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DIMETHYLATED ARSENICS INDUCE DNA STRAND BREAKS IN LUNG VIA THE PRODUCTION OF ACTIVE OXYGEN IN MICE

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SUMMARY: In order to study the genotoxicity of arsenics, we focused our attention on dimethylarsinic acid (DMAA) which was a main metabolite of inorganic arsenics in mammals. ICR mice were orally administered DMAA-Na (1500mg/kg). DNA single-strand breaks occurred specifically in lung at 12h after administration. An *in vitro* experiment indicated that the breaks were not caused directly by DMAA but by dimethylarsine, a further metabolite of DMAA. Furthermore, the dimethylarsine-induced breaks were diminished by the addition of SOD and catalase, suggesting that active oxygen produced by dimethylarsine was involved in the induction of DNA damage.

INTRODUCTION: Epidemiological studies have evidenced that inorganic arsenics are carcinogenic for human subjects, particularly in lung and skin (1). However, experimental studies mainly using arsenate and arsenite have not succeeded in realizing their carcinogenicity, and accordingly its mechanism remains obscure. It is, on the other hand, well known that these inorganic arsenics are metabolized to dimethylated and, in some cases, trimethylated forms in animals (2-8). This metabolic methylation has thought to be a detoxication pathway, which seems likely with respect to general and acute toxicity. We presumed, however, that the methylation might increase the genotoxicity of

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Abbreviations: DMAA, dimethylarsinic acid; DMAA-Na, sodium dimethylarsinic acid; ss-breaks, single-strand breaks; SOD, superoxide dismutase; ESR, electron spin resonance; NBT, nitroblue tetrazolium; SDS, sodium dodecylsulfate; EDTA, ethylenediamine tetraacetic acid; TLC, thin-layer chromatography; EtOAc, ethylacetate; AcOH, acetic acid; AcONa, sodium acetate; EtOH, ethanol; MeOH, methanol; n-BuOH, n-butanol.

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inorganic arsenics, and examined in vivo effect of DMAA, a major metabolite of arsenate and arsenite. Here, we present that the oral administration of DMAA in mice induced DNA strand breaks particularly in lung, a target organ for the carcinogenic action of inorganic arsenics, together with its mechanisms.

MATERIALS AND METHODS: DMAA-Na was obtained from Wako Pure Chemicals Co., SOD and catalase from Sigma Chemical Co. Proteinase K was obtained from E.Merck, Darmstadt. Polycarbonate filters (2.0 and 0.2μm pore size, 25mm diameter) for alkaline elution assay were obtained from Nuclepore Co.
DNA strand breaks in mouse lung: ICR male mice were orally dose with 1500mg/kg of DMAA-Na. The animals were killed and then the organs were homogenized with ice-cold 24mM EDTA containing 75mM NaCl (pH7.5). The homogenates were centrifuged at 200xg for 4min, and the pellets (nuclear fraction) were resuspended in 24mM EDTA containing 75mM NaCl (pH7.5). An aliquat (5ml) of diluted nuclear suspension (ca.$2x10^5$ nuclei/ml) was applied on a 2.0μm filter. Alkaline elution assay was carried out as follows. The nuclei were immediately lysed and digested with a mixture of 2% SDS, 25mM EDTA and 0.1M glycine containing proteinase K (0.5mg/ml). After 30min, the remaining solution was gently aspirated off, and the filter was washed with 3ml of 20mM EDTA (pH10.0) and eluted with a solution containing 20mM EDTA and tetrapropylammonium hydroxide (pH12.1 or 12.6) at a flow rate of 3ml/h. DNA in the eluted fraction (3ml) was precipitated by the ice-cold 95% EtOH containing 55mM AcOH and 0.15M AcONa. The precipitates, left for 2h at -20°C, were collected on the filter (0.2μm). The amount of DNA on the filter was determined by the fluorometric assay (9).
DNA strand breaks on an in vitro experiment: The nuclei isolated from lung of mice were collected on polycarbonate filter (2.0μm) and lysed and digested with SDS and proteinase K in the same manner as described above. The filter was added to 100μl of DMAA-Na solution and allowed to stand for 1h for 20°C. In the case of exposure to dimethylarsine, a solution (1ml) containing 0.1-0.5mmol DMAA-Na was added to 10ml of 6N HCL, and then 5% sodium borohydride (5ml) was slowly added. The generated gas containing dimethylarsine was introduced into an apparatus through a KOH pellet-trap. DNA was exposed to the gas for 1h at 20°C. After exposure to DMAA or dimethylarsine, DNA was eluted with 20mM EDTA (pH12.1) in the same manner described above. In the case of addition of SOD and catalase, the filters were added to a mixture (100μl) of SOD (1400U) and catalase (4400U) in 10mM phosphate buffer containing 0.85% NaCl (pH7.4). Inactived SOD and catalase were prepared by heating for 15min at 100°C.
Arsenics in expired air: Five ICR male mice (ca.25g) were orally administered DMAA-Na at a dose of 1500mg/kg which corresponded to 17.6mg As/25g. The animals were placed in a metabolic cage for collecting the expired air for 24h. The expired air was trapped in 5% hydrogen peroxide (100ml). The sample was concentrated by heating and then digested with a mixture of nitric acid (10ml), sulfuric acid (3ml) and 60% perchloric acid (5ml). Hydride vapor generator attached to a flame atomic absorption spectrometer was used for determination of arsine generated by adding sodium borohydride. For identification of arsenic trapped in 5% hydrogen peroxide, TLC was carried out on cellulose plates No.15035 (E.Merck, Darmstadt). Arsenic on the TLC plates was detected by treatment with iodine.

