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Perspectives - Minireview

Methylated Organic Metabolites of Arsenic and their Cardiovascular Toxicities

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Recently, arsenic-toxicity has become the major focus of strenuous assessment and dynamic research from the academy and regulatory agency. To elucidate the cause and the mechanism underlying the serious adverse health effects from chronic ingestion of arsenic-contaminated drinking water, numerous studies have been directed on the investigation of arsenic-toxicity using various *in vitro* as well as *in vivo* systems. Neverthless, some questions for arsenic effects remain unexplained, reflecting the contribution of unknown factors to the manifestation of arsenic-toxicity. Interestingly, very recent studies on arsenic metabolites have discovered that trivalent methylated arsenicals show stronger cytotoxic and genotoxic potentials than inorganic arsenic or pentavalent metabolites, arguing that these metabolites could play a key role in arsenic-associated disorders. In this review, recent progress and literatures are summarized on the metabolism of trivalent methylated metabolites and their toxicity on body systems including cardiovascular system in an effort to provide an insight into the future research on arsenic-associated disorders.

Key words: Arsenic, Trivalent arsenic metabolite, MMA^{III}, DMA^{III}, Toxicity

Chemical properties of arsenic. Arsenic is a naturally occurring metalloid, ubiquitously distributed in earth crust. (Smedley and Kinniburgh, 2002). The chemical properties of arsenic are complicated and a variety of chemical forms can be produced. Inorganic arsenic is formed through the reactions with oxygen, chloride and sulfur while organic arsenic is produced by combination with hydrogen or carbon (Oremland and Stolz, 2003). Arsenic can display 4 oxidative states, -3, 0, +3 and +5. Generally, trivalent arsenic is known to exhibit more toxicities than pentavalent arsenic species (Lee *et al.*, 1988). In natural state, arsenic exists in compound forms with copper, nickel and iron but in drinking water, arsenic is present mostly in the forms of inorganic pentavalent arsenate (As^{V}) or trivalent arsenite (As^{III}). Methylated organic arsenic is also found naturally and it is from the metabolism of microorganism (Oremland and Stolz, 2003).

Arsenic exposure to human by drinking water. Generally, the main source of arsenic exposure to human is contaminated drinking water and food (Gebel, 1999). Exposure via the contaminated food is mainly from arsenobetaine, arsenocholine and arsenosugars in sea foods (Edmonds *et al.*, 1997). However, due to their low toxicities and rapid urinary excretion, their effects on human are known to be minor (Buchet *et al.*, 1994). On the contrary, chronic arsenic exposure via contaminated drinking water is drawing an enormous public attention worldwide. (Abernathy *et al.*, 2003; Mead, 2005). US Environmental Protection Agency (EPA) and World Health Organization (WHO) are regulating the arsenic level in drinking water up to 10 μg/l (ppb) in an effort to prevent chronic arsenic exposure but the reports of arsenic exposure through contaminated drinking water are continuing throughout world. In a certain region of China, 0.09~1.86 mg/l level of arsenic was detected in the drinking water from a natural well (Xia and Liu, 2004) and in Bengal Delta Plain surrounded by India, West Bengal and Bangladesh, extremely high levels of arsenic ranging up to 3.2 mg/l have been reported (Bhattacharyya *et al.*, 2003). 3.05 mg/l arsenic level was detected in the underground water from a region of Vietnam (Berg *et al.*, 2001), and 0.01~1.82 mg/l of arsenic level was reported in some regions of Taiwan (Bates *et al.*, 1992). These epidemic arsenic

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contaminations have been also reported in other parts of world such as Latin America including Chile, Argentina and Mexico (Cebrian *et al.*, 1983; Hopenhayn-Rich *et al.*, 1996; Caceres *et al.*, 2005,), America and Europe (Lewis *et al.*, 1999; Lamm *et al.*, 2004).

Distribution, metabolism and excretion of arsenic. Arsenic in drinking water is mainly inorganic form and it can be easily absorbed through gastrointestinal tract to systemic blood circulation. Orally administered arsenic is found to be rapidly absorbed and cleared from body in human volunteer through urine for 3~4 days. However, some remain in body and are slowly excreted through hair, nail and sweat (Buchet *et al.*, 1981).

