria (Naranmandura et al., 2011) would appear consistent with the proposal that As\(^{III}\)-induced mitochondrial ROS formation in hepatocytes occurs secondarily to the activation of Nox2 (Li et al., 2014). Notably, Nox2-induced mitochondrial ROS formation (for review, see Dikalov, 2011) may be an important control mechanism in the coronary microcirculation of patients with CAD. For example, flow-induced dilation of these vessels requires \(H_2O_2\) release from the endothelium to activate VSMC hyperpolarizing large conductance \(Ca^{2+}\)-activated \(K^+\) channels (Li et al., 2011b), and the accumulation of ROS in coronary ECs exposed to shear stress is prevented by either Nox2ds-tat or rotenone (unpublished data). Furthermore, it is now apparent that MAA\(^{III}\) and DMA\(^{III}\) induce oxidative stress through direct targeting of mitochondria (Naranmandura et al., 2011) and the endoplasmic reticulum (Naranmandura et al., 2012), respectively. Indeed, in cultured human urothelial cells, MAA\(^{III}\) (although much more potent) takes considerably longer than As\(^{III}\) to induce ROS (30 minutes compared with less than 5 minutes at levels as low as 50 \(nmol/l\) and 1 \(\mu\)mol/l, respectively), prompting these authors to speculate that MAA\(^{III}\) induces oxidative stress through a mechanism distinct to that of As\(^{III}\) (Ebbin et al., 2006). How these findings collectively relate to the chronic development of oxidative stress in the vasculature in vivo (i.e., under exposure to all three trivalent arsenicals) is unclear, but observations that individuals carrying polymorphisms in either \(p22^{phox}\) (gain of function) or Mn-SOD (loss of function) are at increased risk of cardiovascular disease (Kukongviriyapan et al., 2011) suggest that this may be preserved by \(H_2O_2\) in chronic human disease states, the fact that the anti-inflammatory and antithrombotic activity of NO is lost (and that \(H_2O_2\) is proinflammatory) has prompted some researchers to question the long-term benefits of such a substitution in dilator molecule activity (Liu and Gutterman, 2009; Beyer and Gutterman, 2012; Durand and Gutterman, 2013).

Furthermore, observations that arsenic potently disrupts gap junctional communication through downregulation of all three dominant vascular connexins (Chou et al., 2007) and in a tissue-specific manner modulates levels of epoxygenase- and epoxyeicosatrienoic acids through alterations in epoxygenase and soluble epoxide hydrolase expression (Anwar-Mohamed et al., 2012, 2013, 2014) should also motivate further investigations of the effects of arsenic on resistance artery function. Indeed, epoxyeicosatrienoic acids and gap junctions play an integral role in EDH in many vascular beds (for reviews, see Campbell and Fleming, 2010; Ellinsworth et al., 2014a,b). Clarification of these mechanisms will have potentially far-reaching implications in relation to the understanding of iatrogenic effects of arsenic during treatment of acute promyeocyte leukemia, and also the initiation and progression of hypertension and vascular disease due to environmental exposure. Notably, heavy metal environmental contaminants such as mercury, cadmium, and chromium are all associated with endothelial dysfunction and cardiovascular risk through mechanisms involving oxidative stress and Nox2 (Wiggers et al., 2008; Almenara et al., 2013; Kukongviriyapan et al., 2014).

Authorship Contributions
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Conclusions and Future Directions
This review has described the complex effects of acute and chronic arsenic exposure on the function of the vascular endothelium, specifically in relation to regulation of vascular tone. Compelling evidence suggests that inorganic arsenic acutely activates and chronically upregulates Nox2, promotes oxidative stress, reduces NO bioavailability, and suppresses NOS activity and expression. Future research should focus on the related effects of arsenic in the microcirculation, where endothelium-dependent vasodilation is less reliant on NO and instead mediated primarily by endothelium-derived hyperpolarization (EDH) (de Wit and Griffith, 2010; Ellinsworth et al., 2014a). Indeed, in rabbit iliac arteries \(H_2O_2\) formed after the dismutation of As\(^{III}\)-induced \(O_2^-\) potentiates EDH-type relaxations and offsets pharmacologically induced NOS inhibition (Edwards et al., 2013). However, in this study only the effects of extremely high As\(^{III}\) concentrations were analyzed (Edwards et al., 2013). Nevertheless, the idea that \(H_2O_2\) plays a compensatory role is intriguing, because in the microcirculation of patients with CAD, \(H_2O_2\) replaces NO as a major dilator molecule to almost fully preserve endothelial function (Miura et al., 2001; Phillips et al., 2007; Larsen et al., 2009; Liu et al., 2011b). However, although vascular function may be preserved by \(H_2O_2\) in chronic human disease states, the fact that the anti-inflammatory and antithrombotic activity of NO is lost (and that \(H_2O_2\) is proinflammatory) has prompted some researchers to question the long-term benefits of such a substitution in dilator molecule activity (Liu and Gutterman, 2009; Beyer and Gutterman, 2012; Durand and Gutterman, 2013).

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Address correspondence to: David C. Ellinsworth, Bristol Heart Institute, University of Bristol, Queen Building Level 7, Bristol Royal Infirmary, Upper Maudlin Street, Bristol, BS2 8HW, UK. E-mail: davidellinsworth@gmail.com