RESULTS AND DISCUSSIONS: Figure 1 shows the alkaline elution profiles of DNA in various tissues after DMAA administration in mice. In lung (Fig.1a), arsenic-accumulating organ (10), the ss-breaks of DNA appeared markedly at 12h after administration. The ss-breaks were then diminished partially at 15h and fully at 24h,

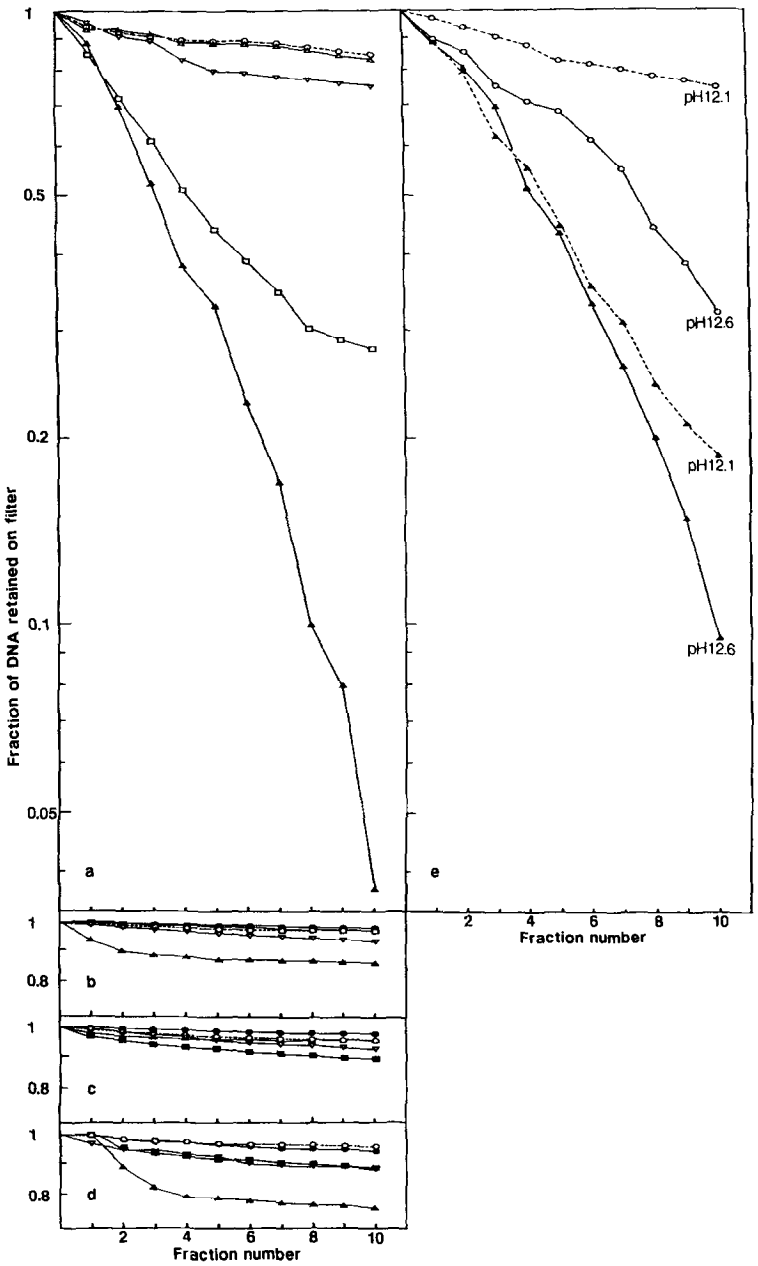

Figure 1. Single-strand breaks (a-d) and alkali-labile sites (e) of DNA in lung (a,e), liver (b), kidney (c) and spleen(d) of mice after DMAA administration. The symbols are: control(○); 6h(●); 9h(△); 12h(▲); 15h(□); 18h(■); 24h(▽) after administration of DMAA.

suggesting that the breaks were readily repairable. On the other hand, in liver, kidney and spleen, which also accumulate arsenics but less than lung (10-12), the ss-breaks detected at 12h were much less than in lung (Fig.1b-d). The lung-specific ss-breaks were also observed in rats, similarly to mice, at 12h after oral administration of DMAA[2]. These results strongly suggest that lung is a target organ for the induction of DNA damage by DMAA administration. Alkali-labile sites of pulmonary DNA after DMAA administration in mice were also determined by eluting at pH12.6. As shown in Fig.1e, the elution patern was similar to that by eluting at pH12.1 (ss-breaks) at the initial phase (fraction no.1-6). This pH independence in DNA breaks indicates that alkali-labile sites were little produced and suggests that the type of DNA damage induced by DMAA administration is not that by alkylating agents, which form alkali-labile DNA adducts, but that by ionizing radiation (13).

In order to reveal whether the pulmonary DNA damage was induced by DMAA itself or its further metabolites, we analyzed volatile metabolites of DMAA in the expired air of mice. As is well known with some microorganisms, DMAA is reduced to dimethylarsine (14) and, occasionally, further methylated to trimethylarsine (15,16), both of which are volatile substances having strong garlic odor. That