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Endonuclease III, Formamidopyrimidine-DNA Glycosylase, and Proteinase K Additively Enhance Arsenic-Induced DNA Strand Breaks in Human Cells

Tsu-Shing Wang,[†] Chiao-Hui Chung,[†] Alexander S. S. Wang,[‡] Da-Tian Bau,[‡] Thangavel Samikkannu,[‡] Kun-Yan Jan,*[,‡] Yang-Ming Cheng,[§] and Te-Chang Lee[§]

Department of Life Sciences, Chung Shan Medical University, Taichung, 402, Taiwan, ROC, and Institute of Zoology and Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan, ROC

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We report here that sequential digestion with endonuclease III, formamidopyrimidine-DNA glycosylase, and proteinase K in Tris buffer markedly increased the sensitivity for detecting DNA damage in arsenic-treated cells. These three enzymes increased DNA strand breaks in an additive manner. By using this sequential-enzyme-digestion comet assay, we demonstrated that trivalent inorganic arsenic induced more DNA damage than monomethylarsonous acid, monomethylarsonic acid, and dimethylarsinic acid in human blood cell lines. However, trivalent inorganic arsenic was far less potent than monomethylarsonous acid in inhibiting pyruvate dehydrogenase activity. Therefore, different mechanisms are involved in inhibiting pyruvate dehydrogenase activity and inducing DNA damage. Our results also indicate while trivalent inorganic arsenic induced more endonuclease III-digestible adducts, monomethylarsonous acid and monomethylarsonic acid induced more proteinase K-digestible adducts. These results suggest there is a difference in the mechanism for inducing DNA damage between inorganic and organic methylated arsenic compounds.

Introduction

Recent studies suggest that arsenic may cause genetic damage through the generation of reactive oxygen species. This notion is supported by the observation that arsenite induces more micronuclei in cells deficient in glutathione peroxidase and catalase (*1, 2*); arsenite-induced apoptosis is triggered by reactive oxygen species (*3, 4*); arsenite activates NADH oxidase to increase superoxide production and to cause oxidative DNA damage (*5*); *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg),[1] an endonuclease known to catalyze the excision of oxidized bases, increases the amount of DNA strand breaks in arsenite-treated cells (*5*−*7*).

Arsenic exposure is associated with various human vascular disorders, including angiosarcomas (*8*), atherosclerotic plaques (*9*), and hypertension (*10*). Since oxidative stress has been shown to be an important cause of human vascular disorders (*11*), it is tempting to speculate that arsenic may induce human vascular disorders via oxidative DNA damage. However, the arsenic concentration in human blood is very low. Certain inhabitants of Inner Mongolia, China, who continued drinking well water containing high concentrations of inorganic arsenic (0.41 μg/mL), had a mean total arsenic concentration in blood of 42.1 ng/mL (about 0.56 μM) (*12*). With the help of Fpg, oxidative DNA damage was detected in human vascular smooth muscle cells treated with 1 μM arsenite for 4 h (*5*), in human lymphocytes with 10 μM arsenite for 2 h (*6*), and in the human leukemia cell lines NB4 and HL60 with 0.25 μM arsenite for 4 h (*7*). The evidence would be more convincing if oxidative DNA damage could be detected in human blood vessel cells treated with arsenic below 0.5 μM. To this end, we have attempted to improve the sensitivity of detecting DNA damage. We found that the DNA strand breaks in arsenite-treated human cells could be increased substantially by comet assay in which the slides were digested with endonuclease III (EnIII), then with Fpg, and finally with proteinase K (PK) in Tris buffer.

The arsenic compounds in drinking water are mainly in inorganic pentavalent forms. Upon ingestion, the pentavalent inorganic arsenic compounds are reduced to trivalent inorganic arsenite, which is then metabolized through a series of methylation. However, inorganic trivalent arsenic (iAs^{III}), monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), monomethylarsonous acid (MMA^{III}), and dimethylarsenous acid (DMA^{III}) are all detectable in the human body (*13*). Methylation was previously recognized as an arsenic detoxification pathway, but recent studies indicate that some methylated arsenic compounds are more toxic than inorganic trivalent arsenic. For example, DMA^{V} has been shown to induce 8-oxo-2′-deoxyguanosine in mice, whereas, arsenite is unable to do so (*14*). Using the standard comet

* Address correspondence to this author at the Institute of Zoology, Academia Sinica, Taipei 11529, Taiwan, ROC. Tel: 886-2-27899513; Fax: 886-2-27858059; E-mail: ZOJKY@Sinica.edu.tw.

