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INDUCTION OF DNA DAMAGE BY DIMETHYLARSINE, A METABOLITE OF INORGANIC ARSENICS, IS FOR THE MAJOR PART LIKELY DUE TO ITS PEROXYL RADICAL

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SUMMARY: To reveal the mechanisms of previously reported lung-specific DNA strand scissions in murine after oral administration of dimethylarsinic acid (DMAA), a main metabolite of inorganic arsenics in mammals, the ultimate substance causing DNA lesion was investigated using dimethylarsine which was a further metabolite of DMAA. The alkaline elution assay using ^{3}H-labeled DNA showed that a major portion of the strand breaks was not suppressed by SOD and catalase, suggesting an ultimate substance other than active oxygen participated in the DNA damage. By ESR analysis, a radical estimated to be $(CH_3)_2AsOO\bullet$ was detected as a reaction product of dimethylarsine and molecular oxygen. This peroxyl radical, rather than active oxygen, was assumed to play a major role in DNA damage.

Human carcinogenicity of inorganic arsenics, evidenced by epidemiological surveys (1), has not been fully proved by experimental studies (2). As an approach to resolve this conflict, we have focused on the genotoxic action of dimethylated arsenics which are mammalian metabolites of inorganic arsenics. Our previous studies revealed that the oral administration of dimethylarsinic acid (DMAA), a main metabolite of inorganic arsenite and arsenate, induced lung-specific DNA damage,

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Abbreviations: DMAA, dimethylarsinic acid; O_2^-, superoxide; ESR, electron spin resonance; DMPO, 5,5-dimethyl-1-pyrrolin-N-oxide; TdR, thymidine; MEM, minimum essential medium; DMAA-Na, sodium dimethylarsinic acid; SOD, superoxide dismutase; DABA, 3,5-diaminobenzoic acid.

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particularly single-strand breaks, in mice and rats (3). We further indicated that the ultimate substances causing the breaks were the reaction products of molecular oxygen and dimethylarsine which was metabolically formed from DMAA (4). As one of these products, we estimated the production of superoxide anion in lung (4). This seems to be reasonable because dimethylarsine is a potent one electron donor to molecular oxygen to produce superoxide anion, which is known to cause the strand breaks of intracellular DNA possibly leading to carcinogenesis (5,6).

Here, we present that, other than superoxide anion, dimethylarsenic peroxyl radical is formed from dimethylarsine and molecular oxygen and that this radical is also and rather mainly responsible to DNA damage.

MATERIALS

DMAA-Na was obtained from Wako Pure Chemicals Co., and SOD, catalase and cytochrome c from Sigma Chemical Co. L-132 cells were purchased from Flow Labolatories Inc. DMPO and [methyl-^{3}H]TdR were obtained from Daiichi Pure Chemicals Co. and NEN Research Products, respectively.

METHODS

L-132 cells were grown in monolayer culture in Eagle's MEM supplemented with 10% fetal bovine serum in the presence of [methyl-^{3}H]TdR (1μCi/ml medium) at 37°C for ca. 20h.

The alkaline elution assay was based on the method described in our previous report (4). L-132 cells (1.5×10^6 cells) were lysed and digested with a mixture (5ml) of 2% SDS, 25mM EDTA and 0.1M glycine containing proteinase K (Merck, Darmstadt, 0.5mg/ml) on 2.0μm polycarbonate filter, and then washed with 20mM EDTA (pH10.0). The filter was further washed with 10mM phosphate buffer containing 0.85% NaCl (pH7.4). Immediately, a mixture (100μl) of SOD (1400 units) and catalase (4400 units) in 10mM phosphate buffer containing 0.85% NaCl (pH7.4) was applied on the filter. The filter was exposed for 1h at 20°C to dimethylarsine generated by the reduction of DMAA with sodium borohydride. After alkaline elution, 200μl of each fraction of ^{3}H-labeled DNA was withdrawn, and then dissolved in 10ml of a scintillation cocktail (Aquasol-2, NEN Research Products). The radioactivity was measured in a liquid scintillation counter (Packard, model 2000CA). The determination of non-labeled DNA was carried out by the method described previously (7).

Dimethylarsine was prepared as follows. 20mmol DMAA-Na in 25ml of 6N HCl was reduced by adding 10% sodium borohydride (20ml) under dry nitrogen. The generated gas containing dimethylarsine was passed through KOH-pellet trap and then trapped in an acetone-dry ice bath. Head-spaced air in trapping tube was exchanged with dry nitrogen and kept at -80°C until use.

The spin trapping method was carried out in an ESR spectrometer (JOEL JES-FE3AX X-band spectrometer). Dimethylarsine was added to acetonitrile containing 10μl of DMPO. After mixing rapidly, the ESR spectrum was immediately measured at room temperature.