Arsenic distribution in blood has been investigated through the bioanalysis of arsenic concentration in plasma, serum and erythrocytes of arsenic exposed human (Heydorn, 1970; Concha *et al.*, 1998). Normal healthy people without arsenic exposure have the blood arsenic concentration less than 5.1 μg/l while arsenic-exposed group showed arsenic concentration of around 32.7 μg/l in erythrocytes and 15.4 μg/l in plasma (Heydorn, 1970). In addition, female population exposed to approximately 200 μg/l of arsenic contaminated drinking water showed average blood arsenic level of 11 μg/l (Concha *et al.*, 1998). Even after 2 years of wash-out period from arsenic exposure, plasma and blood concentration of arsenic were determined to be 7.48 μg/l and 26.3 μg/l, respectively (Mandal *et al.*, 2004). Other study in the population exposed to 5~410 μg/l of arsenic contaminated drinking water showed blood arsenic concentration of 2~42.1 μg/l (Olguin *et al.*, 1983; Pi *et al.*, 2000).

Other than blood, arsenic is mainly distributed into liver, hair and nails. Arsenic is mainly metabolized in liver and plasma undergoing reduction from +5 to +3 and subsequent oxidative methylation (Aposhian, 1997; Vahter, 2002; Aposhian *et al.*, 2004). Pentavalent oxidative state, arsenate (As^{V}) is transported into cell via phosphate carrier system and trivalent oxidative state, arsenite (As^{III}) is delivered via aquaglyceroporin (Liu *et al.*, 2002). Recently new metabolic pathways of inorganic arsenic have been suggested by Hayakawa *et al.* (2005). In cell, As^{V} is reduced into As^{III} in the presence of endogenous inosine or free thiol by purine nucleoside phosphorylase (PNP), glutathione-S-transferase (GST-omega) and unknown mitochondria enzymes (Radabaugh and Aposhian, 2000; Gregus and Nemeti, 2002; Nemeti and Gregus, 2002). Reduced As^{III} forms a complex with glutathione (GSH) producing arsenic triglutathione (ATG), and ATG is methylated to monomethylarsenic diglutathione (MADG) and dimethylarsinic glutathione (DMAG) by arsenic methyltransferase (Cyt 19) using cofactor, S-adenosylmethione (SAM). MADG and DMAG are converted to MMA^{III} and DMA^{III} depending on the GSH concentration, and MMA^{III} and DMA^{III} are oxidized to MMA^{V} and DMA^{V} (Fig. 1).

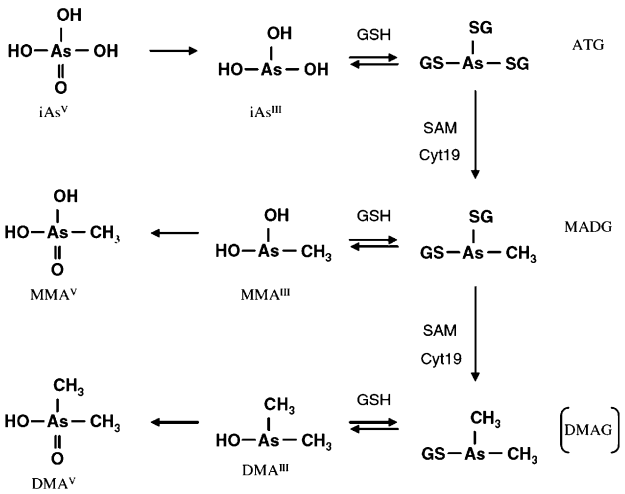

Fig. 1. Biotransformation of inorganic arsenic. DMA^{III}, dimethylarsinous acid; DMA^{V}, dimethylarsinic acid; MMA^{III}, monomethylarsonous acid; MMA^{V}, monomethylarsonic acid. ATG, arsenic triglutathione; MADG, monomethylarsenic diglutathione; DMAG, dimethylarsinic glutathione. Adapted from Hayakawa *et al.* (2005).

Major arsenic metabolite in urine is known to be DMA^{V} but trivalent state was recently identified to be another major metabolite of arsenic in urine (Aposhian *et al.*, 2000a; Aposhian *et al.*, 2000b; Mandal *et al.,* 2004). In human urine, arsenic metabolite contents were measured to be in the order of $DMA^{III} > DMA^{V} > As^{III} > As^{V} > MMA^{III} > MMA^{V}$ (Valenzuela *et al.*, 2005). Although the actual level of trivalent methylated metabolites in environment and human tissue is still poorly investigated, several reports have dealt with the exposure level using *in vitro* and *in vivo* models. Upto now, MMA^{III} and DMA^{III} are known to be present in contaminated water (Hasegawa, 1994), hamster liver tissue (Sampayo-Reyes *et al.*, 2000), cultured human hepatocyte (Del Razo *et al.*, 2001), and human urine (Le *et al.*, 2000a, b; Del Razo *et al.*, 2001). In addition, the presence of MMA^{III} in bile acid were reported in rats (Suzuki *et al.*, 2001). Tissue distribution of radioactive DMA^{III} was also examined in mouse model (Hughes *et al.*, 2008).

