Induction of oxidative DNA damage by arsenite and its trivalent and pentavalent methylated metabolites in cultured human cells and isolated DNA

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Even though a well-known human carcinogen the underlying mechanisms of arsenic carcinogenicity are still not fully understood. For arsenite, proposed mechanisms are the interference with DNA repair processes and an increase in reactive oxygen species. Even less is known about the genotoxic potentials of its methylated metabolites dimethylarsinous acid [DMA(III)] and dimethylarsinic acid [MMA(III)], monomethylarsonous [MMA(III)] and dimethylarsinic acid [DMA(V)]. Within the present study we compared the induction of oxidative DNA damage by arsenite and its methylated metabolites in cultured human cells and in isolated PM2 DNA, by frequencies of DNA strand breaks and of lesions recognized by the bacterial formamidopyrimidine–DNA glycosylase (Fpg). Only DMA(III) (≥10 μM) generated DNA strand breaks in isolated PM2 DNA. In HeLa S3 cells, short-term incubations (0.5–3 h) with doses as low as 10 nM arsenite induced high frequencies of Fpg-sensitive sites, whereas the induction of oxidative DNA damage after 18 h incubation was rather low. With respect to the methylated metabolites, both trivalent and pentavalent metabolites showed a pronounced induction of Fpg-sensitive sites in the nanomolar or micromolar concentration range, respectively, which was present after both short-term and long-term incubations. Furthermore MMA(III) and DMA(V) generated DNA strand breaks in a concentration-dependent manner. Taken together our results show that very low physiologically relevant doses of arsenite and the methylated metabolites induce high levels of oxidative DNA damage in cultured human cells. Thus, biomethylation of inorganic arsenic may be involved in inorganic arsenic-induced genotoxicity/carcinogenicity.

Introduction

The majority of humans are chronically exposed to low levels of arsenic through ingestion of food and water and due to inhalation of arsenic in the ambient air. Even though the commercial use of arsenicals has been partly forbidden or reduced, arsenic contamination of drinking water is still a problem in many parts of the world. Thus, epidemiological evidence strongly supports an association between chronic exposure to arsenic via drinking water and increased risk for cancer of skin, liver, lung, kidney, prostate and bladder (for recent analysis of available literature see refs 1,2). However, the underlying mechanisms of arsenic carcinogenicity are still not fully understood. Arsenite is not significantly mutagenic, neither in bacterial test systems nor in mammalian cells in culture. In contrast, the clastogenic potential of arsenic compounds, generating mainly sister chromatid exchanges and chromatid-type chromosomal aberrations, is well documented. For arsenite, proposed mechanisms are the interference with DNA repair processes and the induction of oxidative stress (summarized in refs 3,4). With respect to DNA repair inhibition, arsenite interacts with enzymatic incision and ligation at low micromolar concentrations. Concerning the induction of oxidative DNA damage, some recent studies reported the generation of DNA damage, including DNA strand breaks (5–11), oxidative DNA base modifications (6,7,9,11) and DNA–protein crosslinks (5,11,12) at rather low concentrations of arsenite.

In humans, like in many mammalian species, inorganic arsenic is almost quantitatively reduced from pentavalent to trivalent arsenic in plasma and subsequently methylated to the trivalent and pentavalent methylated metabolites in the liver (summarized in ref. 13). Dimethylarsinic acid [DMA(V)] is the main urinary metabolite, with normal urinary excretion profiles of 10–20% inorganic arsenic, 10–20% monomethylarsenic acid [MMA(V)] and 60–80% DMA(V) (14). Generally, the in vivo biomethylation and excretion of inorganic arsenic as MMA(V) and DMA(V) has long been thought to be one major detoxification process as the pentavalent methylated metabolites are less reactive towards cellular macromolecules and are eliminated more rapidly (14,15). In support of this assumption, Moore et al. (16) demonstrated that MMA(V) and DMA(V) are less cytotoxic, mutagenic and clastogenic as compared with arsenite and arsenate in the L5178Y/TK±/– mouse lymphoma assay. However, for DMA(V) there is some evidence for being a complete carcinogen in rats (reviewed in ref. 17). Furthermore, it has been shown to induce DNA damage via formation of dimethylarsenic peroxyl radical, superoxide anion or hydroxyl radicals in laboratory animals (18–21) and cultured cells (22–25) but effects were restricted mainly to high, in the case of cellular systems, millimolar concentrations. Only one study showed increased DNA migration in single cell gel electrophoresis (comet assay) in lymphocytes treated with micromolar concentrations of MMA(V) and DMA(V), indicating DNA strand break formation (26).