our experimental animals smelled garlic-like after DMAA administration with an interval of several hours suggested the production of these volatile metabolites. In fact, by trapping the expired arsenics in 5% hydrogen peroxide which oxidized them to DMAA or trimethylarsine oxide, 0.0032% of total administered dose of As was recovered within 24h in the expired air (Table 1a). A thin-layer chromatographic analysis indicated that the oxidized form of expired arsenic trapped in 5% hydrogen peroxide was DMAA, and neither trimethylarsine oxide nor methanearsonic acid was detected (Table 1b). Because DMAA is not volatile, the trapped DMAA possibly originated from gaseous dimethylarsine. Therefore, a major metabolite of DMAA in the expired air of mice is very likely to be dimethylarsine.

To determine whether DMAA itself or dimethylarsine is responsible for DNA strand breaks, an in vitro DNA-strand scission experiment was performed. The nuclei isolated from mouse lung cells were lysed and digested with SDS and proteinase K,

[2]Unpublished data.

Table 1 Arsenic excreted in the expired air after DMAA administration in mice

(a) Arsenic in expired air

	Arsenic (μg As)	% dose
Exp. 1	3.79	0.0041
Exp. 2	2.42	0.0026
Exp. 3	2.54	0.0028
Mean(±S.E.)	2.92±0.44	0.0032

(b) Rf values of 5% hydrogen peroxide-trapped arsenic on TLC

	Methanearsonic acid	DMAA	Trimethylarsine oxide
	EtOAc:AcOH:Water(3:3:1)		
Authentic	0.71	0.81	0.89
Sample	N.D.*	0.82	N.D.
	n-BuOH:AcOH:Water(4:2:1)		
Authentic	0.69	0.76	0.81
Sample	N.D.	0.74	N.D.
	MeOH:EtOAc:AcOH(10:5:1)		
Authentic	0.78	0.82	0.88
Sample	N.D.	0.82	N.D.

*N.D., Not detected.

respectively, on polycarbonate filters, and then exposed to DMAA or gaseous dimethylarsine which was generated by reducing DMAA with sodium borohydride. As shown in Fig.2, dimethylarsine induced ss-breaks of DNA (Fig.2a), while DMAA not (Fig.2b). This strongly suggests that DNA breaks in lung were induced by dimethylarsine which was metabolically produced from DMAA.

