Monomethylarsonous Acid Inhibits Endothelial Nitric Oxide Synthase Activity

Daigo Sumi, Keiko Taguchi, Yang Sun, Yasuhiro Shinkai, and Yoshito Kumagai*

Department of Environmental Medicine, Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1–1–1, Tenno-dai, Tsukuba, Ibaraki 305–8575, Japan

(Received August 3, 2005; Accepted August 13, 2005; Published online August 17, 2005)

Inorganic arsenic undergoes extensive reduction and oxidative methylation in cells to yield a reactive metabolite of inorganic arsenic monomethylarsonous acid (MMA^{III}), 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 MMA^{III} on endothelial NO synthase (eNOS) activity. With the membrane fraction of bovine aortic endothelial cells (BAEC), it was found that MMA^{III} with an IC_{50} 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 MMA^{III}-induced inhibition of eNOS activity. This report is the first to suggest that MMA^{III} 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 atherogenesis, 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 (iAs^{V}) undergoes extensive reduction and oxidative methylation in the body to convert into arsenite (iAs^{III}), followed by monomethylarsonate acid (MMA^{V}), monomethylarsonous acid (MMA^{III}) and dimethylarsenic acid (DMA^{V}). Among these chemicals, like phenylarsine oxide, a trivalent organoarsenical such as MMA^{III} 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 MMA^{III} 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 MMA^{III}.

MATERIALS AND METHODS

Materials —— MMA^{III} 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), monomethylarsonate acid and dimethylarsinic acid from Torikemikaru Institute Ltd. (Yamanashi, Japan), arginine from Sigma (St. Louis, MO, U.S.A.), [^{3}H]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 Pharmaceutical Industrial, Tokyo, Japan) were cultured and the membrane fraction of BAEC was prepared as an enzyme source of eNOS as described previously.[8]

*To whom correspondence should be addressed: Department of Environmental Medicine, Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1–1–1, Tenno-dai, Tsukuba, Ibaraki 305–8575, Japan. Tel. & Fax: +81-29-853-3133; E-mail: yk-em-tu@md.tsukuba.ac.jp

eNOS Activity —— NO production was determined as product formation of L-citrulline from L-arginine as described previously.[8] Protein concentration was determined by the Bradford method with bovine serum albumin as the standard.

Statistical Analysis —— Student's *t*-tests were performed between the different groups, and differences with two-sided *p*-values less than 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

As shown in Fig. 1, MMA^{III} inhibited eNOS enzyme activity in a concentration-dependent manner. IC_{50} value estimated by a non-linear regression analysis was 2.1 μM. eNOS activity was suppressed by approximately 90% by MMA^{III} at 10 μM. Under this condition, however, little appreciable change in eNOS activity by inorganic arsenic (iAs^{III} and iAs^{V}) and the pentavalent metabolites (MMA^{V} and DMA^{V}) was seen (Fig. 2). These results suggest that among the arsenics examined, MMA^{III} 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 MMA^{III} was effectively blocked by dithiothreitol (DTT) as shown in Fig. 2, suggesting that the inhibitory action of MMA^{III} on eNOS occurs through covalent attachment to vicinal thiols of the protein.

It has been shown that MMA^{III} 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 BAEC[12] and some of the reactive sulfhydryls play a role in the maximal catalytic activity of eNOS.[8] Thus, we speculate that such nucleophiles readily undergo modification by MMA^{III}, 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).[4] Our present study suggests the possibility that MMA^{III} generated during biomethylation of arsenic in endothelial cells could alter the systemic NO level by inhibiting eNOS activity.

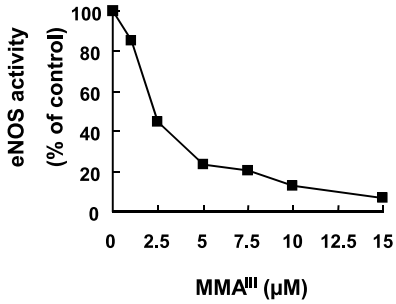

Fig. 1. Inhibition of eNOS Activity by MMA^{III}

eNOS activity was determined in the presence of 0–15 μM MMA^{III} at 37°C for 5 min. Each point is the average of two determinations.