[†] Chung Shan Medical University.

[‡] Institute of Zoology, Academia Sinica.

[§] Institute of Biomedical Sciences, Academia Sinica.

[1] Abbreviations: Fpg, formamidopyrimidine-DNA glycosylase; EnIII, endonuclease III; PK, proteinase K; iAs^{III}, inorganic trivalent arsenic; MMA^{V}, monomethylarsonic acid; DMA^{V}, dimethylarsinic acid; MMA^{III}, monomethylarsonous acid; DMA^{III}, dimethylarsenous acid; PDH, pyruvate dehydrogenase.

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assay without enzyme digestion, the relative potencies of various arsenic compounds in inducing DNA strand breaks in human lymphocytes were reported to be: $DMA^{III} > MMA^{III} \gg iAs^{V} \sim iAs^{III} > MMA^{V} > DMA^{V}$ (*15*). However, this view is not supported by using the sequential enzyme digestion to analyze the DNA damage induced by various arsenic compounds.

Materials and Methods

Cells. HL60 cells (kindly provided by Dr. C. Y. Liu of Veterans Hospital, Taipei) were cultured in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum, penicillin (100 units/mL), streptomycin (100 μg/mL), and 0.03% glutamate. Cultures were incubated at 37 °C in a water-saturated atmosphere containing 5% CO_2. Cells were subcultured every 3 days, and cultures were maintained within a cell density range of $(2-10) \times 10^5$/mL.

N4 is an immortal lymphoblastoid cell line, prepared from a human peripheral blood sample. The blood from a healthy female volunteer was drawn with a heparinized syringe, and lymphocytes were isolated by centrifugation in a Ficoll-Paque (Pharmacia Biotech, Swiss) cushion. They were washed with phosphate-buffered saline and resuspended in RPMI 1640 medium containing 10% fetal calf serum and 1% PSN antibiotic mixture. The lymphocytes were then transformed by using Esptein-Barr virus (*16*).

Chemicals. EnIII and Fpg were purchased from Trevigen (Gaithersburg, MD). Sodium arsenite (>98.5%, iAs^{III}) and dimethylarsinic acid sodium trihydrate (>97%, DMA^{V}) were purchased from Merck (Darmstadt, Germany). MMA^{V} was purchased from Chemical Services (West Chester, PA). MMA^{III} was prepared by reacting MMA^{V} with SO_2 as described by Cullen et al. (*17*). The purity of CH_3AsO was 99.7% as analyzed by a nuclear magnetic resonance spectrometer (AMX400) and a Heraeus CHN-OS rapid element analyzer.

Comet Assay. The standard comet assay without enzyme digestion has been described previously (*7*). For enzyme digestion, slides after lysis treatment were washed with distilled water and then incubated at 37 °C for 30 min in enzyme reaction buffer. Following this, 0.5 unit of EnIII, 1 unit of Fpg, or 5 ng of PK in 10 μL of enzyme reaction buffer was added to the center of each half of the gel. A coverslip was applied, and the slides were incubated at 37 °C for 2 h in a sealed box containing wet tissue paper. The slides were prepared for electrophoresis as previously described (*7*). The migration of DNA from the nucleus of each cell was measured with a computer program using the parameter of comet moment (*18*). The comet moment was calculated using the formula: $\sum_{0\rightarrow n}$[(amount of DNA at distance X) × (distance X)]/total DNA. In initial experiments, we used phosphate-buffered saline as PK digestion buffer, and the enzyme buffers supplied by Trevigen, i.e., 10 mM HEPES−KOH (pH 7.4), 100 mM KCl; 10 mM EDTA, 0.1 mg/mL bovine serum albumin as Fpg digestion buffer; 10 mM HEPES−KOH (pH 7.4), 100 mM KCl, and 10 mM EDTA as EnIII digestion buffer. In the latter experiments 10 mM Tris-HCl (pH 7.5) was used as digestion buffer for all three enzymes.