However, in addition to the pentavalent metabolites, both monomethylarsonous [MMA(III)] and dimethylarsinous acid [DMA(III)] have been identified as intermediates in the metabolic pathway and have been detected in cultured human cells treated with inorganic arsenic (summarized in ref. 27). Also, Mandal et al. (28) reported the presence of MMA(III) (2–5% of urinary arsenic) and DMA(III) (4–21% of urinary arsenic) in the urine of people chronically exposed to inorganic arsenic.
via drinking water in West Bengal, India. Trivalent methylated metabolites are more cytotoxic in cultured mammalian cells (29,30) and more potent inhibitors of the activities of some important enzymes as compared with arsenite (summarized in ref. 27). Nevertheless, only a few data are available with respect to the genotoxicity of the trivalent methylated metabolites. Applying the comet assay, Mass et al. (31) demonstrated that DMA(III) and MMA(III) were more potent in generating DNA strand breaks in human lymphocytes as compared with arsenite. Furthermore, there is evidence that DMA(III) (31,32) and at very high concentrations (30 mM) MMA(III) (31) are able to nick isolated DNA without enzymatic or chemical activation.

The aim of the present study was to compare the induction of DNA strand breaks and oxidative DNA base modifications by arsenite and its trivalent and pentavalent metabolites MMA(III), DMA(III), MMA(V) and DMA(V) in cultured human cells at low, non-cytotoxic concentrations after short- and long-term incubations to elucidate potential contributions of methylated metabolites in arsenic-induced genotoxicity. Furthermore, comparative studies with isolated PM2 DNA were performed to clarify the role of cellular reactions involved in DNA damage induction.

Materials and methods

Caution

Inorganic arsenic is classified as a human carcinogen. The following chemicals are hazardous and should be handled carefully: sodium arsenite, methyloxoarsine, diiodomethylarsine, iododimethylarsine, MMA(V) and DMA(V).

Materials

Ham’s F12 nutrient mixture, fetal bovine serum, trypsin, penicillin–streptomycin solution, trizma base and ficoll 400 are products of Sigma (Deisenhofen, Germany). The culture dishes were supplied by Biochrom (Berlin, Germany). Triton X-100 was bought from Pierce (Oud-Beijerland, The Netherlands), Germany. The culture dishes were supplied by Biochrom (Berlin, Germany). The Fpg protein was a kind gift of Serge T.Schwerdtle et al. (University of British Columbia, Vancouver, Canada). All three materials and methods

Induction of oxidative DNA damage in isolated PM2 DNA

PM2 is a bacteriophage with a circular DNA of 10 kb, which was purified as described elsewhere (34). PM2 DNA prepared by this procedure retained ~90% supercoiled molecules. Linear DNA fragments were not detected.

To investigate the induction of DNA strand breaks and Fpg-sensitive sites in PM2 DNA by the arsenicals, PM2 DNA (30 μg/ml) was dissolved in buffer (40 mM sodium phosphate, 100 mM NaCl, pH 7.4) and incubated with the respective arsenic compound for 60 min at 37 °C. Afterwards PM2 DNA was precipitated with ethanol/125 mM sodium acetate for 30 min and centrifuged for 5 min at 7000 × g. After washing the DNA pellet twice with ethanol/125 mM sodium acetate, removal of the supernatant and resuspension of the DNA pellet (10 μl; 200 ng/sample) was incubated with Fpg (final concentration 1 μg/ml; 30 μl/sample) for 30 min at 37 °C. For the detection of DNA strand breaks only, Fpg was omitted in the last step. The reaction was terminated by adding 7 μl drop solution (0.25% bromophenol blue, 0.25% xylene cyanol, 15% Ficoll 400). Supercoiled and open circular forms of PM2 molecules were separated by electrophoresis in a 1% agarose gel in buffer (890 mM Tris base, 890 mM boric acid, 10 mM EDTA) for 2.5 h at 90 V. After staining with ethidium bromide, the density of the bands was measured using a Herolab gel detection system (EASY win 32). The number of DNA strand breaks and Fpg-sensitive sites were calculated as described before (34,35).

