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Research Article

Impact of arsenic on nucleotide excision repair: XPC function, protein level, and gene expression

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The ubiquitous occurrence of the human carcinogen arsenic results in multiple exposure possibilities to humans. The human diet, especially drinking water, is the primary source of inorganic arsenic intake in the general population. The ingested arsenic is metabolized to methylated derivatives; some of these metabolites are today considered to be more toxic than the inorganic species. Various modes of action have been proposed to contribute to arsenic carcinogenicity; inhibition of nucleotide excision repair (NER), removing DNA helix distorting DNA adducts induced by environmental mutagens, is likely to be of primary importance. Here, we report that arsenite and its metabolite monomethylarsonous acid (MMA(III)) strongly decreased expression and protein level of Xeroderma pigmentosum complementation group C (XPC), which is believed to be the principle initiator of global genome NER. This led to diminished association of XPC to sites of local UVC damage, resulting in decreased recruitment of further NER proteins. Additionally Xeroderma pigmentosum complementation group E protein (*XPE*) expression was reduced, which encodes for another important NER protein and similarly to XPC is regulated by the activity of the transcription factor p53. In summary, our data demonstrate that in human skin fibroblasts arsenite and even more pronounced MMA(III) interact with *XPC* expression, resulting in decreased XPC protein level and diminished assembly of the NER machinery.

Keywords: Arsenic / Biomethylation / Gene expression / Nucleotide excision repair / XPC

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1 Introduction

The human diet is the primary source of arsenic intake in the general population. Whereas organic arsenic (*e.g.*, arsenobetaine) predominates in seafood, levels of inorganic arsenic are highest in grains and drinking water [1]. Regarding worldwide public health, the most important medium for inorganic arsenic exposure is drinking water, producing a wide range of adverse health effects, among others neurotoxicity, liver injury, the endemic "blackfood disease," and especially many types of cancer. Uptake of inorganic arsenic *via* contaminated drinking water has been associated with the induction of bladder, kidney, lung, and skin cancer (reviewed in ref. [2–5]). In summary, the ability of arsenic to induce cancer in humans is beyond dispute, but how arsenic induces cancer is the subject of much conjecture. Various modes of action have been proposed for arsenic carcinogenicity including the induction of oxidative stress, induction of genetic damage, altered DNA methylation patterns, enhanced cell proliferation, suppression of the tumor suppressor protein p53, diminished DNA repair, and recently biomethylation (summarized in ref. [6–11]). One possible underling molecular mechanism for arsenic toxicity may lie in its ability to react with thiols, for example, in zinc binding structures prevalent in many transcription factors, cell cycle control and DNA repair proteins [9]. In humans and many other mammals, inorganic arsenic is converted to its trivalent and pentavalent methylated metabolites, monomethylarsonous (MMA(III)) and dimethylarsinous (DMA(III)) acid, monomethylarsonic (MMA(V)) and dimethylarsinic (DMA(V)) acid; two possible metabolic pathways have been proposed yielding the same methylated metabolites [12–

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Abbreviations: GG-NER, global genome NER; **NER,** nucleotide excision repair; **MMA(III),** monomethylarsonous acid; **UV-DDB,** UV-damage DNA binding complex; **XPC,** Xeroderma pigmentosum complementation group C; **XPE,** Xeroderma pigmentosum complementation group E protein, also known as **p48**

14]. Biomethylation has long been thought to be a detoxification process, yet nowadays it is reasonable to conclude that some adverse health effects seen in humans chronically exposed to inorganic arsenic are in fact caused by these metabolites. With respect to the trivalent metabolites MMA(III) and DMA(III), numerous recent studies have shown that they are as toxic, or even more toxic, compared to inorganic arsenic [15 – 20]. Therefore, they may at the least contribute to inorganic arsenic induced genotoxicity and presumably carcinogenicity.

With respect to DNA repair inhibition, several studies point to an interaction of arsenic with various DNA repair pathways, which may in turn decrease genomic integrity (reviewed in ref. [7, 21]). Especially nucleotide excision repair (NER) is strongly inhibited by arsenic. NER is capable of removing a wide variety of bulky, DNA helix distorting lesions, caused, *e.g.*, by UV-irradiation or environmental mutagens. NER works through a "cut- and -patch" mechanism, after DNA damage recognition, incisions at sites flanking the lesion occur and a oligonucleotide (24 – 32 nucleotides) containing the lesion is excised, followed by subsequent restoration of the original DNA sequence by polymerization/ligation using the nondamaged strand as a template. Global genome NER (GG-NER) is a rather complex mechanism, in total over 30 proteins are involved and detection of the lesion is known to be the crucial step in the GG-NER initiation. As the first GG-NER DNA damage recognition proteins the Xeroderma pigmentosum complementation group C (XPC) protein as well as the UV-damage DNA binding complex (UV-DDB), a heterodimer of the Xeroderma pigmentosum complementation group E protein (XPE, also known as p48) and p127 proteins, have been identified (recently summarized in ref. [22 – 24]).

Surveying the impact of arsenic on NER, numerous studies have shown that inorganic arsenic inhibits repair of bulky DNA adducts induced by UV-irradiation [25 – 30] or benzo[*a*]pyrene in cultured cells and laboratory animals [31 – 33]; additionally arsenite has been shown to down-regulate expression of some NER genes in cultured human cells [34, 35]. In humans, arsenic exposure *via* drinking water was correlated in a dose dependent manner to decreased expression of some NER genes and diminished repair of lesions in lymphocytes [36, 37].