PDH Activity. Pyruvate dehydrogenase (PDH) from porcine heart was purchased from Sigma (St. Louis, MO). The activity of PDH was measured by the method of Robertson (*19*), with some modification. PDH (30 milliunits) was mixed with various concentrations of arsenic and incubated at 37 °C for 0.5 h. They were then mixed with reaction buffer containing Tris-HCl (pH 7.4), 4 mM $NADP^+$, 0.5 mM thiamin pyrophosphate, 1 mM $MgCl_2$, 0.5 mM CoA, 0.5 mM dithiothreitol, and 5 mM pyruvate in a total volume of 1000 μL. After standing for 30 min in room temperature, the NADPH formation was detected by an ELISA reader at 340 nm.

Statistical Analysis. Results are expressed as mean ± standard deviation throughout the paper. Statistical analyses were performed with Student's two-tailed unpaired *t*-test. Values of $p < 0.05$ were considered to be statistically significant and are indicated by *.

Table 1. Effect of Different Buffers in Supporting Enzyme Digestion[a]

	Trevigen EnIII buffer	Trevigen Fpg buffer	Tris buffer
EnIII	8.9 ± 0.4*	6.5 ± 0.8*	19.7 ± 1.4
Fpg	4.1 ± 0.2*	9.9 ± 0.4*	12.7 ± 1.0
EnIII/Fpg	5.6 ± 0.4*	8.3 ± 0.4*	26.9 ± 0.4
EnIII→Fpg	8.9 ± 0.4*	16.8 ± 0.4*	28.2 ± 1.0
Fpg→EnIII	9.5 ± 0.4*	14.2 ± 1.0*	26.8 ± 0.8
EnIII/Fpg→PK	7.5 ± 0.4*	16.3 ± 0.8*	33.4 ± 2.8
EnIII→Fpg→PK	15.5 ± 0.8*	26.1 ± 0.4*	55.3 ± 2.6
Fpg→EnIII→PK	12.5 ± 0.6*	23.3 ± 1.0*	36.1 ± 4.2

[a] The comet moments of untreated cells without enzyme digestion and when digested with EnIII, with Fpg, with PK, and with EnIII→Fpg→PK were 2.0 ± 0.7, 2.2 ± 0.4, 2.5 ± 0.5, 2.1 ± 0.3, and 2.3 ± 0.6, respectively. The data in the table are the comet moment induced by treating HL60 cells with 1 μM $NaAsO_2$ for 4 h. For simplicity, the comet moment of untreated cells has been subtracted. Without enzyme digestion, the comet moment of iAs^{III}-treated cells was 3.8 ± 0.1. (/) Simultaneous digestion; (→) sequential digestion; *$p < 0.05$ compared with that obtained by using Tris buffer.

Results

Sequential Digestion with Fpg Then EnIII, and Then PK in Tris Buffer Gave the Highest Arsenite-Induced Comet Moment. In an attempt to improve the sensitivity for detecting arsenic-induced DNA damage, we have incubated the slides containing sodium arsenite-treated cells with EnIII and Fpg simultaneously, but did not observe an increase in comet moment as compared to incubation with either of the enzymes alone. In this experiment, the enzyme buffers supplied by Trevigen were used; however, we noticed that Tris buffer was used for simultaneous digestion with EnIII and Fpg (*20*). We then tested different buffers for their efficiency in supporting digestion with EnIII and Fpg. To our surprise, Tris buffer gave a much higher arsenite-induced comet moment than did the Trevigen buffers in single-enzyme digestion, simultaneous EnIII and Fpg digestion, and sequential two-enzyme digestion (Table 1). The results in Table 1 also indicate that sequential digestion with EnIII and then Fpg gave higher arsenite-induced comet moment than did sequential digestion with Fpg then EnIII, or simultaneous EnIII plus Fpg digestion. Moreover, sequential digestion with EnIII then Fpg, and then PK gave the highest arsenite-induced comet moment compared to sequential digestion with Fpg then EnIII, and then PK, or simultaneous EnIII plus Fpg digestion followed by PK digestion.