Results

Incubation conditions

To elucidate the induction and persistence of oxidative DNA damage, both short-term (0.5–3 h) and long-term (18 h) incubations were performed with all arsenic compounds. Concerning the trivalent methylated metabolites, we applied methyloxoarsine, diiodomethylarsine and iopidomethylarsine. Methyloxoarsine and diiodomethylarsine have recently been shown to form MMA(III) [CH3As(III)(OH)2] in solution (36), and by extension of similar chemical considerations, iopidomethylarsine is presumed to form DMA(III) in aqueous solution. To exclude that the effects of the trivalent methylated metabolites were caused by the iodine, comparative studies of methyloxoarsine and diiodomethylarsine were performed with respect to their cytotoxicity in HeLa S3 cells and the induction of oxidative DNA damage. Methyloxoarsine and diiodomethylarsine showed the same cytotoxicity in HeLa S3 cells (data not shown). Furthermore, in isolated PM2 DNA as well as in cultured HeLa S3 cells both compounds showed very similar effects on the induction of oxidative DNA damage (data not shown). Thus, the results presented in this study are derived from experiments with diiodomethylarsine and...
iododimethylarsine, abbreviated with MMA(III) and DMA(III), respectively.

Cytotoxicity
The cytotoxicity was determined by investigating the effect of the respective arsenic compound on colony forming ability after 18 h incubation. The trivalent methylated metabolites MMA(III) and DMA(III) exerted higher cytotoxicity as compared with arsenite and especially to the pentavalent methylated metabolites MMA(V) and DMA(V) (Figure 1).

Induction of DNA strand breaks and oxidative DNA base modifications in HeLa cells
To assess the induction of DNA strand breaks and oxidative DNA base modifications by the arsenicals, HeLa S3 cells were incubated with the respective arsenic compounds for short- and 18 h incubation and lesion frequencies were quantified by the alkaline unwinding technique in combination with the bacterial formamidopyrimidine–DNA glycosylase (Fpg) (33) as described in the Material and methods. Fpg recognizes 7,8-dihydro-8-oxoguanine (8-oxoguanine), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-Gua), 4,6-diamino-5-formamido-pyrimidine (Fapy-Ade) and to a smaller extent 7,8-dihydro-8-oxoadenine (8-oxoadenine) as well as apurinic/apyrimidinic sites (AP sites) and converts them into DNA strand breaks by its associated DNA endonuclease activity (37,38).

Arsenite
In a first approach, HeLa S3 cells were incubated for 0.5, 1 or 3 h with 0.001–1.0 µM arsenite. For all incubation times and concentrations applied we observed only low levels of DNA strand breaks up to 0.2 lesions/10⁶ bp (data not shown). Next, the generation of Fpg-sensitive sites was investigated. The steady-state level of 0.2 Fpg-sensitive sites/10⁶ bp was significantly increased at very low concentrations of arsenite in a time- and dose-dependent manner (Figure 2A). Whereas only a few additional Fpg-sensitive sites were detectable at 0.001 µM arsenite after 1 and 3 h incubation, up to 1.34 Fpg-sensitive sites/10⁶ bp were induced by 0.01 µM. To ensure that the induction of Fpg-sensitive sites by arsenite is not restricted to HeLa S3 cells, a second cell line, human lung adenocarcinoma cells (A549), was applied. Again, non-cytotoxic concentrations of 0.1–10 µM arsenite induced only a few DNA strand breaks but up to 0.8 Fpg-sensitive sites/10⁶ bp after 3 h incubation (data not shown).

In contrast to shorter incubation times, only a slight but significant induction of up to 0.18 Fpg-sensitive sites/10⁶ bp and almost no DNA strand breaks were observed after 18 h incubation in HeLa S3 cells (Figure 2B).

Trivalent methylated metabolites
After 3 h incubation both trivalent methylated metabolites MMA(III) and DMA(III) induced only a few DNA strand breaks, but Fpg-sensitive sites at very low, non-cytotoxic concentrations in a dose-dependent manner starting at 0.1 µM (Figures 3A and 4A). In contrast to arsenite, considerable amounts of DNA lesions were still detectable after 18 h incubation. MMA(III) generated DNA strand breaks and to a
Comparable after 3 and 18 h incubation for all concentrations, dependent induction of Fpg-sensitive sites after 18 h incubation. However, the total lesion frequency was decreased in colony forming ability after 3 h incubation with MMA(III). The data represent mean values of at least six determinations ± SD. For assessment of DNA damage, logographically growing cells were treated with MMA(III) for 3 or 18 h and the frequencies of DNA strand breaks and Fpg-sensitive sites were determined by alkaline unwinding as described in the Materials and methods. Steady-state levels of Fpg-sensitive sites were derived from control cells. Shown are mean values of at least six determinations ± SD. Statistically significantly different from control: \( P < 0.05 \), \( * P < 0.01 \), \( ** P < 0.001 \) as determined by Student’s t-test.