In the present study, we searched for potential molecular targets for the observed NER inhibition by arsenic. For the first time gene expression, total protein level and localization of proteins during NER were investigated in one study, at the same time comparing inorganic arsenite and its biomethylated metabolite MMA(III).

2 Materials and methods

2.1 Materials

Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin, and penicillin – streptomycin solutions were obtained from Sigma – Aldrich (Steinsheim, Germany). Genetecin (G418) was purchased from Invitrogen (Karlsruhe, Germany). The culture dishes were supplied by Biochrom (Berlin, Germany). Methylarsine oxide ($CH_3As(III)O$, ≥99% purity) was kindly provided by Prof. Dr. W. R. Cullen (University of British Columbia, Vancouver, Canada) and was used as the precursor to MMA(III). Sodium(meta)arsenite (≥99% purity) was purchased from Fluka Biochemika (Buchs, Germany). Polyclonal rabbit antibody against XPC (H-300), polyclonal rabbit antibody against XPA (Fl-273), monoclonal mouse antibody against XPG (847), and rabbit polyclonal antibody against actin were obtained from Santa Cruz Biotechnology (Santa Cruz, USA). Monoclonal mouse antibody against human p53 (DO-7) was purchased from Dako (Glostrup, Denmark). The secondary HRP-conjugated antibodies were from Santa Cruz Biotechnology, the secondary Cy3-conjugated antibody from Jackson Immunoresearch Laboratories (Westgrove, USA), the secondary Alexa Fluor 488-conjugated antibody from Invitrogen (Paisley, UK), and Vectashield mounting medium containing DAPI (1 μg/mL) from Vector Laboratories (Burlingame, USA). Triton X-100 and BSA were obtained from Fluka Biochemika, formaldehyde solution (37%) from Sigma – Aldrich. Tween-20, TEMED, Tris (>99%), acrylamide (37.5:1), and Giemsa dye were purchased from Roth (Karlsruhe, Germany). All other chemicals were of proanalysis grade and from Merck (Darmstadt, Germany). Enhanced chemiluminescence (ECL) reagent and PVDF membranes were from GE Healthcare (München, Germany). Isopore membrane filters (8 μm) were purchased from Millipore (Bradford, USA). RNA isolation kit (MN Nucleo Spin II) was supplied by Machery-Nagel (Düren, Germany), the protein assay reagent solution, cDNA preparation kit (iSript™ cDNA Synthesis kit), and IQ SYBR Green Supermix for PCR analysis were supplied by BioRad (München, Germany). Primers were designed using primer3 Software Version 0.4.0 and obtained from MWG Biotech AG (Ebersberg, Germany).

2.2 Cell culture and incubation with the arsenic compounds

Normal human diploid skin fibroblasts derived from a healthy individual were immortalized by telomerase transfection (VH10hTert) [38]. VH10hTert cells were grown in tissue culture dishes as monolayers in DMEM supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL), and G418 (25 μg/mL). The cells were incubated at 37°C with 5% CO_2 in air and 100% humidity. For repair experiments, confluent cells were used to prevent post-treatment replication. Cells were treated with the respective arsenical compound as described for the respective experiments. Arsenic stock solutions were prepared in deionized water. To minimize oxidation of MMA(III), stock solution was prepared shortly before each experiment,

arsenite stock solution was stored at 4°C not exceeding 3 wk.

2.3 Cell number and colony forming ability

Confluent cells were incubated as indicated for 24 h with arsenite or MMA(III), washed with PBS, trypsinized and counted, and 500 cells/dish were seeded for determination of colony forming ability. After 10 days of incubation, colonies were fixed with ethanol, stained with Giemsa, counted and calculated as percent of control. Untreated controls exhibited colony forming abilities of about 33%.

2.4 XPC and p53 protein level

We applied Western blot analysis to study the impact of the arsenicals on total protein content of both XPC and the transcription factor p53. UVC-irradiation did not alter total XPC and p53 level as measured 10 min after irradiation (data not shown), therefore impact of arsenic on XPC and p53 level was analyzed without UVC-irradiation. Forty-eight hours after seeding, confluent cells were incubated for 24 h with the respective arsenicals, trypsinized, collected by centrifugation, and sonicated in RIPA-buffer (0.01 M Tris, pH 7.6, 0.15 M NaCl, 0.001 M EDTA, 0.001 M PMSF, 1% Triton X-100, 1% sodium desoxycholate, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1% DOC, 50 mM NaF, 0.1% SDS, 1 mM Na_3VO_4). After centrifugation and quantification of cellular proteins in the supernatant (Bradford analysis), cell extracts with 60–75 μg proteins were dissolved in Laemmli buffer (0.08 M Tris, 2% SDS, 8% glycerin, 2% mercaptoethanol, 0.01% bromphenol blue), denatured 5 min at 95°C, and separated by a 12% denaturating SDS-PAGE. Proteins were transferred to a PVDF membrane by semidry blotting, membranes were blocked with 5% milk solution in PBS, containing 0.05% Tween-20 at room temperature, incubated with a primary antibody against XPC, p53 or actin as loading control, over night at 4°C, followed by incubation with an HRP-conjugated secondary antibody for 1 h at room temperature. After each antibody incubation, membranes were washed three times for 10 min with PBS. Antibodies were visualized by ECL reaction and detected by a chemiluminescence imaging system (LAS 3000, raytest, Straubenhardt, Germany). Protein levels were quantified by densitometric analysis with AIDA Image Analyzer software (Version 4.13, raytest) and normalized to controls. In all experiments proteins were successively analyzed, starting with XPC, followed by p53 and actin.

