

BRCA2-dependent homologous recombination is required for repair of Arsenite-induced replication lesions in mammalian cells

Songmin Ying¹, Katie Myers¹, Sarah Bottomley¹, Thomas Helleday² and Helen E. Bryant^{1,*}

¹The Institute for Cancer Studies, University of Sheffield, Sheffield S10 2RX and ²Gray Institute for Radiation Oncology & Biology, University of Oxford, Oxford, OX3 7DQ, UK

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ABSTRACT

Arsenic exposure constitutes one of the most widespread environmental carcinogens, and is associated with increased risk of many different types of cancers. Here we report that arsenite (As[III]) can induce both replication-dependent DNA double-strand breaks (DSB) and homologous recombination (HR) at doses as low as 5 μM (0.65 mg/l), which are within the typical doses often found in drinking water in contaminated areas. We show that the production of DSBs is dependent on active replication and is likely to be the result of conversion of a DNA single-strand break (SSB) into a toxic DSB when encountered by a replication fork. We demonstrate that HR is required for the repair of these breaks and show that a functional HR pathway protects against As[III]-induced cytotoxicity. In addition, BRCA2-deficient cells are sensitive to As[III] and we suggest that As[III] could be exploited as a therapy for HR-deficient tumours such as BRCA1 and BRCA2 mutated breast and ovarian cancers.

INTRODUCTION

Arsenic is considered to be a significant hazard in our environment and chronic exposure has been linked to skin, lung, urinary bladder, kidney and liver cancer (1–6). The primary route of exposure is via contaminated drinking water. In some areas of the world contaminated water levels can be up to several thousand times the WHO maximum safe level of 0.01 mg/l. In West Bengal, India, the concentration of arsenic in contaminated water ranges from 0.05 to 1.055 mg/l (7). Arsenic occurs commonly in the earth's crust but rarely in its pure elemental state. It is

usually found in the form of highly toxic and unstable sulphides, oxides and arsenates of potassium, sodium or calcium. Once ingested inorganic arsenic is processed through successive oxidative methylation and reduction steps to its trivalent and pentavalent mono- and dimethylated metabolites (8). The increased cancer risk posed by contaminated water is thought to be due to the presence of inorganic trivalent arsenite (As[III]⁺) (9), although later reports have shown that the trivalent methylated metabolites MMA[III] and DMA[III] are just as, if not more, toxic (10). Arsenic trioxide has also been used as a chemotherapeutic against certain cancers for example chronic myeloid leukaemia (11,12). In this respect, arsenic resembles several other anti-cancer drugs that as well as being able to treat cancers are also able to induce cancer themselves. A common feature of carcinogenic anti-cancer drugs is that many of them result in DNA damage; a low dose results in mutations and cancer, and a high dose causes toxicity and cell death.

Many mechanisms have been suggested for the carcinogenic effects of arsenic, including inhibition of the DNA repair enzymes involved in nucleotide excision repair and base excision repair (13–15), induction of DNA–protein adducts and oxidative damage (16–20), altered DNA methylation (21,22), increased cell proliferation through changes in the expression of a number of genes associated with cell growth (such as c-fos, c-jun and EGR-1) as well as cell-cycle arrest [such as GADD153 and GADD45 (23)], p53 suppression (24,25) and p53 activation (26,27).

Here we investigate in more detail the type of DNA lesions formed by arsenic. We find that doses as low as 5 μM produce DNA double-strand breaks (DSBs) that can be repaired when As[III] is removed. We show that As[III] induces homologous recombination (HR), the pathway known to play an important role in repair of the replication associated DSBs. Furthermore, when compared to wild-type, HR-deficient cell lines were more

*To whom correspondence should be addressed. Tel: +44 114 2712993; Fax: +44 114 2795320; Email: h.bryant@sheffield.ac.uk
Present address:

Songmin Ying, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK.

sensitive, exhibited greater numbers of DSBs and underwent increased apoptosis upon As[III] treatment. DSBs were not induced in the absence of replication, suggesting that they result from replication fork collapse. Together these results suggest that HR plays an important role in preventing As[III]-induced tumorigenesis and suggest a potential therapeutic use for As[III] in the treatment of HR-deficient tumours.

MATERIALS AND METHODS

Chemicals and treatment conditions

Sodium meta-arsenite (NaAsO_2) (As[III]) >99% purity was purchased from Sigma-Aldrich (Gillingham, UK). All treatments were performed in Dulbecco's modified Eagle's Medium (DMEM) with 10% foetal bovine serum and penicillin (100 U/ml) and streptomycin sulphate (100 $\mu\text{g}/\text{ml}$) at 37°C under an atmosphere containing 5% CO_2 .

Cells and cell culture

The AA8, V79-4, irs1SF (28), V3-3 (29), EM9 (30), PXR3 (31), irs1 (32) and irsX2.2 (33) cell lines were provided by Larry Thompson (Livermore, CA). Malgorzata Zdzienicka generously provided the V-C8 and V-C8 + B2 cell lines (34). All cell lines in this study were grown in DMEM with 10% foetal bovine serum and penicillin (100 U/ml) and streptomycin sulphate (100 $\mu\text{g}/\text{ml}$) at 37°C under an atmosphere containing 5% CO_2 . SPD8 cells were maintained in 5 $\mu\text{g}/\text{ml}$ 6-thioguanine (6TG) to suppress spontaneous reversion.