lesser extent Fpg-sensitive sites in a concentration-dependent manner starting at 0.1 \( \mu M \) (Figure 3B). Compared with 3 h incubation the frequency of Fpg-sensitive sites was about two to three times lower after 18 h incubation, except for the highest concentration, 5 \( \mu M \) MMA(III), which induced a comparable amount of ~0.3 Fpg-sensitive sites/10^6 bp at both time points. However, the total lesion frequency was about the same (0.1 and 0.5 \( \mu M \)) or even higher (1.0 and 5 \( \mu M \)) after 18 h incubation.

Like MMA(III), DMA(III) showed a concentration-dependent induction of Fpg-sensitive sites after 18 h incubation (Figure 4B). The generation of Fpg-sensitive sites was comparable after 3 and 18 h incubation for all concentrations, except for 5 \( \mu M \), where more Fpg-sensitive sites were present after 18 h incubation. However, in contrast to MMA(III), DMA(III) generated only a few DNA strand breaks.

Pentavalent methylated metabolites

Comparable with arsenite and the trivalent methylated metabolites, DMA(V) and MMA(V) induced only low levels of DNA strand breaks in HeLa S3 cells after 3 h incubation (Figures 5A and 6A), but pronounced levels of Fpg-sensitive sites in a time-dependent manner, reaching values of 0.8 and 0.9 Fpg-sensitive sites/10^6 bp for 250 \( \mu M \) MMA(V) or DMA(V) after 3 h, respectively.

After 18 h incubation MMA(V) induced almost no DNA strand breaks (Figure 5B). In contrast, DMA(V) induced small but significant amounts of DNA strand breaks in a dose-dependent manner up to ~0.35 lesions/10^6 bp (Figure 6B). Both MMA(V) and DMA(V) induced considerable levels of Fpg-sensitive sites at non-cytotoxic concentrations at this time point.

Induction of DNA strand breaks and Fpg-sensitive sites in isolated PM2 DNA

To investigate whether arsenite and/or the trivalent and pentavalent metabolites damage isolated DNA directly, we assessed the effect of the arsenicals on the induction of DNA strand breaks and Fpg-sensitive sites after 1 h incubation of isolated PM2 DNA at 37°C. Neither arsenite, the pentavalent metabolites MMA(V) and DMA(V), nor MMA(III) generated DNA strand breaks or Fpg-sensitive sites in isolated PM2 DNA up to 10 mM (data not shown). However, DMA(III)
observed after 18 h treatment as compared with 3 h, total damage. Whereas in the case of arsenite, less damage was the duration of treatment markedly affected the extent of DNA modifications, whereas the other arsenicals showed no effects with respect to all types of DNA damage investigated. In cells, generated DNA strand breaks and no oxidative DNA base modifications. In isolated DNA, only DMA(III) in intact cells, all five arsenic compounds induced oxidative DMA(V) induced considerable amounts of DNA strand breaks what higher but still non-cytotoxic concentrations of MMA(V) and DMA(V) starting at 10⁻⁵ M in the absence MMA(III) and DMA(III) showed cytotoxic effects in the low micromolar concentration range, both pentavalent methylated metabolites were only slightly cytotoxic even at concentrations up to 500 µM. These findings are in agreement with several new studies investigating the cytotoxicity of arsenic compounds in primary rat and human cells (30) and human hepatocytes (29). One reason for these differences may be the lower cellular uptake/retention of the pentavalent metabolites as compared with the trivalent compounds (39). Altogether, for cytotoxicity of the arsenicals the oxidation state appears to have a higher impact than the degree of methylation.

Concerning the cytotoxicity, the trivalent methylated metabolites were more cytotoxic as compared with arsenite, with MMA(III) being the most cytotoxic compound in HeLa S3 cells. Whereas MMA(III) and DMA(III) showed cytotoxic effects in the low micromolar concentration range, both pentavalent methylated metabolites were only slightly cytotoxic even at concentrations up to 500 µM. These findings are in agreement with several new studies investigating the cytotoxicity of arsenic compounds in primary rat and human cells (30) and human hepatocytes (29). One reason for these differences may be the lower cellular uptake/retention of the pentavalent metabolites as compared with the trivalent compounds (39). Altogether, for cytotoxicity of the arsenicals the oxidation state appears to have a higher impact than the degree of methylation.