2.5 Determination of relative XPC and XPE gene expression

For gene expression analysis, total RNA from cells was isolated after 24 h incubation with the respective arsenic compound. Thereafter, the medium was removed, cells were trypsinized, washed with PBS, and total RNA was isolated applying an RNA isolation kit according to the manufacturer's instructions. Total RNA concentration was determined by absorbance at 260 nm. The integrity was checked by denaturing agarose gel electrophoresis using 1 μg total RNA with the ribosomal RNA species appearing as sharp bands with the 28S RNA band containing approximately twice the amount of the 18S RNA band, indicating intact RNA. For cDNA synthesis, 1 μg RNA was reverse transcribed according to the manufacturer's instructions (Bio-Rad) using oligo-dT primers and randomhexamer primers. Real time reverse transcriptase PCR analysis was performed using the iCycler™ IQ detection system. An annealing temperature of 60°C was optimized by a temperature gradient for highly purified salt free primers for the target genes *XPC* and *XPE* and the reference gene *GAPDH*. For the PCR reaction, a master mix of the following components was prepared: 12.5 μL IQ SYBR Green Supermix, 10.5 μL water, 0.5 μL forward primer (10 pmol/μL), 0.5 μL reverse primer (10 pmol/μL), 1 μL of the respective cDNA. The forward and reverse primers sequences are shown below (Table 1). The thermal cycling program consisted of the following steps: 1.5 min at 95°C to activate the polymerase then, 40 cycles of 30 s at 95°C, 1 min at 60°C, and 15 s at 72°C. Fluorescence was measured during the 72°C elongation steps.

Specifity of amplification products was checked by melting curve analysis. The relative expression ratio R was calculated based on the efficiency E, the threshold cycle (C_t), and the reference gene *GAPDH* according to the following equation:

$$R = \frac{(1 + E_{\text{targetgene}})^{(C_t\,\text{Control, target gene} - C_t\,\text{Sample, target gene})}}{(1 + E_{\text{GAPDH}})^{(C_t\text{Control,GAPDH} - C_t\text{Sample,GAPDH})}}$$

The efficiency was calculated according to $E = 10^{-\frac{1}{m}} - 1$ by performing a serial dilution of the template after plotting the C_t values as a function of log concentration of the template.

2.6 Impact on XPC association at damage sites after local UVC-irradiation

To examine the impact of arsenic on damage association of XPC during NER local UVC-irradiation combined with fluorescent antibody labeling was applied in human fibroblasts attached to coverslips.

Forty-eight hours after seeding cells on coverslips were preincubated with the respective arsenical for 24 h followed by local UVC-irradiation. For local UVC-irradiation, cells on coverslips were washed once with PBS, covered with an UVC-blocking isopore polycarbonate filter with a pore size of 8 μm and UVC-irradiated. Cells were locally irradiated with a noncytotoxic dose of 30 J/m^2 at a wavelength of

Table 1. Primer sequences of the selected genes

Genes of interest	Forward primer	Reverse primer
XPC	5′-CTG CAC CAC CAA CTG CTT AG-3′	5′-GGC ATG GAC TGT GGT CAT GAG-3′
XPE	5′-CCT GAA CCC ATG CTG TGA TTG-3′	5′-GCT GGC TTT CCC TCT AAC CTG-3′
GAPDH	5′-CGT GGA CGG GAA GGT GC-3′	5-GGC CAC GCG GTG TAG AT-3′

254 nm, applying a General Electric germicidal lamp (Bioblock Scientific, Illkirch, France). Doses were checked before each experiment by a dosimeter (UV-radiometer211, PRC Krochmann, Berlin, Germany). After irradiation, the filter was removed, and cells were postincubated for 10 min in the continued presence of arsenic. The fluorescent labeling was performed as described before [39] with minor modifications. Briefly, cells were washed twice with cold PBS, fixed and lysed with PBS containing 1% formaldehyde and 0.2% Triton X-100 for 30 min on ice, washed again twice with cold PBS and incubated with 3% bovine albumin in PBS for 30 min at room temperature. Primary and secondary antibodies were incubated for 1 h at 37°C and room temperature, respectively, in washing buffer (WB: PBS, 0.5% bovine albumin, 0.05% Tween-20). To achieve low background signaling, after each antibody incubation, cells were washed three times for 5 min with WB. Finally cells were postfixed with PBS with 2.6% formaldehyde and mounted in Vectashield mounting medium containing DAPI. Assembly of proteins was measured on a Zeiss Axio Imager M1 wide field fluorescence microscope, equipped with a 63 × Plan Apochromat oil immersion lens and a 100 W adjustable mercury arc lamp (Zeiss, Oberkochen, Germany). Images were recorded with a cooled CCD camera (AxioCam MRm, Zeiss).

