Monomethylarsonous Acid Inhibits Endothelial Nitric Oxide Synthase Activity

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Inorganic arsenic undergoes extensive reduction and oxidative methylation in cells to yield a reactive metabolite of inorganic arsenic monomethylarsonous acid (MMAIII), which has a high reactivity toward vicinal thiols. Our epidemiological study in an endemic area of chronic arsenic poisoning and in experiments with rabbits exposed to arsenic revealed that arsenic exposure results in a reduction of systemic nitric oxide (NO) production. In this study, we examined the effect of MMAIII on endothelial NO synthase (eNOS) activity. With the membrane fraction of bovine aortic endothelial cells (BAEC), it was found that MMAIII with an IC50 value of 2.1 μM was a potent inhibitor of eNOS, whereas inorganic arsenic and their methylated metabolites had no effect on eNOS activity. Interestingly, addition of dithiothreitol markedly blocked the MMAIII-induced inhibition of eNOS activity. This report is the first to suggest that MMAIII interacts with eNOS protein through presumably vicinal thiols, leading to decreased eNOS activity.

Key words —— monomethylarsonous acid, arsenic, endothelial nitric oxide synthase

INTRODUCTION

Arsenic is a naturally occurring metalloid that causes endothelial cell destruction and atherosclerosis, resulting in peripheral vascular dysfunction, hypertension and atherosclerosis.1) Nitric oxide (NO) is a signal molecule that plays a role in vascular tone. Decreased NO bioactivity in endothelium is implicated in the pathophysiology of several diseases.2)

We reported previously that prolonged exposure of humans and rabbits to inorganic arsenic in vivo caused a reduction in systemic NO levels.3,4) Although it is postulated that such decreased NO levels caused by inorganic arsenic exposure may be attributable to the metalloid-mediated activity loss of endothelial NO synthase (eNOS), this issue remains to be elucidated.

Inorganic arsenate (iAsV) undergoes extensive reduction and oxidative methylation in the body to convert into arsenite (iAsIII), followed by monomethylarsonic acid (MMAV), monomethylarsonous acid (MMAIII) and dimethylarsenic acid (DMAV). Among these chemicals, like phenylarsine oxide, a trivalent organoarsenical such as MMAIII is thought to have a chemical reactivity with protein vicinal thiols.5,6) With 9,10-phenanthraquinone, which can selectively interact with proximal protein thiols as a probe,7) we found that decreased eNOS activity caused by 9,10-phenanthraquinone was at least partially associated with thiol modification of this protein.8) Indeed, this suggests that there are proximal protein thiols in eNOS that may interact with MMAIII as well, thereby diminishing the enzyme activity.

In this communication, we show that eNOS activity was decreased in protein extracts from bovine aortic endothelial cells (BAEC) by MMAIII.

MATERIALS AND METHODS

Materials ——— MMAIII was synthesized and characterized as previously described.9) Chemicals were purchased as follows: sodium arsenite from Wako Pure Chemical Industries, Ltd (Osaka, Japan), sodium arsenate from Kishida Chemical Co. Ltd. (Osaka, Japan), monomethylarsonic acid and dimethylarsinic acid from Torikemikaru Institute Ltd. (Yamanashi, Japan), arginine from Sigma (St. Louis, MO, U.S.A.), [3H]arginine from Dupont/NEN research products (Boston, MA, U.S.A.) and AG50W-X8 resin from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Calmodulin (CaM) was purified from bovine brain. All other reagents and chemicals used were of the highest grade available.

Cell Culture ——— BAEC (Dainippon Pharmaceutica Industrial, Tokyo, Japan) were cultured and the membrane fraction of BAEC was prepared as an enzyme source of eNOS as described previously.8)
**RESULTS AND DISCUSSION**

As shown in Fig. 1, MMAIII inhibited eNOS enzyme activity in a concentration-dependent manner. IC_{50} value estimated by non-linear regression analysis was 2.1 µM. eNOS activity was suppressed by approximately 90% by MMAIII at 10 µM. Under this condition, however, little appreciable change in eNOS activity by inorganic arsenic (iAsIII and iAsV) and the pentavalent metabolites (MMAV and DMAV) was seen (Fig. 2). These results suggest that among the arsenics examined, MMAIII is the sole chemical showing potent inhibitory action on eNOS activity. It is well recognized that trivalent organoarsenicals exhibit high affinity for vicinal thiols.5,6) Consistent with this notion, decreased NOS activity caused by MMAIII was effectively blocked by dithiothreitol (DTT) as shown in Fig. 2, suggesting that the inhibitory action of MMAIII on eNOS occurs through covalent attachment to vicinal thiols of the protein.

It has been shown that MMAIII is a reactive metabolite of inorganic arsenic that inhibits glutathione reductase,10) thioredoxin reductase,11) and now eNOS. There are 28 cysteine residues of eNOS in BAEC12) and some of the reactive sulfhydrys play a role in the maximal catalytic activity of eNOS.8) Thus, we speculate that such nucleophiles readily undergo modification by MMAIII, resulting in a reduction of the enzyme activity.

In a cross-sectional study in an endemic area of chronic arsenic poisoning in Inner Mongolia, we found that systemic NO productions (as evaluated by plasma NO metabolites levels) in arsenic-exposed residents were approximately half of those in the controls.3) Such diminished NO levels were also observed in rabbits exposed to inorganic arsenate in drinking water (5 ppm, 18 weeks).13) Our present study suggests the possibility that MMAIII generated during biomethylation of arsenic in endothelial cells could alter the systemic NO level by inhibiting eNOS activity.

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