With Enzyme Digestion, the Levels of DNA Strand Breaks Induced by iAs^{III} Were Higher than Those of MMA^{III}. Although many reports indicate that trivalent inorganic arsenic is more toxic than organic arsenic, several recent studies show that methylated arsenic compounds can be more toxic than trivalent inorganic arsenic. We have then used the above-described sequential-enzyme-digestion comet assay to compare the relative potencies of various arsenic compounds in inducing DNA damage. The results indicate that the relative potency in HL60 cells was: $MMA^{III} > iAs^{III} > MMA^{V} > DMA^{V}$ without enzyme digestion (Figure 1A). Enzyme digestion substantially increased the DNA strand breaks in all the treatments (Figure 1A,B). With enzyme digestion, the levels of DNA strand breaks induced by treatment with

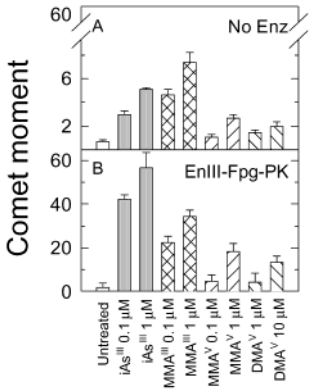

Figure 1. Induction of DNA damage by iAs^{III}, MMA^{III}, MMA^{V}, and DMA^{V}. HL60 cells were treated for 4 h with 0.1 and 1 μM $NaAsO_2$, 0.1 and 1 μM MMA^{III}, 0.1 and 1 μM MMA^{V}, and 1 and 10 μM DMA^{V}. DNA strand breaks were analyzed using comet assay without enzyme digestion (A), or with digestion by EnIII then Fpg, and then PK in Tris buffer (B).

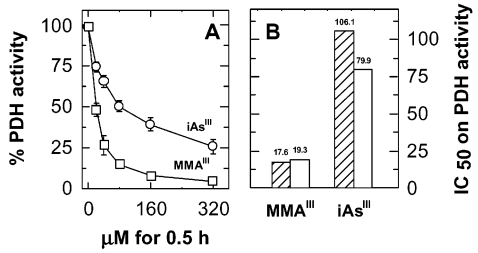

Figure 2. Effect of treatment with iAs^{III} and MMA^{III} on PDH activity. (A) Commercial PDH (30 milliunits) was mixed with arsenic and incubated at 37 °C for 0.5 h. The activity of PDH was measured as described under Materials and Methods. (B) The IC values estimated from (A) (open bars) were compared with that of Petrick et al. (*21*) (shaded bars)

iAs^{III} were higher than that of MMA^{III}, MMA^{V}, and DMA^{V} (Figure 1B). Since MMA^{III} was reported to be more potent in inhibiting the activity of PDH than iAs^{III} (*21*), we have examined the inhibition of PDH activity to check the quality of MMA^{III} used in this investigation. The results indicate that MMA^{III} was indeed far more potent than iAs^{III} in inhibiting PDH activity (Figure 2). To confirm that iAs^{III} is indeed more potent than MMA^{III} in inducing DNA damage, we reexamined the DNA damage in HL60 cells by treatment with arsenic for 1−4 h. The results indicate that DNA damage was increased with increasing arsenic treatment time, and this phenomenon was more obvious with enzyme digestion than without (Figure 3A,C). Without enzyme digestion, only a small amount of DNA damage was detected, and the difference between the levels of DNA damage induced by iAs^{III} and MMA^{III} was not very obvious. Enzyme digestion markedly increased the level of DNA damage, and the level of DNA damage induced by iAs^{III} was apparently higher than that of MMA^{III}. Further confirmation was made by using a lymphoblastoid cell line, because in human lymphocytes MMA^{III} is 77 times more potent in inducing DNA damage than iAs^{III} (*15*). The results again indicate that more DNA damage was induced by iAs^{III} than MMA^{III} at the concentration of 0.1 or 1 μM with treatment for 1, 2, or 4 h (Figure 3B,D).