With respect to DNA damage our data support the hypothesis raised by Mass et al. (31) that the trivalent methylated arsenic species are genotoxic and may contribute to

Discussion

The data presented in this paper reveal oxidative DNA damage at surprisingly low concentrations of arsenite starting at 1 nM, by MMA(III) and DMA(III) starting at 100 nM and at somewhat higher but still non-cytotoxic concentrations of MMA(V) and DMA(V) starting at 10 µM. Whereas only MMA(III) and DMA(V) induced considerable amounts of DNA strand breaks in intact cells, all five arsenic compounds induced oxidative DNA base modifications. In isolated DNA, only DMA(III) generated DNA strand breaks and no oxidative DNA base modifications, whereas the other arsenicals showed no effects with respect to all types of DNA damage investigated. In cells, the duration of treatment markedly affected the extent of DNA damage. Whereas in the case of arsenite, less damage was observed after 18 h treatment as compared with 3 h, total DNA damage generated by all methylated metabolites was similar or even higher after 18 h incubation.
DNA damage including DNA strand breaks, DNA–protein discoloration for arsenite, Fpg-sensitive sites may be a more systems, millimolar concentrations (23–25); nevertheless as these results seem to contradict most previous reports where the frequency of DNA strand breaks was detected using arsenite concentrations as low as 0.25 mM incorporated into the comet assay, significant strand breakage was observed at even lower, nanomolar concentrations. Altogether, these results show that DNA strand break assays in the absence of oxidative DNA base modifications taking place in the presence of arsenicals, and the methylated metabolites. Thus, far less lesions were detected after 18 h incubation with arsenite as compared with 3 h incubation, but no Fpg-sensitive sites in isolated PM2 DNA whereas in cells mainly Fpg-sensitive sites were detected. This indicates that on cellular conditions lesions are generated not by DMA(III) itself but rather by reactive species formed inside the cell. In contrast to DMA(III), arsenite, MMA(III), DMA(V) and DMA(V) showed no effects on isolated DNA up to 10 mM, indicating again the importance of cellular components for lesion induction. With respect to DMA(V), electron spin resonance studies provided evidence that besides the superoxide radical anion a dimethylarsenic peroxyl radical was formed by the reaction of molecular oxygen with dimethylarsine, a product in the further metabolic processing of DMA(III). Furthermore, the addition of human liver ferritin increased the DNA damage on isolated pBR322 DNA by DMA(III). Nevertheless, whether this mechanism is relevant in intact cells has to be further elucidated. In cellular systems, in addition to a direct increase in ROS, arsenite and especially some of the methylated metabolites may induce oxidative DNA damage indirectly by inhibition of important detoxifying enzymes. Thus, both trivalent methylated metabolites MMA(III) and DMA(III) are more potent inhibitors of isolated glutathione reductase (summarized in ref. 27) as compared with arsenite, which may be due to the interaction of trivalent arsenic with critical thiol groups and may alter the cellular redox status.

One very interesting outcome of this study concerns the persistence of oxidative DNA damage, which was clearly different for arsenite and its trivalent and pentavalent methylated metabolites. Thus, far less lesions were detected after 18 h incubation with arsenite as compared with 3 h incubation, whereas both trivalent and pentavalent methylated metabolites increased the extent of oxidative DNA damage also after 18 h incubation at non-cytotoxic concentrations in HeLa S3 cells. As the frequency of Fpg-sensitive sites resembles a steady-state between damage induction and repair, removal of oxidative DNA base modifications taking place in the presence of low arsenite concentrations might be a plausible explanation, whereas the methylated metabolites may interfere with the sensitive indicator of oxidative DNA damage after treatment with arsenicals.

However, the underlying mechanisms for the induction of oxidative DNA damage by the different arsenic compounds investigated still remain unclear. Recent studies have provided evidence that arsenite can induce DNA damage by promoting the formation of reactive oxygen species, particularly superoxide radical anions and hydrogen peroxide (6,10,11). Furthermore, reactive oxygen species scavengers such as superoxide dismutase, catalase, glutathione peroxidase and DMSO counteracted the formation of deletion mutations in human chromosome 11 in a human–hamster hybrid cell line (40), of micronuclei and of sister chromatid exchanges (41–43).