Recombination assay

A total of 1.5×10^6 SPD8 cells were inoculated into 100 mm dishes, in media without 6TG, 4 h prior to a 24 h treatment with As[III]. After treatments, the cells were rinsed three times with PBS and 10 ml media added before allowing the cells to recover for 48 h. After recovery, cells were released by trypsinisation and counted. HPRT⁺ revertants were selected by plating 3×10^5 treated cells per dish in the presence of HaST (50 μM hypoxanthine, 10 μM L-azaserine, and 5 μM thymidine). To determine cloning efficiency, two dishes were plated with 500 cells each without selection. The colonies obtained were stained with methylene blue in methanol (4 g/l), following 7 days (in the case of cloning efficiency) or 10 days (for reversion) of incubation. Reversion/recombination frequency = number of revertants/(3×300000) \times cloning efficiency.

Immunofluorescence

Cells were plated onto coverslips allowed to settle for 4 h and grown for 24 h in the presence or absence of treatments as indicated. Medium was then removed and coverslips rinsed once in PBS at 37°C. Cells were fixed in 3% paraformaldehyde in PBS containing 0.1% Triton X-100 for 20 min at room temperature and coverslips then extensively washed (2 \times 15 min in PBS containing 0.1% Triton X-100 and 0.15% bovine serum albumin, 1 \times 10 min in PBS containing 0.3% Triton X-100 and 1 \times 15 min in

PBS containing 0.1% Triton X-100 and 0.15% bovine serum albumin) prior to incubation with rabbit polyclonal anti Rad51 antibody (H-92, Santa Cruz) or rabbit polyclonal anti γH2AX (Cell Signalling) at a dilution of 1:1000 for 16 h at 4°C. The coverslips were subsequently washed (as above) followed by 1 h incubation at room temperature with Cy-3-conjugated goat anti-rabbit IgG antibody (Zymed) at a concentration of 1:500 and finally washed again as above. Coverslips were washed briefly in PBS, DNA stained with 1 $\mu\text{g}/\text{ml}$ To Pro (Molecular Probes) and finally mounted in SlowFade Antifade (Molecular Probes).

Images were obtained with a Zeiss LSM 510 inverted confocal microscope using planapochromat 63 \times /NA 1.4 oil immersion objective and excitation wavelengths 488, 546 and 630 nm. Through focus maximum projection images were acquired from optical sections 0.50 μm apart and with a section thickness of 1.0 μm .

The frequencies of cells containing Rad51 foci were determined in three separate experiments. At least 300 nuclei were counted on each slide. Nuclei containing more than 5 foci were classified as positive.

Clonogenic survival assay

Five hundred cells were plated in triplicate onto 100 mm dishes 4 h prior to treatment with increasing doses of arsenic or MMS as indicated. 10 days later, when colonies could be observed, they were fixed and stained with methylene blue in methanol (4 g/l). Colonies consisting of more than 50 cells were subsequently counted. Each colony was assumed to represent one cell surviving from the original 500 and surviving fraction for each dose calculated. Lines of best fit were plotted and IC50 values determined. For survival in the presence or absence of aphidicolin 500 cells plated the day before were pre-treated for 1 h with 3 μM aphidicolin, then exposed to 200 μM arsenic in the presence or absence of 3 μM aphidicolin for 6 h. Plates were then washed and normal media left on the cells for 10 days. Colonies were stained and counted as above.

Pulsed field gel electrophoresis

A total of 1.5×10^6 cells were plated onto 100 mm dishes and allowed 4 h for attachment. Exposure to arsenic with or without 3 μM aphidicolin at the indicated dose was for 24 h after which cells were trypsinised and 10^6 cells melted into each 1% agarose insert. These inserts were incubated in 0.5 M EDTA, 1% N-laurylsarcosyl, proteinase K (1 mg/ml) at room temperature for 48 h then washed four times in TE buffer prior to loading onto a 1% agarose (chromosomal grade) gel. Separation by Pulsed-field gel electrophoresis was for 24 h (BioRad; 120° angle, 60 to 240 S switch time, 4 V/cm). The gel was subsequently stained with ethidium bromide for analysis or transferred to Hybond N+ transfer membrane (Amersham, UK) and Southern blotting was carried out using [α -³²P]dCTP-labelled fragmented total genomic DNA as a probe.

For repair assays agarose inserts were left for increasing lengths of time in media prior to transfer to the EDTA/laurylsarcosyl/proteinase K buffer.

Annexin-V assay

A total of 1×10^5 cells were washed in PBS before for staining using ApoTarget Annexin-V kit (Biosource International) according to manufacturer's instructions. Briefly, cells were mixed with FITC conjugated Annexin-V protein to assay apoptosis and PI to assay cell viability. Samples were analysed by flow cytometry (Becton-Dickenson FACSort, 488nm laser) and percentage of apoptotic cells determined as the fraction of live cells (those that excluded PI) that had Annexin-V bound.