EnIII, Fpg, and PK Revealed DNA Adducts in a Nonoverlapping Manner. In an attempt to understand the reason DNA damage could be markedly increased by a sequential digestion with EnIII then Fpg and then PK, we observed that these three enzymes revealed DNA

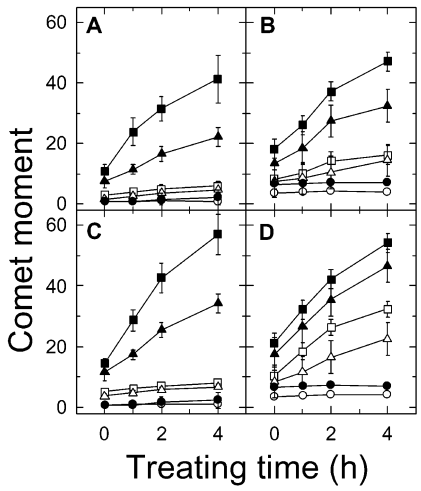

Figure 3. Comparison of DNA damage induced by iAs^{III} and MMA^{III}. HL60 (A, C) or N4 cells (B, D) were treated with 0.1 (A, B) or 1 μM (C, D) arsenic for 0, 1, 2, and 4 h. DNA strand breaks were analyzed by comet assay without enzyme digestion (open symbols), or with sequential enzyme digestion (closed symbols). Circles, untreated; triangles, MMA^{III}; squares, iAs^{III}.

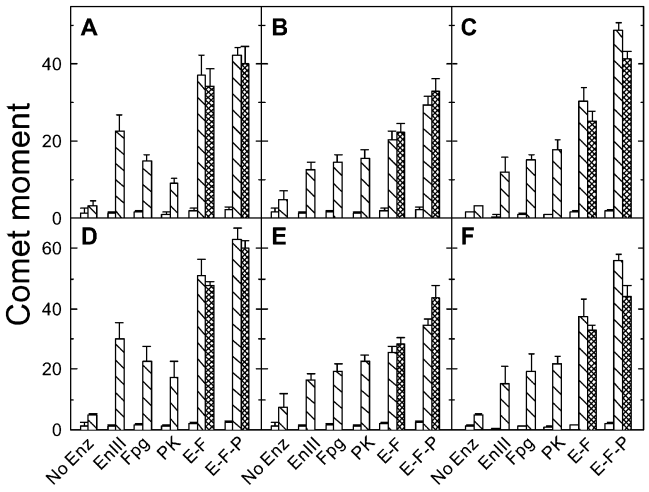

Figure 4. Effect of enzyme digestion on the level of DNA strand breaks in cells treated with iAs^{III}, MMA^{III}, and MMA^{V}. HL60 cells were treated for 4 h with 0.1 (A) and 1 μM iAs^{III} (D), 0.1 (B) and 1 μM MMA^{III} (E), or 1 (C) and 2 μM MMA^{V} (F). DNA strand breaks were analyzed by using comet assay without enzyme digestion (No Enz), or digestion with either EnIII, Fpg, or PK alone, or with EnIII then Fpg (E−F), or with EnIII then Fpg and then PK (E−F−P), in Tris buffer. Open bars, untreated; hatched bars, treated with arsenic; cross-hatched bars, expected additive values (comet moment of EnIII + Fpg − Untreated; or EnIII + Fpg + PK − 2 Untreated).

adducts in a nonoverlapping manner. The levels of DNA strand breaks revealed by EnIII then Fpg were more or less equal to sum of those by EnIII and Fpg alone (Figure 4). And the levels of DNA strand breaks revealed by EnIII then Fpg then PK were more or less equal to the sum of that by EnIII, Fpg, and PK alone. We also noticed that the relative level of DNA damage revealed by these three enzymes was: EnIII > Fpg > PK in iAs^{III}-treated cells. Whereas, in MMA^{III}- and MMA^{V}-treated cells, the relative level was: PK > Fpg > EnIII (Figure 4).