Using Axio Vision (Version 4.5) imaging software, the relative fluorescence intensity of XPC in spots of local damage and the relative fluorescence intensity of XPC in nuclei without spots were measured; DAPI staining was used to identify cell nuclei. Since the used antibodies showed hardly unspecific binding, fluorescence was mainly caused by the XPC protein. To quantify the actual fluorescence intensities of DNA-damage associated XPC the relative fluorescence of XPC in nuclei without spots was subtracted from the relative fluorescence intensity of XPC in spots of local damage. At least 20 images were taken *per* coverslip harboring at least 55 spots. For statistical analysis, a Student's *t*-test was performed comparing the mean relative fluorescence intensities of at least three independent experiments with each three coverslips to controls.

3 Results

3.1 Cytotoxicity of the arsenicals

The cytotoxicity was determined by investigating the effect of the respective arsenic compound on cell number and colony forming ability after 24 h incubation. For both arsenicals, stronger effects were seen on colony forming ability as compared to cell number as described for other cell lines before (*e.g.*, [32]). This indicates that in case of 24 h incubation with arsenic, cell number is not a sensitive indicator of arsenic cytotoxicity. Colony forming ability is more susceptible, taking into account the long-term viability of exposed cells. Regarding both endpoints, the trivalent methylated metabolite MMA(III) exerted higher cytotoxicity as compared to arsenite (Fig. 1).

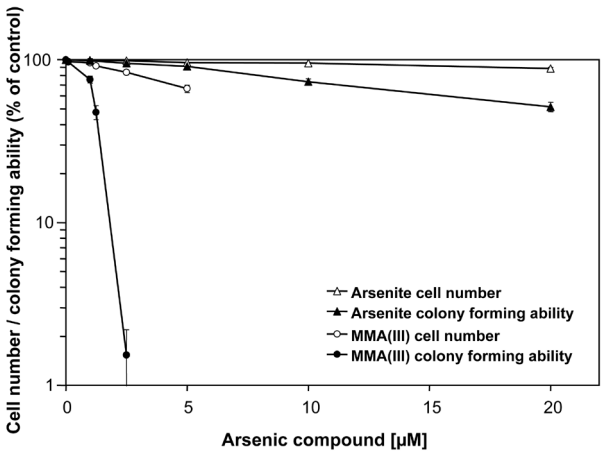

Figure 1. Cytotoxicity of arsenite or MMA(III) in VH10hTert cells after 24 h incubation. Cytotoxicity was determined by a decrease in cell number (open symbols) and colony forming ability (closed symbols). The data represent mean values of at least 3 (cell number) or 9 (colony forming ability) determinations ± SD.

3.2 Impact on XPC and p53 protein level

Western blot experiments demonstrated a concentration dependent decrease of total XPC protein level in VH10hTert cells after 24 h incubation with noncytotoxic concentrations of arsenite (Fig. 2A) or MMA(III) (Fig. 2B). Quantification showed stronger effects by MMA(III) at five times lower concentrations (Figs. 2C and D).

Concerning p53, which is a transcriptional factor for XPC and XPE [40, 41], interestingly both arsenic compounds increased total p53 protein level (Figs. 2A and B).

3.3 Impact on XPC and XPE gene expression

To elucidate whether the arsenic induced decrease of total XPC protein content is due to lowered *XPC* gene expres-