RESULTS

Arsenic has been shown to induce many types of DNA damage including oxidative adducts, DNA strand breaks and DNA-protein cross links (16,35), however, the mechanism by which each lesion is formed is not clear. Here we used the Chinese hamster ovary cell line, AA8, to look for As[III]-induced DNA damage. γ H2A.X forms foci upon DNA damage and is often taken as a marker of DNA DSBs. We saw that doses as low as $5 \mu\text{M}$ As[III] could induce γ H2A.X foci (Figure 1B), while by $50 \mu\text{M}$ a large increase can be seen (Figure 1A and B). It is not clear exactly which types of damage induce γ H2A.X foci so we used pulsed field gel electrophoresis (PFGE) to look directly at DSB formation (36). By conventional ethidium bromide staining DSBs were not detectable until $25\text{--}50 \mu\text{M}$ As[III] (Figure 1C), however, by Southern analysis, DSBs could be visualized at doses as low as $5 \mu\text{M}$ (0.65 mg/l), which is less than two orders of magnitude above the WHO safety level (Figure 1C). It is estimated that $50 \mu\text{M}$ As[III] produces a similar amount of DSBs as a 6 Gy dose of ionizing radiation (Figure 1D).

To study if As[III]-induced DSBs could be repaired after release from the drug, we followed the disappearance of DSBs by PFGE at different time points after 24 h of treatment with $50 \mu\text{M}$ As[III] (Figure 1E). Repair occurred between 2 h and 12 h after removal of As[III]. These results indicate that As[III] induces DSBs that are repaired in wild-type cells.

DSBs are substrates for the DSB-repair machinery, including non-homologous end joining (NHEJ) and homologous recombination (HR) (37). To investigate if HR is triggered by As[III] we looked for Rad51 foci formation. RAD51 is involved in the strand transfer reaction of HR (38) and relocates into stable nuclear foci during HR repair (39,40). We saw that 24 h of treatment with $10 \mu\text{M}$ As[III] could increase the number of cells containing Rad51 foci ~ 3 -fold over spontaneous levels (Figure 2A). To test directly whether As[III] can induce HR we used the SPD8 cell line described previously (41). Here, a partial duplication of exon 7 of the hypoxanthine guanine phosphoribosyl transferase (*hprt*) gene that arose spontaneously in SPD8 cells, leads to expression of non-functional HPRT protein and reversion to wild type by homologous recombination can be selected for in HaST media. Colonies formed following selection are therefore indicative of HR. We found a dose-dependent increase in HR in the *hprt* gene when SPD8 cells were treated with As[III] (Figure 2B). In this system, following a 24 h

treatment with $20 \mu\text{M}$ As[III] there was a 5-fold induction in HR compared to spontaneous levels.

As DSBs and HR are induced following As[III] treatment, it seems likely that HR is responsible for repair of As[III]-induced DSBs. To test this we investigated the level of As[III]-induced DSBs in the BRCA2-deficient cell line V-C8. BRCA2 is involved in regulating Rad51 mediated HR (42) and thus these cells are HR deficient. Consistent with a role in repair we saw that BRCA2-deficient cells had higher levels of DSBs than BRCA2 complemented (V-C8 B2) cells (Figure 3A). In the V-C8 cells breaks were visible by ethidium bromide staining at $1 \mu\text{M}$ As[III], 50 fold less As[III] than is needed to produce a similar amount of DSBs in the BRCA2 complemented cells. In addition DSBs were examined 24 h after removal of As[III]. As expected repair had occurred in BRCA2 complemented cells, but in the BRCA2-deficient cells breaks were still present (Figure 3B), confirming that HR is responsible for a portion of DSB repair following As[III]-induced damage. The persistence of DSBs can lead to apoptosis; therefore we compared apoptosis in V-C8 and V-C8 B2 cells. Following 24 h treatment with $10 \mu\text{M}$ As[III], V-C8 cells were undergoing significant levels of apoptosis compared to both untreated and As[III]-treated V-C8 B2 cells (Figure 3C and D). Together these data suggest that As[III] induces DNA DSBs, which are repaired by HR in wild-type cells.

Considering the high level of As[III]-induced apoptosis in the BRCA2 defective cells we tested the possibility that As[III] could be used to selectively kill HR-deficient cells. HR defective cell lines containing mutations in BRCA2 (V-C8) XRCC2 (*irs1*) and XRCC3 (*irs1SF*) were examined for their survival in increasing doses of As[III] relative to parental (V79-4 and AA8) and corrected cell lines (V-C8 B2, *irSX2.2* and *PXR3*) (Figure 4A, B and C). BRCA2, XRCC2 and XRCC3-deficient cell lines were all sensitive to As[III]-induced cytotoxicity. A small number of DSBs were repaired even in the V-C8 cell line (Figure 3B). So to determine whether an alternative pathway for DSB repair – non-homologous end joining (NHEJ), is also involved in repair of As[III]-induced DSBs, we determined the survival of the DNA-PK-deficient cell line (V3.3) in As[III] relative to the parental (AA8) and DNA-PK complemented [V3.3(hYAC)] cell lines. Unlike the HR-deficient cells the DNA-PK-deficient line showed only a small increase in sensitivity to As[III], suggesting that NHEJ has less of a role in repairing DNA DSBs induced by low doses of As[III]. Interestingly there was a difference in sensitivity at higher doses. Taken together these results suggest HR is required for repair of As[III]-induced DNA damage. The specific sensitivity of HR-deficient cell lines to As[III] opens up the possibility that As[III] may have potential therapeutic value in the treatment of HR-deficient tumours.