Toxicity of trivalent arsenic metabolites. Biological activities of MMA^{V} and DMA^{V} are known to be inferior to those of inorganic arsenic and therefore, methylation pathways have been regarded as the detoxification of

inorganic arsenic (Vahter and Marafante, 1983; Eguchi *et al.*, 1997; Del Razo *et al.*, 2001). Although trivalent methylated metabolites, MMA^{III} and DMA^{III} had been known to be chemically reactive (Cullen *et al.*, 1984), their toxicological roles were questioned due to the absence of knowledge about their biological concentrations. With the recent discovery of trivalent methylated metabolites, MMA^{III} and DMA^{III} as major human metabolites of inorganic arsenic and their strong toxicities, however, a renewed view of arsenic methylated metabolites as toxic metabolites is gaining more and more strength (Petrick *et al.*, 2000, 2001; Styblo *et al.*, 2000; Sakurai *et al.*, 2002). Petrick *et al.* (2000) showed that in Chang human hepatocyte, MMA^{III} showed 10 times stronger toxicities than As^{III} and potent toxicites of MMA^{III} and DMA^{III} could be also observed in other cell types such as normal human hepatocyte, epidermal keratinocyte, bronchial epithelial cell and urinary bladder UROtsa cell (Styblo *et al.*, 2000). In addition, toxicities of MMA^{III} and DMA^{III} were shown in various cancer cell lines including human hepatocellular carcinoma, human bladder transient carcinoma, human acute promyelocytic leukemia and human osteocarcinoma (Styblo *et al.*, 2002) and inhibition potency of MMA^{III} against glutathione reductase, glutathione peroxidase and thioredoxin reductase was also the strongest among arsenic species (Chouchane and Snow, 2001; Lin *et al.*, 2001). DMA^{III} is known to have neurotoxic potential in contrast to DMA^{V} (Kruger *et al.*, 2007). Furthermore, trivalent methylated arsenic species are known to directly react with DNA to induce genotoxicity (Mass *et al.*, 2001) and they have been identified as clastogen in human lymphocyte and mouse lymphoma cell (Kligerman *et al.*, 2003). Indeed, after intraperitoneal injection, MMA^{III} showed strong acute toxicities in hamsters. LD_{50} level of MMA^{III} was estimated to be around 29.3 mmol/kg which is 5 times lower than that of inorganic arsenic (Petrick *et al.*, 2001).

The reason for the potent toxicities of trivalent methylated metabolites over inorganic or pentavalent methylated metabolites has not been fully elaborated, but several explanations such the higher affinity to free thiol (Shiobara *et al.*, 2001) and the strong potential of oxidative stress (Nesnow *et al.*, 2002) of trivalent methylated metabolite have been suggested. Some reports on the thiol-bound arsenicals suggested that thiol-bound form, which is referred as arsenothiol, could be responsible for the toxicity of methylated metabolites (Styblo *et al.*, 1997). Supporting this view, a recent paper has shown that low-concentration of dithiol compounds potentiate the toxicity of trivalent methylated metabolites (Jan *et al.*, 2006). In addition, higher intracellular accumulation of MMA^{III} over As^{III} might be suggested as well for the strong toxicity of MMA^{III} (Hirano *et al.*, 2004).

Effects of trivalent arsenic metabolites on cardiovascular system. Based on the epidemiological reports on the link between chronic ingestion of arsenic-contaminated drinking water and diverse cardiovasular diseases inculding peripheral vascular disease, ischemic heart disease, atherosclerosis and hypertension (Yu *et al.*, 2002; Abernathy *et al.*, 2003; Tseng *et al.*, 2003; Simeonova and Luster, 2004; Yoshida *et al.*, 2004; Kwok *et al.*, 2007), numerous studies have been directed to elucidate the effects of arsenic on cardiovascular system using *in vitro* as well as *in vivo* assays. It is now well-established by substantial number of evidences that the inorganic arsenic including arsenite and arsenate has deleterious effects on cardiovascular system. Exposure to arsenite and arsenate could lead to the development of atherosclerosis, increase of reactive nitrogen species, thrombus formation, and decrease of nitric oxide and cyclic GMP *in vivo* (Lee *et al.*, 2002; Simeonova *et al.*, 2003; Bunderson *et al.*, 2004). In isolated vascular tissues, alteration of vascular tone was induced by arsenite-induced excessive contraction as well as impaired relaxation (Lee *et al.*, 2003, 2005). In vascular cells like vascular smooth muscle cells and endothelial cells, inorganic arsenic could induce oxidative stress, DNA damage and apoptosis with the expression of several proteins by activation of transcription factors (Hirano *et al.*, 2003; Tsou *et al.*, 2003; Kumagai and Pi, 2004; Soucy *et al.*, 2004). Inorganic arsenic also enhances the activation of platelets, potentiating agonist-induced platelet aggregation and procogulant activity through phosphatidylserine exposure and microparticle generation (Lee *et al.*, 2002; Bae *et al.*, 2007).