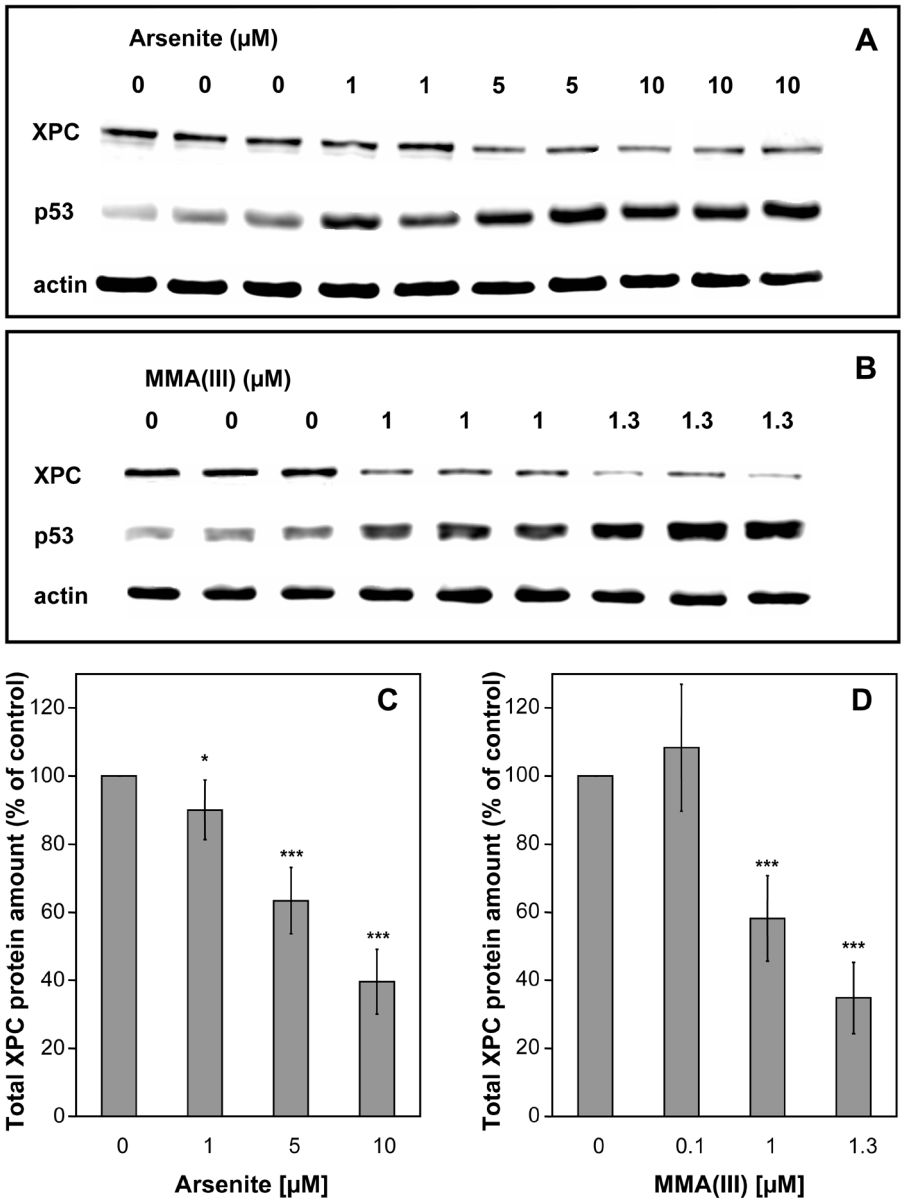

Figure 2. Effect of arsenite or MMA(III) on the XPC and p53 protein level. Confluent VH10hTert cells were incubated for 24 h with the respective arsenic compound. Subsequently, cell extracts were prepared and Western blot analysis was performed as described in Section 2. (A, B) Representative Western blots out of at least four independent experiments for each compound. (C, D) Bar graphs showing quantified XPC (C, D) signals normalized to untreated control cells (100%) derived from Western blot experiments. Shown are mean values of at least three independent experiments with double determinations ± SD. Statistically significant different from non-arsenic exposed controls: $^{*}p < 0.05$, $^{***}p < 0.001$ as determined by Student's *t*-test.

sion, effects of the respective arsenic compound on the expression of *XPC* and additionally *XPE* were investigated by real time reverse transcriptase PCR. Both arsenic compounds strongly decreased *XPC* and *XPE* mRNA levels after 24 h incubation; stronger effects were observed on *XPE* (Figs. 3A and B).

3.4 Impact on the assembly of NER proteins

As described earlier [39], in untreated cells or after global UVC-irradiation in the absence of the arsenicals, XPC, XPA, and XPG show a homogenous distribution and strictly nuclear localization (data not shown). Local UVC-irradiation provoked an intranuclear translocation of the NER proteins to the sites of DNA photolesions within minutes. A maximum accumulation of XPC at local damage sites was obtained 10 min after local UVC-irradiation; therefore this postexposure time was used for experiments studying the effects of the arsenicals. At later stages XPC intensities in spots slowly decreased and 6 h after UVC-irradiation spots were no longer visible (data not shown).

A 24 h preincubation with arsenite (Figs. 4A and C) or its methylated metabolite MMA(III) (Figs. 4B and D) significantly decreased XPC accumulation at local UVC-damage sites in a concentration dependent manner. Inhibition started at 1.0 µM arsenite or 0.1 µM MMA(III) and reached around 30% reduction of XPC intensities in spots at noncytotoxic concentrations. Comparable effects occurred by five times lower concentrations of MMA(III) as compared to arsenite; 5 µM arsenite and 1 µM MMA(III) resulted in