DNA double strand breaks can arise indirectly when the replication fork reaches a single strand break or lesion, and causes replication fork collapse (43–47). As[III] has been reported to induce oxidative damage in cells (16,19,48), which could potentially collapse replication forks. We therefore determined whether or not replication

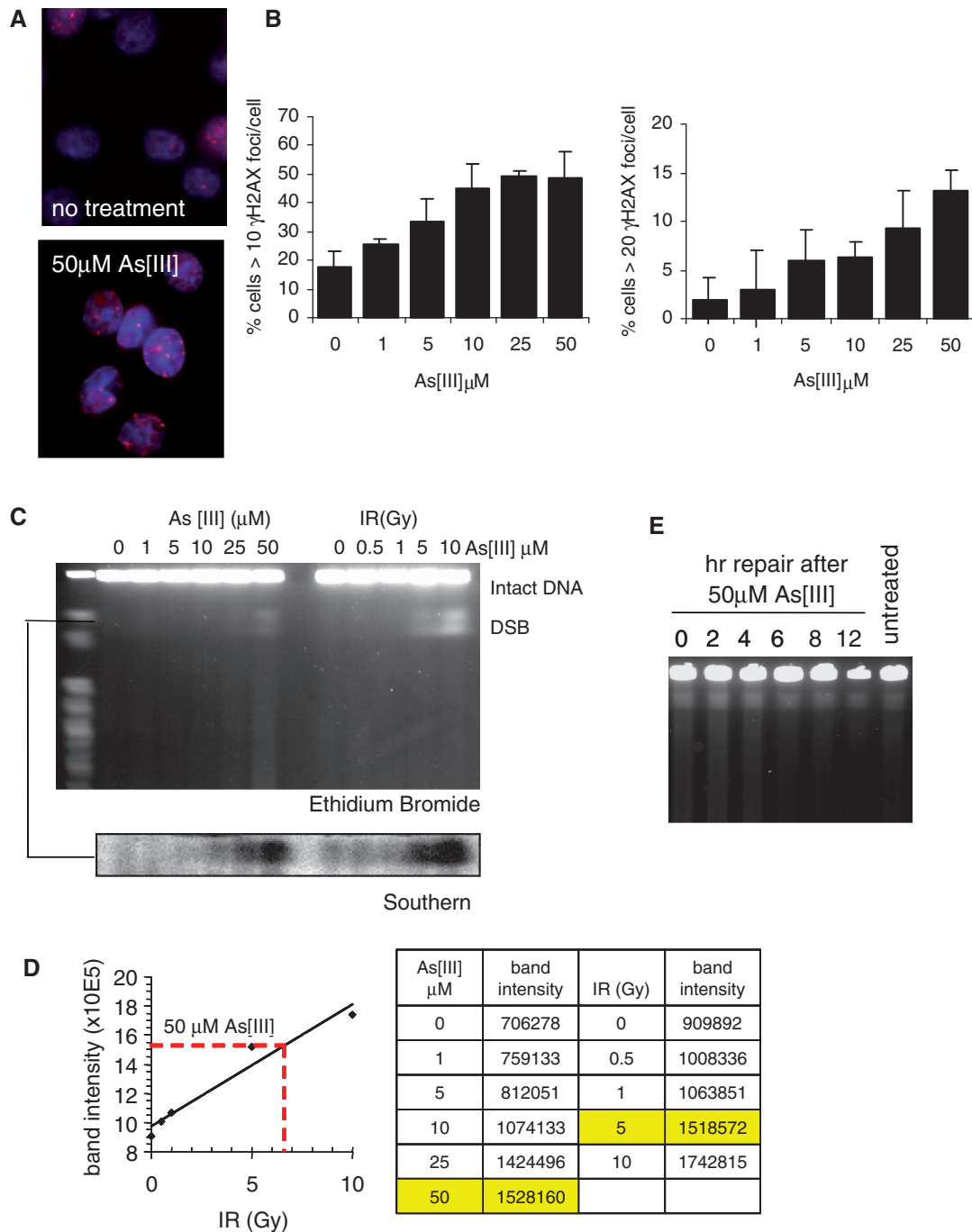


Figure 1. DNA double-strand breaks are induced by As[III] and repaired in AA8 Chinese hamster cells. (A) An example of γ H2A.X foci (red) and DNA (blue) staining with and without As[III] treatment. (B) Quantification of γ H2A.X foci after 24-h treatment with increasing doses of As[III]. Results are the average and standard deviation of three repeats, on each occasion at least 100 cells were counted for each condition. (C) Ethidium bromide staining (upper) and Southern analysis (lower) of DNA double-strand breaks visualized with pulsed-field gel electrophoresis after 24-h treatment with increasing doses of As[III]. Southern analysis used labeled fragmented genomic DNA as a probe. (D) The intensity of each band on the Southern blot. (E) DNA double-strand breaks following a 24 h 50 μ M As[III] treatment and subsequent repair for times indicated.