We, then, postulated the participation in the DNA damage of active oxygen, which is readily produced by the reaction between dimethylarsine and molecular oxygen. An *in vitro* experiment demonstrated that superoxide radical was produced when dimethylarsine and molecular oxygen coexisted; when dimethylarsine was introduced under air to NBT solution, a detecting reagent for superoxide radical (17-19), NBT was reduced and its solution turned to blue color[2]. Therefore, we examined the effects of SOD and catalase on the induction of ss-breaks by dimethylarsine. Either enzyme suppressed the induction of ss-breaks (Fig.3a). When both the enzymes were added together, the

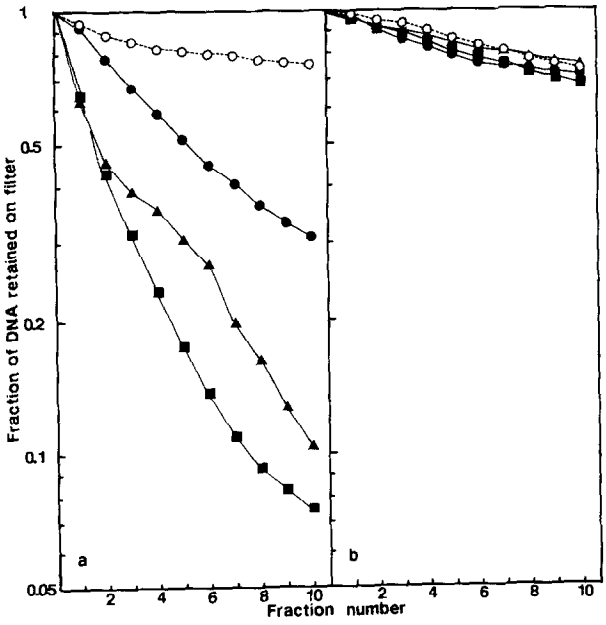

Figure 2. Single-strand breaks of DNA after in vitro exposure to dimethylarsine (a) and DMAA (b). The symbols are: control(○); 0.1mmol DMAA(●); 0.2mmol DMAA(▲); 0.5mmol DMAA(■).

induction was completely inhibited (Fig.3b), while heat-treated enzymes showed no inhibition (Fig.3c). This result may indicate that pulmonary ss-breaks were not directly induced by dimethyl-

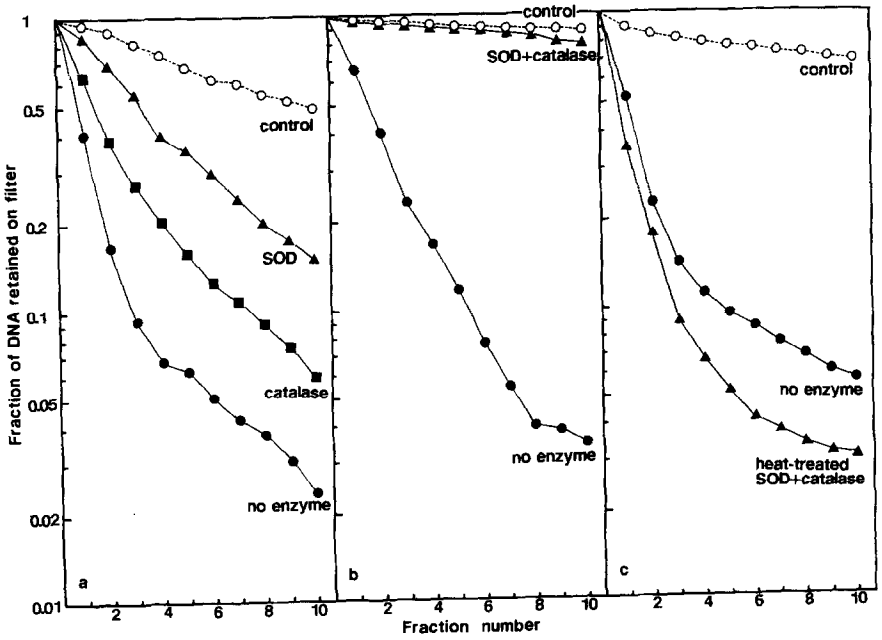

Figure 3. Effect of SOD and/or catalase on DNA strand breaks induced by dimethylarsine.

arsine but by active oxygen produced by the reaction of dimethylarsine with molecular oxygen in lung. The production of active oxygen by this reaction was directly confirmed in an *in vitro* system by ESR spectrometry and the cytochrome c method, which will be reported elsewhere.