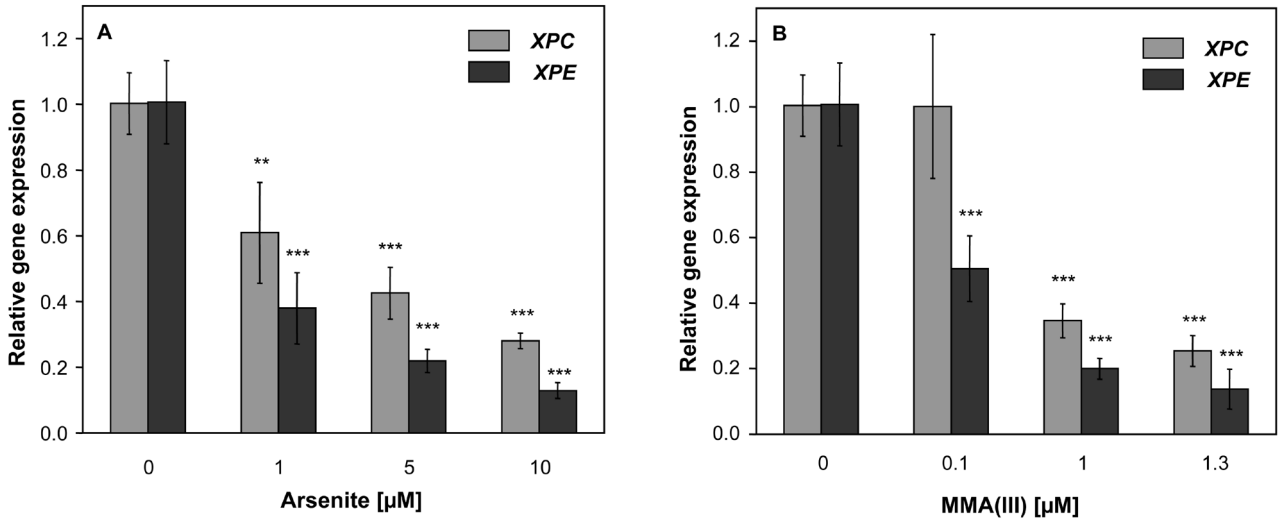

Figure 3. Effect of arsenite or MMA(III) on *XPC* and *XPE* gene expression in VH10hTert cells. Confluent cells were incubated with arsenite (A) or MMA(III) (B) for 24 h. Relative gene expression of *XPC* and *XPE* were determined by real time reverse transcriptase PCR as described in Section 2. Shown are mean values of at least six experiments each referring to the control and normalized to *GAPDH* ± SD. Statistically significantly different from nonarsenic exposed control cells: $^{**}p < 0.01$, $^{***}p < 0.001$ as determined by Student's *t*-test.

significantly ($p < 0.001$) diminished XPC spot intensities of 71 and 76%, respectively. Reduced XPC association at local damage sites resulted in a diminished binding of later NER proteins, among others XPA and XPG (data not shown).

Furthermore relative total nuclear XPC fluorescence was also decreased by the arsenicals in a similar concentration dependent manner as compared to XPC spot intensities, indicating a reduction of XPC protein amount in the nuclei by the arsenicals (Figs. 5A and B).

4 Discussion

The data presented in this study demonstrate for the first time that arsenite and its trivalent methylated metabolite MMA(III) exert its effects at least in part by altering the DNA damage recognition process of NER.

Various studies have identified XPC as the principle initiator of the GG-NER pathway. The functional XPC-DNA-binding domains interact with HR23B to form a complex that recognizes and binds to bulky DNA lesions [39, 42]. This is essential for the recruitment of all subsequent NER proteins [39]. Upon XPC binding, GG-NER is initiated through the sequential recruitment of XPA, TFIIH, XPG, and RPA, forming the so-called preincision complex, which verifies the damage. In the present study, both arsenicals decreased the XPC protein level in the nuclei, which was analyzed by fluorescent antibody labeling in cells, and the total XPC protein content, as measured by Western blot analysis. Lower XPC protein levels are most likely due to an inhibition of *XPC* gene expression by arsenite and MMA(III). Consistently, down-regulation of *XPC* expression by arsenite has been shown previously [34]. In contrast to our data, Shen *et al*. [33] observed no effect of MMA(III) on the XPC protein level. This difference might be caused by different incubation protocols. In our study, cells were incubated for 24 h with the respective arsenicals, allowing time to alter expression of various genes and protein levels. Shen *et al*. investigated effects after short time incubation (1 – 4 h).

Besides lowering *XPC*, both arsenicals strongly decreased *XPE* expression. Together with p127, XPE (p48) forms the heterodimer UV-DDB [43, 44]. In the case of UVC-induced cyclobutane pyrimidine dimers (CPDs) detection by XPC is inefficient and UV-DDB has been suggested as playing a crucial role; additionally UV-DDB was recently implicated in the repair of UVC-induced 6-4 photoproducts (6-4 PPs) [45]. In contrast to later NER proteins [39], UV-DDB does not require XPC for binding to damaged DNA. Transfection studies indicate that XPE rather than p127 is responsible for binding of UV-DDB to damaged DNA [46], in UV-irradiated cells most XPE binds to photolesions, with XPE most probably functioning independently of XPC [47]. Thus, XPE has an important role in GG-NER, probably by preparing chromatin around damaged DNA for assembly of the preincision complex. In summary, the arsenicals decreased the expression of two genes, which encode for the first two GG-NER initiating proteins, XPC and XPE.

For both *XPE* and *XPC*, the tumor suppressor protein p53 has been identified as transcription factor. Following activation by posttranslational modifications such as phosphorylation and acetylation, p53 plays a guarding role in maintaining genome integrity by activating the transcription of