is required for As[III]-induced DSBs. Cells were co-treated with As[III] and the DNA polymerase inhibitor aphidicolin and DSBs measured by PFGE (49). Less As[III]-induced DNA DSBs were released on pulsed-field gel electrophoresis when cells were co-treated with aphidicolin (Figure 5A). This suggests that As[III] may act by increasing the number of lesions that collapse replication forks

during replication. We looked to see if inhibiting replication during exposure to As[III] could protect from As[III]-induced death. AA8 cells were treated for 16 h with increasing doses of As[III] in the presence or absence of aphidicolin and apoptosis determined by Annexin V staining (Figure 5B). As expected higher doses of As[III] induced apoptosis. Treatment with aphidicolin alone

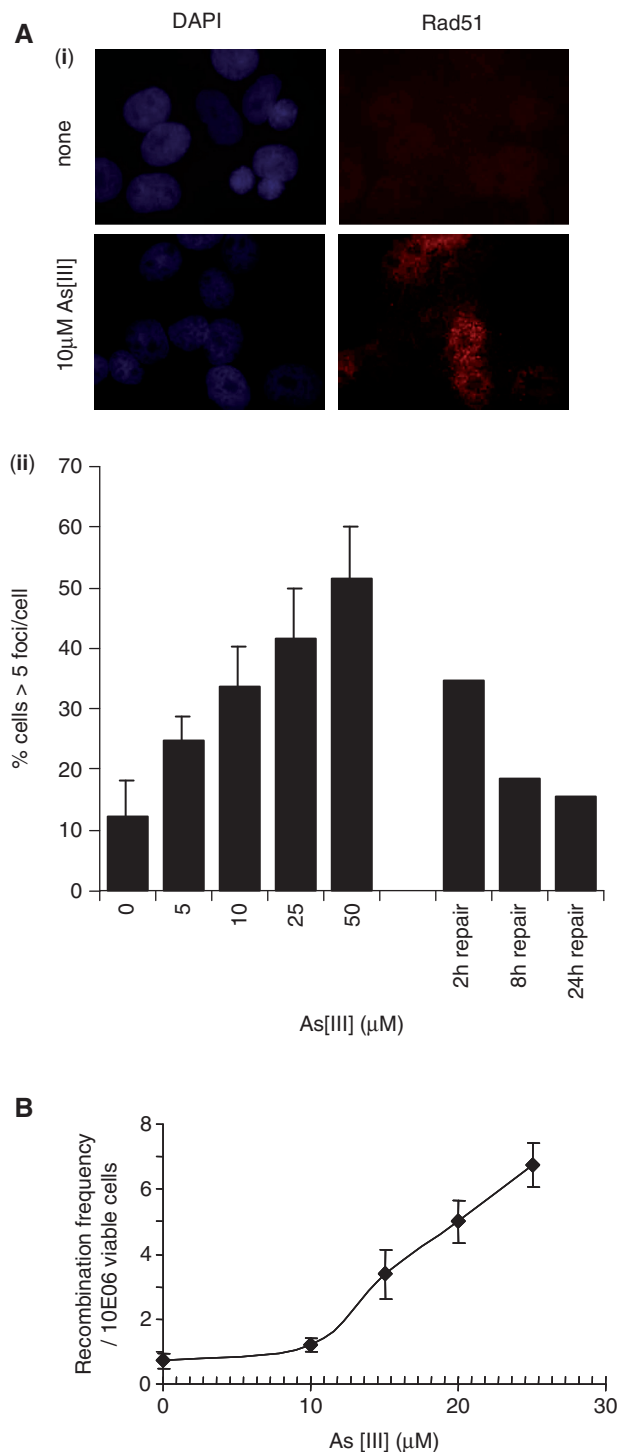


Figure 2. RAD51 foci and homologous recombination are induced by As[III]. (A) i. Examples of Rad51 foci staining. ii. The number of wild-type AA8 cells containing RAD51 foci following a 24-h treatment with As[III] at the doses indicated and remaining after 24-h treatment with 50 μM and repair for the time indicated. Foci after the repair times indicated. (B) Homologous recombination was determined in SPD8 cells. The reversion frequency from a non-functional to a functional *hprt* gene, giving resistance to HaST following a 24 h treatment with As[III] at various concentrations is shown. The average (symbol) and standard deviation (error bars) from three to five experiments are shown.

also induced apoptosis but this level was not further increased when As[III] was added. In addition, cells were treated in the presence or absence of aphidicolin for 6 h with a concentration of As[III] known to be toxic to wild-type cells. They were then replated as for a clonogenic survival assay (Figure 5C). A similar result was found, aphidicolin alone was detrimental to cell survival but addition of arsenic did not cause any further decrease in cell survival. These data together suggest that following exposure to As[III] it is the collapse of replication forks and resultant DSBs which are lethal to cells.