The determination of superoxide anion by the cytochrome c method (8) was carried out as follows: A solution (10μl) of SOD (37500 units/ml) in 10mM phosphate buffer containing 0.85% NaCl (pH7.4) was added to 3ml of $5x10^{-5}$M cytochrome c solution. The solution was exposed to dimethylarsine generated by the reduction of 1mmol DMAA with sodium borohydride. Immediately, the solution was transferred to a cuvette and then absorbance at 550nm was measured at given time intervals for 1h.

RESULTS AND DISCUSSION

The L-132 cells, an established human embryonic cell line of type II alveolar epithelial cell (9), were lysed and digested on a polycarbonate filter (2.0μm) and exposed to dimethylarsine which was generated from 0.2mmol DMAA with sodium borohydride. The single strand breaks of DNA were analyzed by the alkaline elution assay. Figure 1a shows the alkaline elution profiles of DNA based on the fluorescence measurement using DABA which reacts with deoxyribose moieties of DNA. A remarkable strand scissions induced by the exposure were apparently diminished by the addition of SOD and catalase. On the other hand, when the assay was performed by counting ^{3}H-radioactivity of ^{3}H-TdR-labeled L-132 cells, the profile of strand scissions was markedly different from that by the fluorescence analysis; the scissions were hardly diminished by the addition of SOD and catalase (Fig. 1b).

To explain this disagreement, the amount of DNA before and after the exposure to dimethylarsine was analyzed by the two methods. As shown in Table 1, ^{3}H-radioactivity was not changed after the exposure, while the fluorescence decreased remarkably even in the presence of SOD and catalase. This result strongly suggests that the deoxyribose moieties of DNA were attacked by the exposure of dimethylarsine and, consequently, the formation of fluorescent quinaldine from DABA and deoxyribose decreased. Actually, we found that dimethylarsine readily reacted with deoxyribose in DNA and, resultingly, produced the propenal derivatives (e.g., thymine propenal), which could not form fluorescent derivatives with DABA[2]. This is very likely to be the reason for the decrease of fluorescence after dimethylarsine exposure and for the disagreement between the two methods. It may, therefore, be proposed that the radio-tracer measurement is more relevant for the determination of DNA damage than the fluorescence measurement.

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[2]Unpublished data.

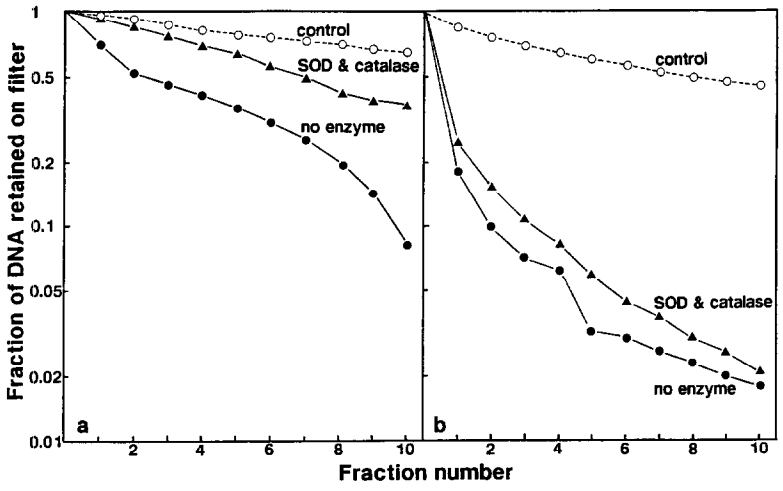


Figure 1 Single-strand breaks of DNA detected by the fluorescence assay (a) and the radioactivity assay (b) after in vitro exposure to dimethylarsine.

The radioactivity assay indicating that SOD and catalase gave little effect on DNA strand scission by dimethylarsine exposure (Fig. 1b) strongly suggests that some radical species other than active oxygen participated in DNA damage. To determine the radical species, we applied electron spin resonance (ESR) analysis by using the spin trapping method with DMPO. Figure 2 shows the ESR spectrum of DMPO spin adduct of a reaction product of dimethylarsine and molecular oxygen. A radical species with the coupling constants of a_N=13.7G and a_H=11.2G was detected ca. 3min after starting the reaction in acetonitrile. These values are different from those reported for the DMPO spin adducts of O_2^- (a_N=14.2G, a_H=12.0G) (10) and •OH (a_N=15.0G, a_H=15.0G) (11). Furthermore, since the coupling constants of DMPO spin adducts

Table 1. Comparison of total amount of DNA by the fluorescence assay and the ^{3}H-radioactivity assay after exposure of dimethylarsine

	Fluorescence (Relative intensity)	^{3}H(dpm)
Control	3.075 (20.5)*	27681
Dimethylarsine		
No enzyme	2.390 (15.9)*	29055
SOD & catalase	1.996 (13.3)*	30659

The values represent total recovered from each fraction of alkaline elution. *The values in parentheses show the amount of DNA(μg) determined by the fluorescence analisis.

