Induction of Lung-specific DNA Damage by Metabolically Methylated Arsenics via the Production of Free Radicals

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To clarify the genotoxicity of inorganic arsenics, we focused on the genotoxic effect of metabolically methylated arsenics in mammals. Oral administration to mice of dimethylarsinic acid (DMAA), a major metabolite of inorganic arsenics, induced lung-specific DNA damage, i.e., DNA single-strand breaks and the clumping of heterochromatin. The lung-specific strand breaks were not caused by DMAA itself, but by dimethylarsine, a further metabolite of DMAA. An *in vitro* experiment indicated that DNA single-strand breaks by dimethylarsine were suppressed by the presence of superoxide dismutase and catalase, suggesting that the strand breaks were induced via the production of free-radical species including active oxygens. Dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\bullet$] and superoxide anion radical produced from the reaction between molecular oxygen and dimethylarsine were detected by electron-spin resonance analysis using a spin-trapping agent and the cytochrome-c method, respectively. Of these two radicals, the dimethylarsenic peroxyl radical rather than the superoxide anion radical is assumed to play the dominant role in causing the DNA damage, at least for DNA single-strand breaks. — Environ Health Perspect 102(Suppl 3):37–40 (1994).

Key words: DNA damage, single-strand breaks, methylated arsenics, free radicals, dimethylarsenic peroxyl radical, dimethylarsine

Introduction

A number of epidemiologic investigations have pointed out that inorganic arsenics, e.g., arsenite and arsenate, are carcinogenic for human lungs and skin (*1*). However, animal experiments have not succeeded in proving their carcinogenicity. In regard to the genotoxicity of inorganic arsenics, Bertolero et al. (*2*) reported that arsenite and arsenate showed transforming activity in BALB/3T3 clone A31-1-1 cells. The morphological transforming activity of inorganic arsenics was also demonstrated in Syrian hamster embryo (SHE) cells (*3*). However, few positive results have been obtained in mammalian genotoxicity tests, and the evidence that inorganic arsenics can cause mutations and allied genotoxic effects in bacteria is so far inconclusive. No conclusive proof has yet been provided for the genotoxicity of inorganic arsenics. On the other hand, as shown in Figure 1, inorganic arsenics are well known to be metabolized to methanearsonic acid and, subsequently, to dimethylarsinic acid (DMAA) in mammals (*4*). These methylated arsenics are known to be less toxic than inorganic arsenics (*5*). However, the genotoxic action of these methylated arsenics has been scarcely investigated.

To evaluate the genotoxicity of methylated arsenics produced during the metabolic processing of inorganic arsenics, we conducted the present study, focusing on DMAA, the major metabolite of inorganic arsenics in mammals. In this article, we summarize our evidence that the oral administration of DMAA induces lung-specific DNA damage (*6,7*). We further estimate that the damage is caused by free radical species, particularly the dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\bullet$], produced in the metabolic processing of DMAA (*8*).

Lung-specific DNA Damage Induced by the Administration of DMAA in Mice

Male ICR strain mice (ca. 25 g) were orally administered the sodium salt of DMAA (1500 mg/kg); 9 to 24 hr later, DNA was isolated from various tissues, and DNA single-strand breaks were determined by the alkaline elution assay according to the procedure of Kohn et al. (*9*). As shown in Figure 2, single-strand breaks in DNA were induced in the lungs after DMAA administration. DNA strand breaks were not seen in liver, kidney, or spleen (*6*). The appearance of these lung-specific strand breaks occurred approximately 12 hr after administration of DMAA. The breaks were partially repaired at 15 hr and fully repaired at 24 hr. The single-strand breaks detected in mouse lung at 12 hr after DMAA administration did not accompany the alkali-labile sites in DNA (*6*); the alkaline elution pattern of DNA obtained by eluting at pH 12.1 was similar to that obtained by eluting at pH 12.6. This suggests that the breaks were directly induced, similarly to the case of ionizing radiation. The fact that the strand breaks occurred specifically in lung tissue might be related to arsenic carcinogenesis in the lung.

Next, we investigated lung-specific DNA damage by morphological evaluation (*7*). Light microscopy analysis within 24 hr after administration of DMAA showed no morphological or pathological changes in liver, spleen, kidney, testis, and bone marrow, or even in the lungs, in which DNA strand breaks were induced. However, by a transmission electron microscopic survey, a remarkable clumping of heterochromatin was observed in the nuclei of endothelial cells of alveolar wall capillaries in mice 12 to 48 hr after administration of DMAA; such clumping was not seen in the sinus endothelium of the liver (*7*). Thus, the

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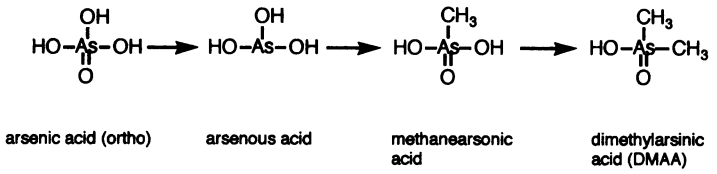

Figure 1. Metabolic pathway of inorganic arsenics in mammals.

morphological changes, as well as DNA single-strand breaks, were also specifically induced in the lung by DMAA administration in mice. These changes in heterochromatin in the lung endothelium might be related to the development of sarcomas such as hemangioendothelial sarcomas, which have been clinically observed after arsenic ingestion (*10*).