Many reports describe oxidative DNA damage as a result of As[III] exposure (16–20). During repair of oxidative base damage SSBs are induced and these can collapse replication forks into DSBs (50). As As[III]-induced DSBs were dependent upon DNA replication, we reasoned that As[III] is inducing single strand lesions. To test this we determined whether or not cells lacking the SSB repair pathway were sensitive to As[III]. The EM9 CHO cell line is deficient in XRCC1 (30) and thus in SSB repair. Surprisingly, in a colony forming assay with increasing doses of As[III], EM9 cells showed similar cytotoxicity as the parental AA8 cell line (Figure 5D). In addition, we treated cells with As[III] and tested sensitivity to the alkylating agent methyl methanesulfonate (MMS). Co-treatment with As[III] increased the MMS sensitivity of AA8 but not EM9 cells (Figure 5E). These data together suggest that As[III] also inhibits repair of lesions. It is likely that it is this increase in unrepaired lesions that leads to replication fork collapse and DSB formation.

DISCUSSION

The way in which As[III] either directly induces DNA damage and/or modifies repair is predicted to play a major role in the molecular mechanism of As[III]-induced carcinogenicity. The DNA lesions that result following As[III] exposure and the processes by which cells deal with them are therefore of interest. The micromolar concentrations of As[III] used in this study are similar to the average arsenic level in whole blood of individuals exposed to contaminated water (51,52). Therefore we believe these experiments replicate the effects that occur in patients exposed to damaging levels of As[III].

We saw that, relative to wild-type cells, the SSB repair (XRCC1)-deficient EM9 cell line was not sensitive to As[III] and suggest that this is because As[III] itself inhibits single strand break repair. This is supported by the finding that, when co-treated with As[III], wild type but not SSB repair-deficient cells showed increased sensitivity to the alkylating agent MMS. Our data are consistent with previous findings that As[III] inhibits SSB repair to prevent repair of oxidative damage (15,53). We also demonstrate that DSBs are induced upon exposure to As[III], even at low and physiologically relevant doses. The appearance of As[III]-induced DSBs was dependent on replication therefore it is likely that As[III] does not directly induce DSBs. Rather we propose that they arise as a result of unrepaired SSBs at replication forks collapsing into DSBs (47).

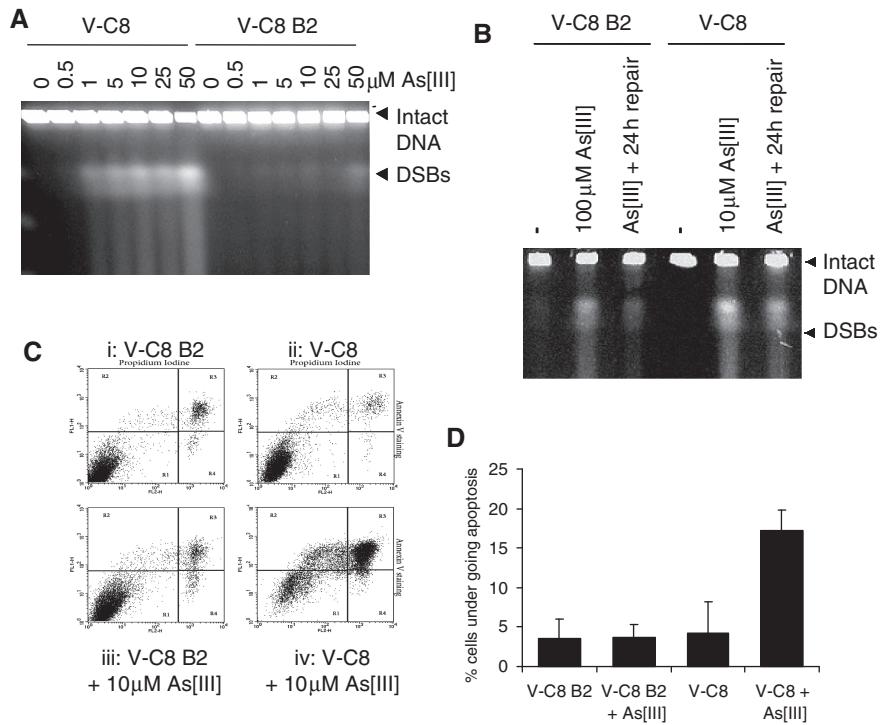


Figure 3. DNA double-strand breaks are induced by As[III] but not repaired in V-C8 Chinese hamster cells. Ethidium bromide staining of DNA DSBs visualized with pulsed-field gel electrophoresis (A) with increasing doses of As[III] and (B) 24h after removal of As[III], in the BRCA2 deficient (V-C8) and complemented (V-C8 B2) cells lines. (C and D) Apoptosis levels as indicated by FACS analysis of annexin V staining after 24h treatment with As[III].