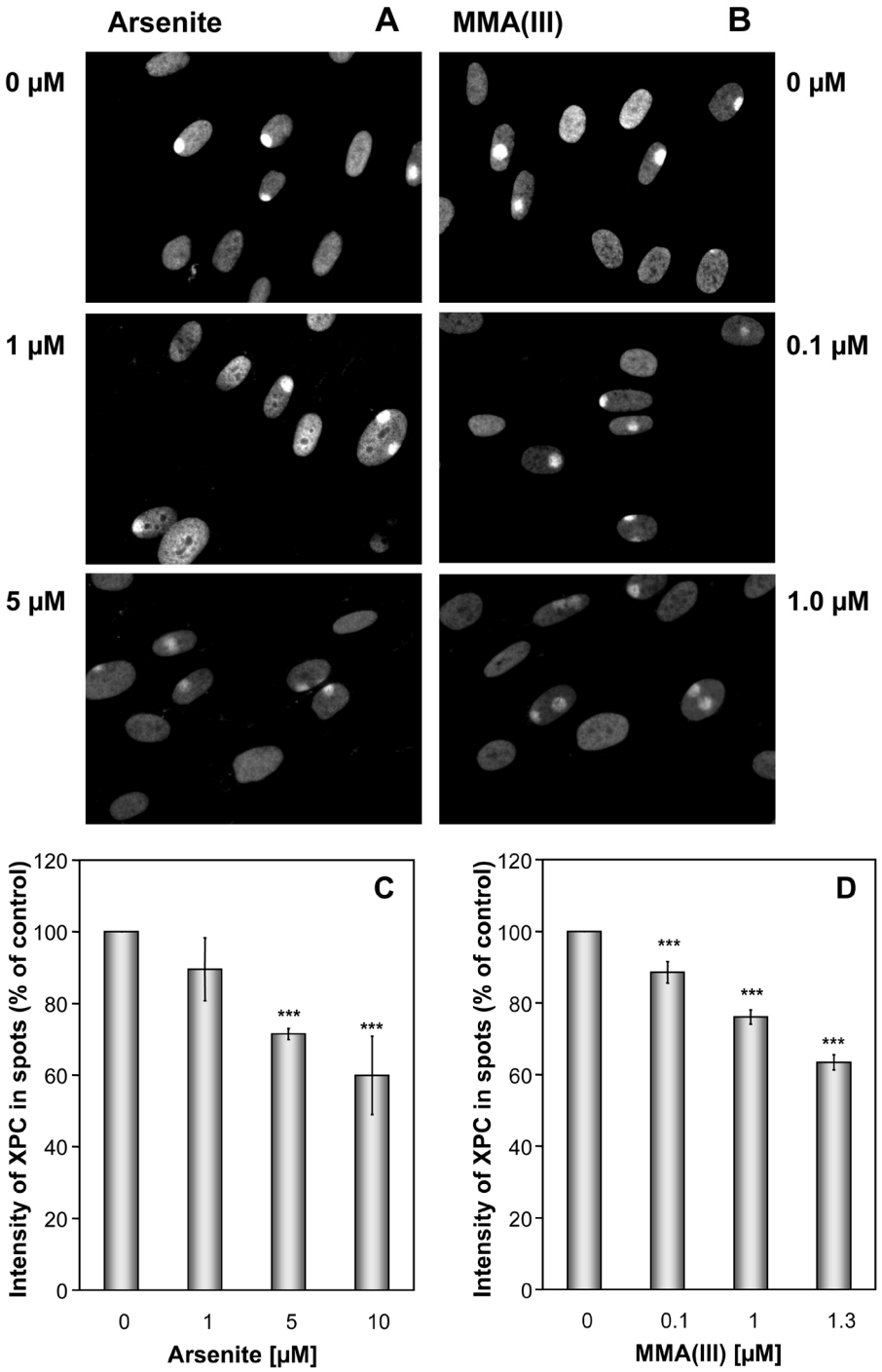

Figure 4. Impact of arsenite or MMA(III) on XPC accumulation in local UVC-damaged spots. VH10hTert cells were preincubated with arsenite (A, C) or MMA(III) (B, D) for 24 h, locally UVC-irradiated through an 8 µm pore size filter and postincubated in the continued presence of arsenic for 10 min. (A, B) Fluorescent immunostaining of XPC in the presence or absence of arsenic. (C, D) Bar graphs showing average intensities of XPC spots after incubation with the arsenicals normalized to control cells without arsenic incubation (100%). Shown are mean values of at least three independent determinations with each three coverslips ± SD. Statistically significant different from nonarsenic exposed controls: ***$p < 0.001$ as determined by Student's *t*-test.

genes involved in cell cycle check points, cellular senescence, apoptosis, and DNA repair. Our current knowledge of p53 response after arsenic exposure is variable; responses are different depending on cell type and incubation times. Cell culture studies have identified that arsenite could induce p53 accumulation through an ATM-dependent pathway [48, 49]. On the other hand arsenite [50] and MMA(III) [33] have been shown to inhibit p53 phosphorylation, reduce overall p53 level and p53 DNA binding activity. In the present study both arsenicals increased total protein level of p53. We observed *XPE* and *XPC* down-regulation, which somehow appears contradictory with regard to the role of p53 as a transcription activator of these genes. However, it is known that for its function, p53 requires its zinc containing, correctly folded wild-type conformation [51, 52]; many cancer-associated mutations cause loss of