Identification of DMAA Metabolites in the Expired Air

To identify the ultimate substances causing DNA single-strand breaks, we examined the further metabolites of DMAA. McBride and Wolfe (*11*) reported that, in microorganisms, DMAA was further metabolized to dimethylarsine and trimethylarsine. This metabolic process has been assumed to exist also in humans and mammals; Solis-Cohen and Githens (*12*) reported that arsenic-intoxicated humans had a garlic-like odor. Since the mice had such an odor as well, after the administration of DMAA we tried to identify the volatile metabolites of DMAA in the expired air of mice after DMAA administration. The metabolites in the expired air were trapped in 5% hydrogen peroxide, which oxidized them to DMAA and trimethylarsine oxide, if any. A thin-layer chromatographic analysis indicated that the oxidized form of the expired arsenic trapped in 5% hydrogen peroxide was only that of DMAA; i.e., no trimethylarsine oxide was detected (*6*). Thus, the volatile metabolite of DMAA excreted in the expired air after DMAA administration is very likely to be dimethylarsine. An *in vitro* experiment using DNA prepared from mouse lung indicated that the induction of DNA strand breaks was due to dimethylarsine and not to DMAA itself (*6*).

Participation of Active Oxygen Species in Lung-specific DNA Damage

Since dimethylarsine is a potent electron donor and rapidly reacts with molecular oxygen, we postulated that the DNA single-strand breaks induced by dimethylarsine were not directly caused by dimethylarsine itself but by active oxygen species produced from the reaction between dimethylarsine and molecular oxygen. An *in vitro* experiment using nitroblue tetrazolium demonstrated that the superoxide anion radical was produced by one-electron reduction of molecular oxygen when the tetrazolium was coincubated with dimethylarsine. We therefore investigated the effects of superoxide dismutase (SOD) and catalase on DNA strand breaks induced by dimethylarsine (*6*). The breaks were decreased significantly by the addition of these enzymes to the reaction (Figure 3). The result supports our hypothesis that the formation of strand breaks by dimethylarsine exposure is induced via the production of active oxygen species.

To further reveal the participation of active oxygen species in the induction of lung-specific DNA strand breaks after DMAA administration in mice, we looked for the appearance of biochemical reactions protective against active oxygens in lungs *in vivo*. Of the enzymes directly catalyzing the protective reactions, the activities of SOD, particularly Mn-SOD, and glutathione peroxidase in pulmonary cells were enhanced by DMAA administration in mice (*19*). For the protective mechanisms against oxidative stress in pulmonary cells, alterations of the cycle of glutathione oxidation/reduction are generally observed (*13*). In fact, the administration of DMAA brought about an increase in glucose-6-phosphate dehydrogenase activity and decreases in reduced glutathione (GSH) and nicotinamide adenine dinucleotide phosphate, reduced (NADPH) levels in pulmonary cells (*19*). These cellular responses support the possibility that active oxygen species, e.g., superoxide anion radical and hydrogen peroxide, are produced in pulmonary cells after DMAA administration in mice.

Formation of Arsenic Peroxyl Radical and its Role in DNA Damage

In the course of determination of DNA single-strand breaks by the alkaline elution method, we noticed that the amount of DNA, determined by quantifying the deoxyribose residues, significantly

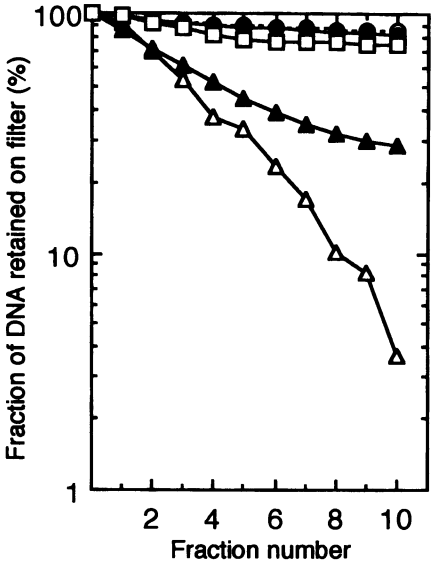

Figure 2. Single-strand breaks in pulmonary DNA induced by DMAA administration in mice. Mice were orally administered the sodium salt of DMAA (1500 mg/kg). The pulmonary DNA was eluted at pH 12.1 by the alkaline elution method. The symbols are as follows: control (○); 9 hr (●); 12 hr (△); 15 hr (▲); 24 hr (□) after administration of DMAA.