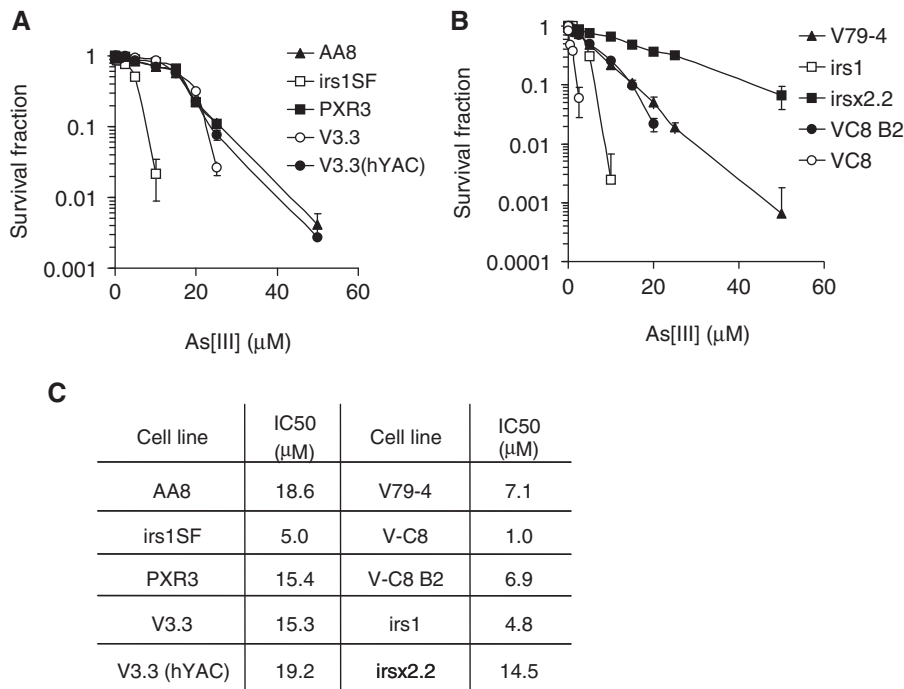


Figure 4. Homologous recombination deficient cell lines are hypersensitive to As[III]. (A) Clonogenic survival of AA8 (wild type), irs1SF (XRCC3 deficient), PXR3 (XRCC3 complemented), V3-3 (DNA-PKcs deficient) and V3.3(hYAC) (DNA-PKcs complemented) cells with increasing doses of As[III]. (B) Clonogenic survival of V79-4 (wild-type), V-C8 (BRCA2 deficient), V-C8 B2 (V-C8 complemented with BRCA2), irs1 (XRCC2 deficient) and irsx2.2 (XRCC2 complemented) cells with increasing doses of As[III]. The average (symbol) and standard deviation (error bars) from three to four experiments are shown. (C) IC₅₀ As[III] values of each cell line.