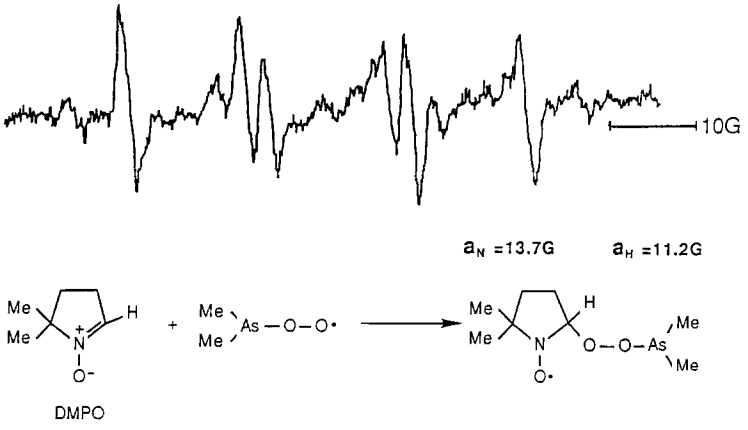


Figure 2 ESR spectrum of the arsenic-peroxyl radical produced by the reaction of dimethylarsine and molecular oxygen.

have reported to be a_N=14-16G and a_H=19-23G for C-radicals, a_N=13-14G and a_H= 6-9G for O-radicals, and a_N=12-15G and a_H=10-12G for alkylperoxyl radicals (12), we assume that the most probable DMPO spin adduct detected here is $(CH_3)_2$AsOO-DMPO. Although DMPO spin adduct of O_2^-, i.e., HOO-DMPO, was not detected by the ESR analysis possibly due to its instability, the production of O_2^- seemed to be also probable because DNA damage was diminished by SOD and catalase (Fig. 1a) (4). Therefore, we applied the cytochrome c method to detect O_2^-. As shown in Figure 3, immediately after the exposure of cytochrome c to dimethylarsine under air, the absorbance of 550nm indicating the reduction of cytochrome c remarkably elevated and further increased

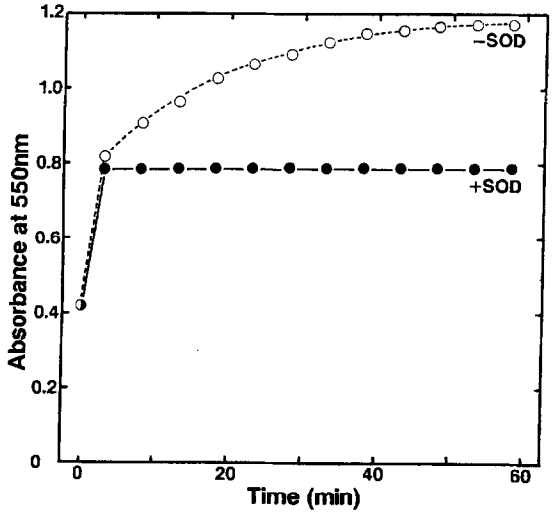
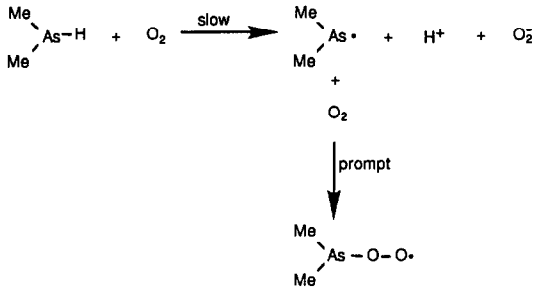


Figure 3 Identification by the cytochrome c method of superoxide generated by the reaction of dimethylarsine and molecular oxygen.



Scheme 1 Reaction pathway of dimethylarsine and molecular oxygen.

for approximately 60min. In the presence of SOD, on the other hand, this continuous reduction of cytochrome c was not observed although the initial elevation of the absorbance was remarkably occurred. This suggests that the initial increase was made by dimethylarsine, a potent reducing agent, and the slow increase afterwards without SOD was by O_2^-.

As represented in Scheme 1, dimethylarsine, which is metastable compared to inorganic arsine (AsH_3), gives an electron to molecular oxygen to form $(CH_3)_2As\bullet$ and O_2^- radicals rather slowly. The former radical promptly reacts with molecular oxygen to produce dimethylarsenic-peroxyl radical ($(CH_3)_2AsOO\bullet$), which is fairly stable, assumingly even in cells, and attacks the deoxyribose residues of DNA to cause strand scissions similarly to •OOH, because the chemical properies of both the radicals seem to be analogous. This dimethylarsenic-peroxyl radical may play a major role in DNA strand scission rather than active oxygen, as presumed from the data shown in Figure 1. Recent works on chemical carcinogenesis have pointed out the dominant role of active oxygen, particularly of hydroxyl radical, in DNA damage in most cases (13). However, in case of arsenics, we would propose that dimethylarsenic-peroxyl radical might be a major ultimate substance to cause DNA lesion rather than active oxygen.

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