Discussion

Tris Buffer Does Not Contain EDTA or Potassium. The results presented in Table 1 clearly indicate

that digestion with EnIII, Fpg, or PK in Tris buffer gave more DNA strand breaks for iAs^{III}-treated cells than digestion with these enzymes in Trevigen buffers. The Trevigen buffers contain HEPES−KOH, 10 mM EDTA, and 100 mM KCl, whereas the Tris buffer does not have these components. The lack of a divalent metal chelator and the decrease in ionic strength of Tris buffer could mean that additional nonspecific nuclease activity might have contributed to the observed increase in comet moment when using Tris buffer. However, this notion is not supported by the observation as indicated in the note for Table 1, that digestion of cells without arsenic treatment with EnIII, Fpg, or PK in Tris buffer did not increase the comet moment appreciably compared to without enzyme digestion. At this moment, we are still unable to give a good explanation why digestion of iAs^{III}-treated cells with EnIII, Fpg, or PK in Tris buffer gave more DNA strand breaks than in Trevigen buffers.

EnIII, Fpg, and PK Each Has a Different Pattern of Substrate Specificity. The present experiments demonstrated that sequential digestion with EnIII, Fpg, and then PK markedly increased the levels of DNA damage induced by iAs^{III}, MMA^{III}, MMA^{V}, and DMA^{V}. EnIII has been shown to cleave thymine glycol, 5,6-dihydrothymine, 5-hydroxydihydrothymine, 5-hydroxycytosine, 5-hydroxyuracil, and uracil glycol (*22*). Fpg protein has been shown to cleave oxidative bases such as 8-oxoguanine, 5-hydroxycytosine, 5-hydroxyuracil, 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine, and 4,6-diamino-5-formamidopyrimidine (*23*). PK is reported to release the DNA strand breaks bound by DNA−protein cross-links (*24*). In addition, arsenite induction of Fpg- and PK-digestible adducts in HL60 cells has shown to be mediated by nitric oxide and superoxide (*7*). Therefore, digestion with these enzymes seems to cleave the adducts induced by arsenic treatment and transform these DNA adducts into breaks, rather than increases the amount of DNA damage induced by arsenic. Since the present results show that the levels of DNA strand breaks increased by these three enzymes were in an additive manner, it implies that there is little overlapping in the adducts removed by each of these three enzymes. Thus, the amount of 5-hydroxycytosine and 5-hydroxyuracil in arsenic-treated human lymphoblastoid and HL60 cells was negligible. These results are also consistent with the view that EnIII removes oxidized pyrimidines and Fpg removes oxidized purines (*25*).

Inorganic and Organic Methylated Arsenic Compounds Induce DNA Damage via Different Mechanisms. The present results also show that while iAs^{III} induced more EnIII-digestible adducts, MMA^{III} and MMA^{V} induced more PK-digestible adducts. Therefore, there is a difference in the mechanism of DNA damage induction between inorganic and organic methylated arsenic compounds. This notion is consistent with the report that while peroxyl radical has been shown to be involved in DMA^{V}-induced DNA damage (*26*), peroxynitrite, hypochlorous acid, and hydroxyl radicals are proposed to be involved in iAs^{III}-induced DNA damage (*7*). Moreover, while MMA^{III} can nick purified ϕX174 DNA directly, the iAs^{III} is unable to do so (*15*). However, the difference in purely chemical reactivity as observed in the ϕX174 DNA system may not be in any way related to the differences in DNA damage seen after treatment of biological systems with inorganic and organic methylated arsenic compounds.

Arsenic Induces DNA Damage and Inhibits PDH Activity via Different Mechanisms. The results show that while iAs^{III} was more potent than MMA^{III} in inducing DNA damage, it was far less potent than MMA^{III} in inhibiting PDH activity. Therefore, different mechanisms are involved in inhibiting PDH activity and inducing DNA damage. Arsenite is a potent inhibitor of PDH (*27*), and the inhibition is believed to be mediated through the covalent binding of arsenite to critical vicinal dithiols on lipoamide moieties of the enzyme (*28*), whereas radicals rather than specific binding to vicinal thiols is involved in the induction of DNA damage (*7*).