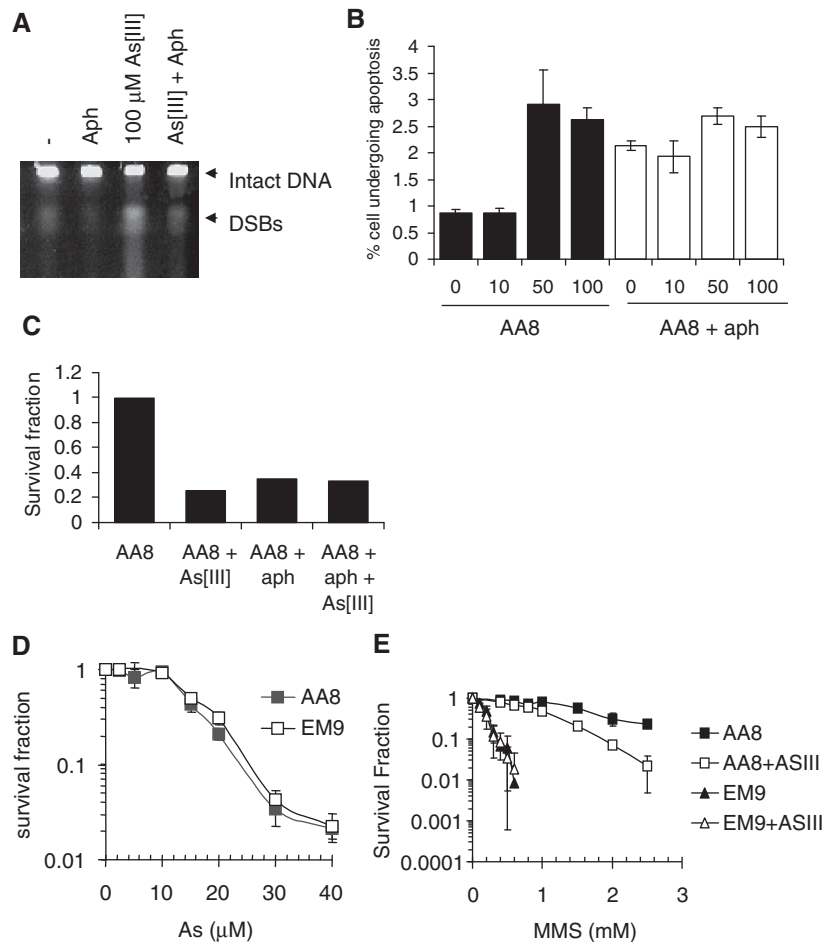


Figure 5. As[III] inhibits single-strand break repair, which causes replication forks to collapse and DSBs to form. (A) Ethidium bromide staining of DNA double-strand breaks visualized with pulsed-field gel electrophoresis after a 6-h treatment of AA8 cells with and without 3 μ M aphidicolin (aph) and/or 100 μ M As[III]. (B) Apoptosis measured by annexin-V staining after a 16-h treatment of AA8 cells with and without 3 μ M aphidicolin (aph) and/or increasing doses of As[III]. (C) Clonogenic outgrowth of AA8 cells treated for 6h with and without 3 μ M aphidicolin (aph) and/or 200 μ M As[III] then left to grow in normal media for 7 days. (D) Clonogenic survival of AA8 (wild type) and EM9 (XRCC1 deficient) cells with increasing doses of As[III]. (E) Clonogenic survival of AA8 (wild type) and EM9 (XRCC1 deficient) cells with and without 20 μ M As[III] and increasing doses of methyl methanesulfonate (MMS).

The lack of increase in sensitivity to As[III] in SSB repair-deficient cells was surprising as As[III] is known to generate reactive oxygen species (ROS) and is reported to induce oxidative damage (54). However, our data do not exclude such damage being induced and are compatible with a model whereby As[III] can both increase damage and inhibit repair. Furthermore, As[III] has also been shown to inhibit nucleotide excision repair (16,48). It is therefore likely that As[III] targets many aspects of DNA repair adding weight to the hypothesis that As[III] mediated inhibition of DNA damage repair contributes to increased replication fork collapse and thus the DSBs observed.

DSBs are lethal to cells and like other DSBs collapsed replication forks are a substrate for homologous recombination (47). We suggest that due to As[III]-induced inhibition of repair, larger numbers of SSBs persist into S-phase and collapse replication forks forming DSBs, which can be the substrate for homologous recombination. This explains the increase in Rad51 foci and

induction of HR seen following As[III] treatment. As replication fork associated DSBs are repaired by HR and mainly produce SCE events (47) this model would explain the increased SCE levels in lymphocytes from people exposed to Arsenic contaminated water (55,56).

We found that cells with a deficiency in HR [irs1, irs1SF and V-C8, deficient in XRCC2 (32), XRCC3 (28) and BRCA2 (34)] were hypersensitive to As[III]-induced damage and could not repair As[III]-induced DSBs, implicating HR in the repair of these lesions. Gene polymorphisms in the HR gene XRCC3 were seen to influence micronuclei (MN) frequency in human peripheral blood lymphocytes of workers exposed to inorganic arsenite compounds (57) and the DNA repair capacity was reduced in arsenic exposed individuals developing pre-malignant hyperkeratosis compared to exposed individuals without skin lesions (58). HR may then be important in protecting against As[III]-induced carcinogenesis and differences in HR capacity in individuals may contribute to their risk following exposure.

Interestingly arsenic derived compounds have been used for centuries in Chinese medicine to treat cancer and have recently been used to efficiently treat acute promyelocytic leukaemia (59) and several solid tumours (60). Arsenic trioxide is well tolerated and the adverse reactions can be managed (59). We have previously shown that PARP inhibitors can be used alone to specifically kill HR-deficient tumours (61), they have also been used to sensitise cells to other DNA damaging agents (62). Here we show that BRCA2-deficient cells are sensitive to As[III]. It is therefore possible that arsenic derived compounds could be used as chemotherapeutic agents either alone or in combination with other agents for this subset of tumours. Arsenite is reported to compete with Zn in binding to the zinc finger of PARP1 in order to inhibit repair of oxidative base damage (53). It can be predicted that As[III] prevents PARP from binding to damaged DNA, this is different to other PARP1 inhibitors currently in clinical trials for treatment of BRCA1/2-associated cancer, which are based on NAD analogues and inhibit the enzymatic action of PARP1. Resistance to PARP inhibitors in cell lines appears to occur via intragenic deletion in BRCA2 (63), however other mechanisms of resistance are possible and inhibiting PARP in a different way may provide a second route of treatment.

Only at concentrations greater than 20 μ M did DNA-PK-deficient cells become sensitive to As[III], suggesting that at higher doses As[III]-induced damage is repaired by the error prone process of non-homologous end joining. This could then explain the increased level of chromosomal aberrations seen in As[III] exposed individuals. Indeed some reports suggest that the level of chromosomal damage in exposed individuals can be used to predict future risk of cancer (64).

In conclusion, we find that As[III] causes replication-dependent repairable DSBs (probably as a result of decreased single strand break repair) and that the HR machinery is activated. HR-deficient cells are highly sensitive to As[III]-induced lesions suggesting that HR is an important pathway for error-free repair of As[III]-induced damage. We suggest that the HR repair pathway is important in preventing As[III]-induced DNA lesions from causing gene rearrangements or mutations that may inactivate tumour suppressor genes or activate proto-oncogenes and thereby cause cancer. In addition, we suggest that arsenic-based therapy may be an efficient way to kill HR-deficient cancers.

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