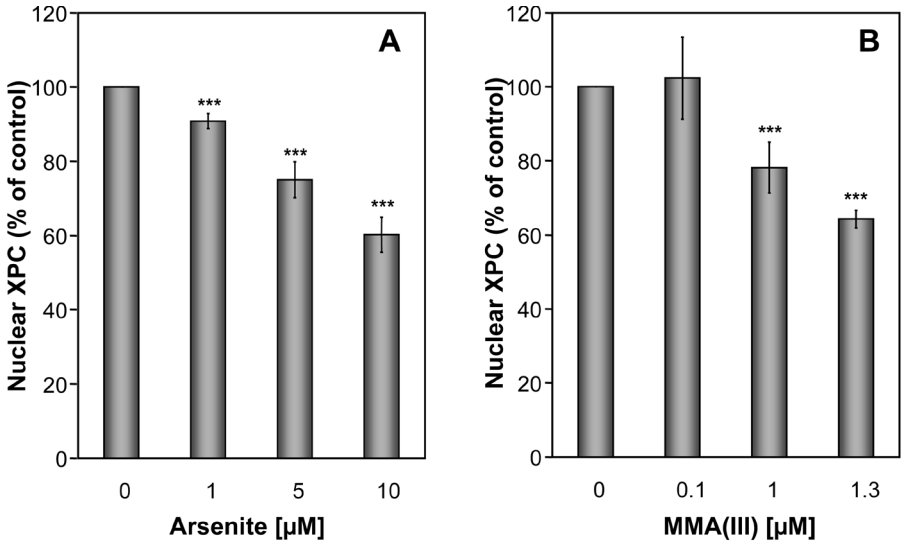

Figure 5. Impact of arsenite or MMA(III) on the amount of total XPC in nuclei. VH10hTert cells were preincubated with arsenite (A) or MMA(III) (B) for 24 h, locally UVC-irradiated through an 8 μm pore size filter and postincubated in the continued presence of arsenic for 10 min. Fluorescence intensity of XPC was measured in nuclei without local damage spots. Shown are mean values of at least three independent determinations with each three coverslips + SD. Statistically significant different from nonarsenic exposed controls: ***$p < 0.001$ as determined by Student's *t*-test.

Table 2. Summary of the observed effects of the arsenicals related to NER

Parameter (applied technique)	Arsenite	MMA(III)
Gene expression (real time reverse transcriptase PCR)	*XPC*↓ *XPE*↓	*XPC*↓ *XPE*↓
Total cellular protein level (Western blot analysis)	XPC↓ p53↑	XPC↓ p53↑
Nuclear protein level (fluorescence microscopy)	XPC↓	XPC↓
XPC association to local damage sites (fluorescence microscopy)	↓	↓

this conformation [53]. A recent study has postulated increased formation of conformational "mutant" p53 protein by arsenite, by the use of a conformation specific antibody specifically recognizing the zinc free unfolded p53 protein [54]. If this effect was caused by direct interactions of arsenite with p53 protein and/or mutation of p53 by arsenite leading to the altered protein structure has to be further elucidated. Furthermore, it is known that the half-life of this p53 protein with unfolded zinc binding domain is increased compared to correctly folded wild-type p53 protein. Additionally this conformational "mutant" p53 protein has been shown to accumulate in cells [55]. Thus the observed increase of total p53 protein level by the arsenicals in the present study may also be due to an increase of conformational mutant p53 protein, which is no longer capable to function as transcription factor for *XPE* and *XPC*. However, decrease of *XPE* and *XPC* expression may also result from arsenic-induced disruption of signal transduction, epigenetic effects, and alterations in involved transcription factors (summarized in ref. [56]).

Despite not knowing the exact mechanisms of how arsenic decreases *XPE* and *XPC* expression, the present study demonstrates for the first time that these effects result in altered DNA damage recognition (summarized in Table 2). Thus, due to decreased XPC protein levels by the arsenicals, we observed reduced XPC localization to UVC-induced DNA damage in cells after incubation with noncytotoxic concentrations of the arsenicals (summarized in Table 2); additionally reduced binding of XPE may partly contribute to the observed effects. Consequently assembly of later preincision GG-NER proteins, such as XPA and XPG, was diminished in locally UVC-irradiated human cells.

Finally, it is clear that the observed impact on XPC is an important mechanism for GG-NER inhibition by arsenic. Concordantly, it has been demonstrated before, that arsenite reduces the frequency of incision events during repair of UVC-induced lesions [28]; additionally, higher concentrations of arsenite were shown to effect the polymerization and ligation step of NER. Besides effects on XPC and XPC-dependent processes, reduced DNA damage recognition and therefore reduced incision frequencies may additionally result from interaction of the arsenicals with XPA function. Subcellular studies previously investigated the impact of arsenite on XPA binding to a UVC- [57] or MMC-damaged [58] oligonucleotide; they demonstrated no decrease of XPA binding or XPA binding inhibition only at high micromolar to millimolar concentrations of arsenite. Consistently, it has recently been shown that in case of a synthesized peptide representing the zinc finger structure of the

human XPA (XPAzf), the zinc containing peptide was totally resistant to equimolar arsenite concentrations. Furthermore a ten-fold excess of arsenite relating to the peptide amount was required to partially oxidize the zinc finger structure. In contrast, equimolar MMA(III) concentrations released Zn^{II} from ZnXPAzf easily, forming mono- and di-arsenical derivatives of XPAzf [59]. However, up to now, it is unknown whether the XPA function is inhibited by arsenic under cellular conditions.

Arsenic is known to enhance the persistence of bulky DNA lesions and consequently the mutagenicity induced by UVC and benzo[*a*]pyrene [60, 61]. Since bulky lesion formation is believed to be responsible for carcinogenicity of these agents, genetic integrity depends largely on efficient NER. Therefore, NER inhibition by arsenic, resulting in comutagenic effects after mixed exposure with environmental agents, is believed to be an important mechanism for arsenic-induced carcinogenicity. In this study, we show that DNA damage recognition during GG-NER is diminished suggesting that bulky lesions induced by environmental agents might persist in the presence of arsenic.

With respect to biomethylation, the data presented in this paper are consistent with other studies showing stronger genotoxicity by the trivalent methylated metabolites as compared to inorganic arsenite (recently summarized in ref. [10]). The observed stronger impact on XPC by MMA(III), is a good model to explain stronger NER inhibition by MMA(III) as compared to arsenite [32, 33]. Finally, these data provide further evidence that in the case of DNA repair inhibition, biomethylation of arsenic increases inorganic arsenic induced genotoxicity and most probably contributes to its carcinogenicity.

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