Arsenic May Induce Oxidative DNA Damage To Cause Atherosclerosis. The present results indicate that iAs^{III}, MMA^{III}, and MMA^{V} at the concentration of 0.1 μM were able to induce DNA adducts in human leukemia and lymphoblastoid cells, and these adducts were sensitive to EnIII and Fpg, which are known to excise oxidized bases. Since a total blood arsenic concentration of 42.1 ng/mL (0.56 μM) was measured in people consuming water containing high levels of arsenic (*12*), these results indicate that arsenic can induce oxidative DNA damage within pathologically meaningful concentrations. Since increasing DNA damage is correlated with the severity of atherosclerotic disease (*29*), it seems that arsenic may induce oxidative DNA damage to cause atherosclerosis. The demonstration of oxidative DNA damage in arsenic-treated human cells also implies that arsenic is capable of inducing reactive oxygen species in human tissues. Since reactive oxygen species are known to damage DNA, proteins, and lipids, the arsenic-induced reactive oxygen species may also cause human disorders via pathways other than oxidative DNA damage. Reactive oxygen species have been shown to be involved in the multistage carcinogenic process, including carcinogen activation, oxidative DNA damage, and tumor promotion (*30*).

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References

(1) Wang, T. S., Shu, Y. F., Liu, Y. C., Jan, K. Y., and Huang, H. (1997) Glutathione peroxidase and catalase modulate the genotoxicity of arsenite. *Toxicology* **121**, 229−237.

(2) Wang, T. S., and Huang, H. (1994) Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. *Mutagenesis* **9**, 253−257.

(3) Gurr, J. R., Bau, D. T., Liu, F., Lynn, S., and Jan, K. Y. (1999) Dithiothreitol enhances arsenic trioxide-induced apoptosis in NB4 cells. *Mol. Pharmacol.* **56**, 102−109.

(4) Wang, T. S., Kuo, C. F., Jan, K. Y., and Huang, H. (1996) Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. *J. Cell. Physiol.* **169**, 256−268.

(5) Lynn, S., Gurr, J. R., Lai, H. T., and Jan, K. Y. (2000) NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. *Circ. Res.* **86**, 514−519.

(6) Li, D., Firozi, P. F., Chang, P., Wang, L. E., Xiong, P., Sturgis, E. M., Eicher, S. A., Spitz, M. R., Hong, W. K., and Wei, Q. (2001) In vitro BPDE-induced DNA adducts in peripheral lymphocytes as a risk factor for squamous cell carcinoma of the head and neck. *Int. J. Cancer* **93**, 436−440.

(7) Wang, T. S., Hsu, T. Y., Chung, C. H., Wang, A. S. S., Bau, D. T., and Jan, K. Y. (2001) Arsenite induces oxidative DNA adducts