Although a wide range of cardiovascular effects of inorganic arsenic is well-characterized, information on the role of trivalent methylated metabolites in arsenic-associated diseases is extremely limited. Hirano *et al.* (2004) showed that MMA^{III} has higher toxicity than other arsenic species in rat heart endothelial cells, and Li *et al.* (2007) have reported that MMA^{III} exposure leads to the inhibition of eNOS activity and alteration of eNOS phosphorylation in endothelial cells. Recently, we investigated the effects of trivalent methylated metabolites on vascular tone using isolated blood vessel, and found that MMA^{III} and DMA^{III} could induce smooth muscle dysfunction through the impairment of agonist-induced blood vessel contraction, which is mediated by the disturbance of calcium regulation (Fig. 2). Dysfunction of smooth muscle cells is well-correlated with the alteration of blood pressure after exposure to MMA^{III} in *in*

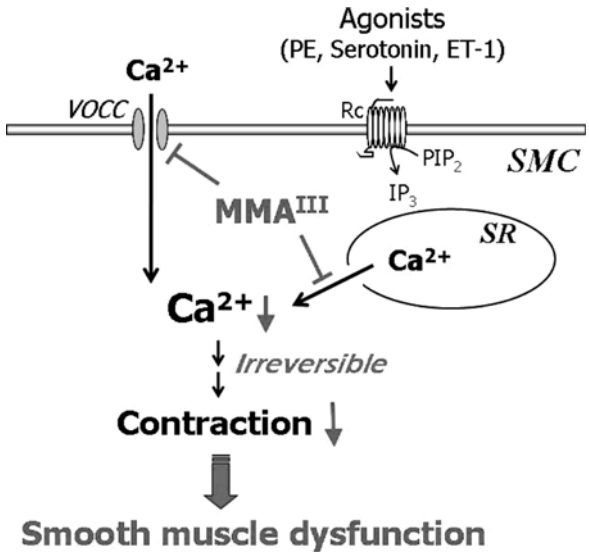

Fig. 2. A suggested mechanism for smooth muscle dysfunction by MMA^{III}. SMC, smooth muscle cells; SR, sarcoplasmic reticulum; VOCC, voltage-operated calcium channel. Adapted from Bae *et al*. (2008).

vivo animal model. Besides vascular cells like smooth muscle cells or endothelial cells, trivalent methylated arsenic metabolites could affect platelet function. In our recent study, MMA^{III} and DMA^{III} were shown to induce apoptotic-like events in platelets, accompanied by phosphatidylserine exposure, resulting in the promotion of procoagulant activity which could be also confirmed in *in vivo* thrombosis model (unpublished data). Notably, procoagulant activity could be increased directly by MMA^{III} and DMA^{III} in the absence of activating agonists, in contrast to the case of inorganic arsenic where agonist was required for the inorganic arsenic-induced procoagulant activity (Bae *et al.*, 2007).

Concluding remarks. Due to the potent toxicity and/or high reactivity of trivalent methylated arsenics *in vitro*, increasing efforts are currently being made to explain the role of these metabolites for the arsenic-associated adverse health effects. Although their actual level in environment and body after the chronic ingestion of arsenic need to be addressed further to confirm their roles, it is a matter of fact that trivalent methylated arsenic metabolites have deleterious effects on the diverse body systems, especially cardiovascular system. Upto now, most of the toxicological studies for the regulatory purposes have been focused on the effects of inorganic arsenic, but now the bottom line for assessment of arsenic-toxicity should be changed to explore the possible involvement of trivalent methylated metabolites. The potent effects of trivalent methylated metabolites with distinct toxic mechanisms from that of inorganic arsenic could give a novel clue for the explanation of the unsolved questions for arsenic-associated health effects.

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