Concerning the methylated metabolites, our data as well as some reports in the literature suggest that no common mechanism for DNA damage induction applies for all compounds investigated. Thus, in the present study only DMA(III) at concentrations as low as 10 μM induced DNA strand breaks but no Fpg-sensitive sites in isolated PM2 DNA whereas in cells mainly Fpg-sensitive sites were detected. This indicates that on cellular conditions lesions are generated not by DMA(III) itself but rather by reactive species formed inside the cell. In contrast to DMA(III), arsenite, MMA(III), DMA(V) and DMA(V) showed no effects on isolated DNA up to 10 mM, indicating again the importance of cellular components for lesion induction. With respect to DMA(V), electron spin resonance studies provided evidence that besides the superoxide radical anion a dimethylarsenic peroxyl radical was formed by the reaction of molecular oxygen with dimethylarsine, a product in the further metabolic processing of DMA(III). Furthermore, the addition of human liver ferritin increased the DNA damage on isolated pBR322 DNA by DMA(III). Nevertheless, whether this mechanism is relevant in intact cells has to be further elucidated. In cellular systems, in addition to a direct increase in ROS, arsenite and especially some of the methylated metabolites may induce oxidative DNA damage indirectly by inhibition of important detoxifying enzymes. Thus, both trivalent methylated metabolites MMA(III) and DMA(III) are more potent inhibitors of isolated glutathione reductase (summarized in ref. 27) as compared with arsenite, which may be due to the interaction of trivalent arsenic with critical thiol groups and may alter the cellular redox status.

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repair of the induced lesions. Thus, in the case of MMA(III) and DMA(V) far more DNA strand breaks were observed after 18 h incubation as compared with short-term treatment, which may be indicative of ongoing or inhibited repair processes. Even though this issue has not been investigated systematically up to now; preliminary and yet unpublished data from our laboratory revealed an inhibition of Fpg by MMA(III) and DMA(III) whereas neither arsenite nor the pentavalent methylated metabolites showed any effect (T.Scherwitzl, I.Walter and A.Hartwig, unpublished observations). Nevertheless, other explanations cannot be excluded. Thus, uptake of the pentavalent metabolites into cells is slower as compared with arsenite and thus DNA damage induction may be shifted towards later time points; nevertheless, uptake of MMA(III) is more efficient and about as fast as arsenite (30).

Taken together, our results demonstrate the generation of oxidative DNA damage by arsenite and all methylated metabolites investigated, even though the underlying mechanisms of DNA damage induction appear to be quite different. Very low concentrations of arsenite lead to DNA damage under conditions where due to the low methylation capacity of HeLa S3 cells (45,46) no relevant formation of methylated metabolites is expected. Furthermore, even if the methylation capacity would be sufficient to metabolize these very low concentrations of arsenite, even higher concentrations of the metabolites would be required to induce comparable amounts of DNA damage. On the other hand, the trivalent metabolites were more potent in generating persistent oxidative DNA damage detected after 18 h incubation as compared with arsenite at still submicromolar concentrations as well. Concerning the pentavalent metabolites, DNA damage was observed at higher but still non-cytotoxic concentrations. Nevertheless, it has to be taken into account that uptake into cells is probably very low, whereas in humans the metabolites are generated inside the cell and may generate DNA damage at much lower concentrations. Thus, further studies are needed to assess the effective intracellular concentrations and speciation after incubation with the respective arsenic compounds in cultured cells.

In summary, the data suggest that biomethylation of arsenite is no prerequisite for the arsenite-induced genotoxicity but may additionally contribute to genetic alterations. The biologically probably most crucial lesion recognized by the Fpg protein is 8-oxoguanine. If not repaired, this lesion has mutagenic properties causing GC to TA transversions (47). The potential relevance of the data presented in this study becomes obvious when comparing the applied concentrations of arsenite with blood concentrations of arsenic on environmental exposure conditions. Total arsenic concentrations in whole blood of people with low known exposure to arsenic cover a range of 4–27 nM, whereas exposure to arsenic in drinking water containing 100, 200 or 400 µg/l arsenic corresponded to mean whole blood concentrations of about 55, 133 or 173 nM, respectively (summarized in refs 1,2). Since in our study, concentrations as low as 10 nM arsenite or 100 nM of the trivalent methylated metabolites induced significant amounts of DNA damage, the findings will help to understand the carcinogenicity of arsenic compounds at extremely low exposure conditions.

During revision of this paper, Wang et al. (48) reported on independently derived data on DNA damage by arsenite and some methylated metabolites determined by comet assay. In agreement with our results, they observed enzyme-sensitive sites generated by comparatively low concentrations of arsenite, DMA(V), MMA(V) and MMA(III) after short-term incubation of HL 60 cells.

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