and DNA-protein cross-links in mammalian cells. *Free Radical Biol. Med.* **31**, 321−330.
(8) Falk, H., Herbert, J. T., Edmonds, L., Heath, C. W., Thomas, L. B., and Popper, H. (1981) Review of four cases of childhood hepatic angiosarcomas—elevated environmental arsenic exposure in one case. *Cancer* **47**, 382−391.
(9) Chen, C. J., Wu, M. M., Lee, S. S., Wang, J. D., Cheng, S. H., and Wu, H. Y. (1988) Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. *Arteriosclerosis* **8**, 452−460.
(10) Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H., and Axelson, O. (1999) Hypertension and arsenic exposure in Bangladesh. *Hypertension* **33**, 74−78.
(11) Martinet, W., Knaapen, M. W. M., De Meyer, G. R. Y., Herman, A. G., and Kockx, M. M. (2001) Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. *Circ. Res.* **88**, 733−739.
(12) 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 Radical Biol. Med.* **28**, 1137−1142.
(13) Aposhian, H. V., Gurzau, E. S., Le, X. C., Gurzau, A., Healy, S. M., Lu, X., Ma, M., Yip, L., Zakharyan, R. A., Maiorino, R. M., Dart, R. C., Tircus, M. G., Gonzalez-Ramirez, D., Morgan, D. L., Avram, D., and Aposhian, M. M. (2000) Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. *Chem. Res. Toxicol.* **13**, 693−697.
(14) Yamanaka, K., Mizoi, M., Kato, K., Hasegawa, A., Nakano, M., and Okada, S. (2001) Oral administration of dimethylarsinic acid, a main metabolite of inorganic arsenic, in mice promotes skin tumorigenesis initiated by dimethylbenz[*a*]anthracene with or without ultraviolet B as a promoter. *Biol. Pharm. Bull.* **24**, 510−514.
(15) Mass, M. J., Tennant, A., Roop, B. C., Cullen, W. R., Styblo, M., Thomas, D. J., and Kligerman, A. D. (2001) Methylated trivalent arsenic species are genotoxic. *Chem. Res. Toxicol.* **14**, 355−361.
(16) Pattengale, P. K., Smith, R. W., and Gerber, P. (1973) Selective transformation of B lymphocytes by E.B. virus. *Lancet* **2**, 93−94.
(17) Cullen, W. R., McBride, B. C., Manji, H., Pickett, A. W., and Reglinski, J. (1989) The metabolism of methylarsine oxide and sulfide. *Appl. Organomet. Chem.* **3**, 71−78.
(18) Kent, C. R., Eady, J. J., Ross, G. M., and Steel, G. G. (1995) The comet moment as a measure of DNA damage in the comet assay. *Int. J. Radiat. Biol.* **67**, 655−660.
(19) Robertson, J. G., Barron, L. L. and Olson, M. S. (1987) Effects of alpha-ketoisovalerate on bovine heart pyruvate dehydrogenase complex and pyruvate dehydrogenase kinase. *J. Biol. Chem.* **261**, 76−81.
(20) Banath, J. P., Wallace, S. S., Thompson, J., and Olive, P. L. (1999) Radiation-induced DNA base damage detected in individual aerobic and hypoxic cells with endonuclease III and formamidopyrimidine-glycosylase *Radiat. Res.* **151**, 550−558.
(21) 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 of pyruvate dehydrogenase. *Chem. Res. Toxicol.* **14**, 651−656.
(22) Sarker, A. H., Ikeda, S., Nakano, H., Terato, H., Ide, H., Imai, K., Akiyama, K., Tsutsui, K., Bo, Z., Kubo, K., Yamamoto, K., Yasui, A., Yoshida, M. C., and Seki, S. (1998) Cloning and characterization of a mouse homologue (mNthl1) of *Escherichia coli* endonuclease III. *J. Mol. Biol.* **282**, 761−774.
(23) Nickoloff, J. A., Spirio, L. N., and Reynolds, R. J. (1998) A comparison of calcium phosphate coprecipitation and electroporation. Implications for studies on the genetic effects of DNA damage. *Mol. Biotechnol.* **10**, 93−101.
(24) Hartmann, A., and Speit, G. (1994) Comparative investigations of the genotoxic effects of metals in the single cells gel (SCG) assay and the sister chromatid exchange (SCE) test. *Environ. Mol. Mutagen.* **23**, 299−305.
(25) Ide, H. (2001) DNA substrates containing defined oxidative base lesions and their application to study substrate specificities of base excision repair enzymes. *Prog. Nucleic Acid Res. Mol. Biol.* **68**, 207−221.
(26) Yamanaka, K., Hoshino, M., Okamoto, M., Sawamura, R., Hasegawa, A., and Okada, S. (1990) Induction of DNA damage by dimethylarsine, a metabolite of inorganic arsenics, is for the major part likely due to its peroxyl radical. *Biochem. Biophys. Res. Commun.* **168**, 58−64.
(27) Hu. Y., Su, L., and Snow, E. T. (1998) Arsenic toxicity is enzyme specific and its affects on ligation are not caused by the direct inhibition of DNA repair enzymes. *Mutat. Res.* **408**, 203−218.
(28) Broome, M. C., Thomas, M. P., Hillier, A. J., and Jago, G. R. (1980) Pyruvate dehydrogenase activity in group N streptococci. *Aust. J. Biol. Sci.* **33**, 15−25.
(29) Botto, N., Rizza, A., Colombo, M. G., Mazzone, A. M., Manfredi, S., Masetti, S., Clerico, A., Biagini, A., and Andreassi, M. G. (2001) Evidence for DNA damage in patients with coronary artery disease. *Mutat. Res.* **493**, 23−30.
(30) Hu, J. J., Dubin, N., Kurland, D., Ma, B. L., and Roush, G. C. (1995) The effects of hydrogen peroxide on DNA repair activities. *Mutat. Res.* **336**, 193−201.

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