Since McBride and Wolfe (14) proposed a metabolic methylation pathway of inorganic arsenics in microoraganisms, similar pathways producing DMAA have been evidenced in many species of organisms including mammals (2-8). We also observed that DMAA was a major metabolite from arsenite in rodents[2]. On the other hand, epidemiologically indicated human lung carcinogenesis by inorganic arsenics is not yet evidenced by *in vivo* experiments with animals. Under these facts, we considered that the methylated arsenics were worth to be investigated on their genotoxicity particularly in lung cells. As a result, we found that lung-specific strand breaks of DNA were occurred by DMAA administration in mice. It has been proposed that the metabolic methylation of inorganic arsenics results in the lowering of their general toxicity. However, the present finding that dimethylarsine which was metabolically produced in mammals induced a significant DNA damage suggest that, so far concerning to their genotoxicity, metabolic methylation may cause arsenics rather toxic. Furthermore, molecular oxygen was found to be neccessary for the occurrence of genotoxicity of methylated arsenics *via* its conversion to active oxygen. This would explain the lung-specific DNA breaks by DMAA administration; molecular oxygen in lung is richer than other organs, *e.g.*, liver, kidney and spleen. The process mentioned above might account for the high risk of lung cancer by arsenics.

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REFERENCES

1. International Agency for Research on Cancer (1980) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 23, pp.39-141, IARC, Lyon.
2. Crecelius, E.A. (1977) Environ. Health Perspect. 19, 147-150.
3. Charbonneau, S.M., Tam, G.K.H., Bryce, F., Zawidska, Z. and Sandi, E. (1979) Toxicol. Lett. 3, 107-113.
4. Tam, G.K.H., Charbonneau, S.M., Bryce, F., Pomroy, C. and Sandi, E. (1979) Toxicol. Appl. Pharmacol. 50, 319-322.

5. Charbonneau, S.M., Hollins, J.G., Tam, G.K.H., Bryce, F., Ridgeway, J.M. and Willes, R.F. (1980) Toxicol. Lett. 5, 175-182.
6. Bertolero, F., Marafante, E., Edel Rade, J., Pietra, R. and Sabbioni, E. (1981) Toxicology 20, 35-44.
7. Buchet, J.P., Lauwerys, R. and Roels, H. (1981) Int. Arch. Occup. Environ. Health 48, 71-79.
8. Vahter, M. (1981) Environ. Res. 25, 286-293.
9. Bradley, M.O., Dysart, G., Fitzsimmons, K., Harback, P., Lewi, J. and Wolf, G. (1982) Cancer Res. 42, 2592-2597.
10. Vahter, M., Marafante, E. and Dencker, L. (1984) Arch. Environ. Comtam. Toxicol. 13, 259-264.
11. Lindgren, A., Vahter, M. and Dencker, L. (1982) Acta Pharmacol. Toxicol. 51, 253-265.
12. Rowland, I.R. and Davis, M.J. (1982) J. Appl. Toxicol. 2, 294-298.
13. Kohn, K.W., Ewig, R.A.G., Erickson, L.C. and Zwelling, L.A. (1981) in DNA Repair, A Laboratory Manual of Researc Procedures (Friedberg, E.C. and Hanawalt, P.C., Eds) Vol.1, Part B, pp. 379-401, Marcel Dekker, New York and Basel.
14. McBride, B.C. and Wolfe, R.S. (1971) Biochemistry 10, 4312-4317.
15. Cullen, W.R., Froese, C.L., Lui, A., McBride, B.C., Patmore, D.J. and Reimer, M. (1977) J. Organomet. Chem. 139, 61-69.
16. Woolson, E.A. (1977) Environ. Health Persp. 19, 73-81.
17. Rajagopalan, K.V. and Handler, P. (1964) J. Biol. Chem. 239, 2022-2026.
18. Miller, R.W. (1970) Can. J. Biochem. 48, 935-939.
19. Beauchamp, C. and Fridovich, I. (1971) Anal. Biochem. 44, 276-287.