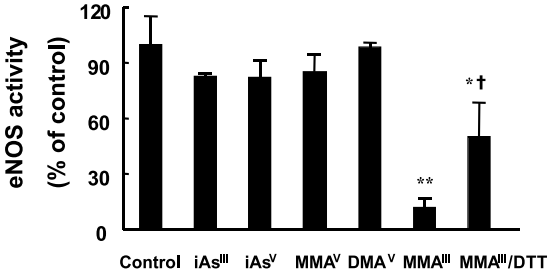

Fig. 2. Inhibition of eNOS Activity by Arsenicals

eNOS activity was determined in the presence of 10 μM iAs^{III}, iAs^{V}, MMA^{V}, DMA^{V}, MMA^{III} or MMA^{III}/DTT [MMA^{III} plus DTT (100 μM)] at 37°C for 5 min, respectively. Each value is the mean ± S.D. of three determinations. *$p < 0.05$ *vs.* control. **$p < 0.01$ *vs.* control. †$p < 0.05$ *vs.* MMA^{III}.

Acknowledgements We are especially grateful to Dr. William R. Cullen for providing MMA and Ms. F. Miyamasu for grammatical correction in the preparation of this manuscript. This work was supported in part by the Japan-China Medical Association to Y. K., the Sumitomo Foundation to Y. K. and the Grants-in-Aids for specific research from the Ministry of Education, Science and Culture of Japan (No. 15406004, Y. K.). K. T. was awarded by the Ishidu Shun Memorial Scholarship.

REFERENCES

1) Wu, M. M., Kuo, T. L., Hwang, Y. H. and Chen, C. J. (1989) Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular disease. *Am. J. Epidemiol*., **130**, 1123–1133.
2) De Meyer, G. R. Y. and Herman, A. G. (2000) *Nitric Oxide*, Academic Press, San Diego.
3) Pi, J., Kumagai, Y., Sun, G., Yamauchi, H., Yoshida, T., Iso, H., Endo, A., Yu, L., Yuki, K., Miyauchi, T.

and Shimojo, N. (2000) Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia. *Free Radic. Biol. Med*., **28**, 1137–1142.

4) Pi, J., Horiguchi, S., Sun, Y., Nikaido, M., Shimojo, N., Hayashi, T., Yamauchi, H., Itoh, K., Yamamoto, M., Sun, G., Waalkes, M. P. and Kumagai, Y. (2003) A potential mechanism for the impairment of nitric oxide formation caused by prolonged oral exposure to arsenate in rabbit. *Free Radic. Biol. Med*., **35**, 102–113.
5) Cullen, W. R., McBride, B. C. and Reglinski, J. (1984) The reaction of methylarsenicals with thiols: Some biological implications. *J. Inorg. Biochem*., **21**, 179–194.
6) Hughes, M. F. (2002) Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett*., **133**, 1–16.
7) Kumagai, Y., Hayashi, T., Miyauchi, T., Endo, A., Iguchi, A., Kiriya-Sakai, M., Sakai, S., Yuki, K., Kikushima, M. and Shimojo, N. (2001) Phenanthraquinone inhibits eNOS activity and suppresses vasorelaxation. *Am. J. Physiol*., **281**, R25–R30.
8) Taguchi, K., Kumagai, Y., Endo, A., Kikushima, M., Ishii, Y. and Shimojo, N. (2001) Phenanthraquinone affects endothelial nitric oxide synthase activity through modification of the thiol group: an alternative inhibition mechanism. *J. Health Sci*., **47**, 571–574.
9) Petrick, J. S., Jagadish, B., Mash, E. A. and Aposhian, H. V. (2001) Monomethylarsonous acid (MMA^{III}) and arsenite: LD_{50} in hamsters and in vitro inhibition pyruvate dehydrogenase. *Chem. Res. Toxicol*., **14**, 651–656.
10) Styblo, M., Serves, S. V., Cullen, W. R. and Thomas, D. J. (1997) Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem. Res. Toxicol*., **10**, 27–33.
11) Lin, S., Del Razo, L. M., Styblo, M., Wang, C., Cullen, W. R. and Thomas, D. J. (2001) Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. *Chem. Res. Toxicol*., **14**, 305–311.
12) Nishida, K., Harrison, D. G., Navas, J. P., Fisher, A. A., Dockey, S. P., Uematsu, M., Nerem, R. M., Alexander, R. W. and Murphy, T. J. (1992) Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest*., **90**, 2092–2096.