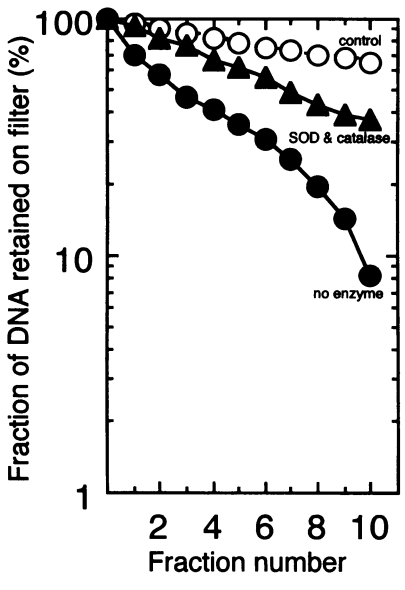

Figure 3. Effect of SOD and catalase on DNA single-strand breaks Induced by dimethylarsine. DNA was exposed at 20° C for 1 hr to dimethylarsine generated from 0.2 mmole (27.6 mg) DMAA with sodium borohydride. After exposure to dimethylarsine, the DNA was eluted at pH 12.1 by the alkaline elution method. The determination of DNA was carried out by the procedure using 3,5-diaminobenzoic acid.

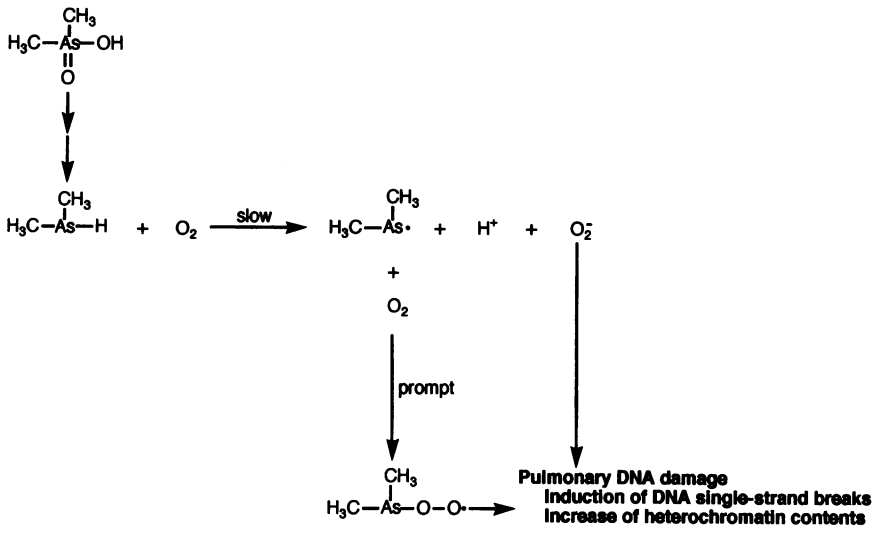

Figure 4. Free radical species formed by the reaction between dimethylarsine and molecular oxygen and its pulmonary DNA damage.

Table 1. Total amount of DNA detected by fluorescence analysis after exposure to dimethylarsine.

	Fluorescence (Relative fluorescence
Control	3.075
Dimethylarsine	
No enzyme	2.390
SOD + catalase	1.996

The values indicate total recovered from each fraction of the alkaline elution (Figure 3).

decreased after dimethylarsine exposure (Table 1). This decrease was not suppressed by the presence of SOD and catalase, suggesting that some radical species other than active oxygen species participated in DNA strand breaks. We therefore tried to determine the radical species by electron-spin resonance (ESR) analysis using the spin-trapping method with 5,5'-dimethyl-1-pyrroline-*N*-oxide (DMPO) (*8*). The coupling constants (a_N = 13.7 G and a_H = 11.2 G) obtained for the DMPO-spin adduct were not in agreement with those reported for the DMPO-spin adducts of superoxide anion radical (*14*) and hydroxyl radical (*15*), but were similar to those of alkyl peroxyl radicals (*15*). We considered that the free radical thus detected was the dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\cdot$]. We also detected, by the cytochrome c method (*8*), superoxide anion radical produced by the reaction of molecular oxygen with dimethylarsine. However, the production of superoxide anion radicals occurred relatively slowly.

Discussion

Inorganic arsenics are well known to be metabolized to methanearsonic acid and DMAA in mammals (*4*). The methylation process of inorganic arsenics has generally been thought to be a detoxication process (*16*). However, Vahter et al. (*17*) reported that DMAA had higher affinity than inorganic arsenics for the cell nucleus. We therefore assumed that DMAA might inflict a more toxic effect on the nucleus, so we investigated the genotoxic effects of the methylated metabolites, particularly those of the dimethylated ones.

Our results are summarized in Figure 4. We conclude that lung-specific DNA damage, i.e., DNA single-strand breaks (*6*) and the clumping of heterochromatin (*7*), induced by DMAA administration in mouse is due to the free radical species produced from the reaction between molecular oxygen and dimethylarsine, a further metabolite of DMAA (*6,19*). The dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\cdot$] may also play a major role in lung-specific DNA damage by DMAA administration in mice (*8*).

Recent studies of chemical carcinogenesis have indicated the dominant role of active oxygen (*20*). Of active oxygen species, the hydroxyl radical and superoxide anion radical are thought to be closely related to carcinogenesis. In the case of arsenics, however, we have shown that the dimethylarsenic peroxyl radical may play a dominant role in genotoxicity. Our proposal might explain the high risk of lung cancer by exposure to inorganic arsenics.

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