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Interaction of arsenic(III) with nucleotide excision repair in UV-irradiated human fibroblasts

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Even though epidemiological studies have identified arsenic compounds as carcinogenic to humans, they are not mutagenic in bacterial and mammalian test systems. However, they increase the mutagenicity and clastogenicity in combination with other DNA damaging agents and there are indications of inhibition of DNA repair processes. We investigated the effect of arsenic(III) on nucleotide excision repair (NER) after UV irradiation in human fibroblasts in detail by using two repair-proficient and one partly repair-deficient xeroderma pigmentosum group C human fibroblast cell lines. The results show that two steps of NER are affected by arsenite. Most severely, the incision frequency is reduced at concentrations as low as 2.5 μM arsenic(III); at higher, cytotoxic concentrations, the ligation of repair patches is also impaired. Furthermore, our results indicate that both the global genome repair pathway and the transcription-coupled repair pathway are affected by arsenite. Repair inhibition may well explain the potentiation of genotoxic effects by arsenic in combination with other DNA damaging agents and may thus be of high relevance for the carcinogenic action of arsenic compounds.

Introduction

Adverse health effects caused by arsenic compounds have long been recognized. They include neurotoxicity, liver injury and peripheral vascular disease, known as 'blackfoot disease', but also increased risk of cancer. Significant exposures to arsenic occur in a variety of workplaces, including copper, zinc and lead smelters, glass works and during the production and use of arsenic-containing agricultural products like pesticides and herbicides. Furthermore, arsenic is still used in semiconductors. Even though the commercial use of arsenicals has been reduced during the last few decades, one area of environmental concern is the occurrence of high levels of arsenic in the drinking water in some regions of Canada, Japan, Argentina, India and Taiwan, due to natural sources. While the inhalation of arsenic compounds increases the risk of lung cancer, the ingestion of arsenic has been primarily associated with increased incidences of skin cancer, although lung, kidney, bladder and liver tumors have been described as well (1–4). However, the mechanism underlying this carcinogenic action is not clear, since arsenic compounds are not mutagenic in bacterial test systems nor in mammalian cells in culture (5–7). In contrast, their clastogenic potential to produce mainly chromatid-type chromosomal aberrations and sister chromatid exchanges are well documented, with arsenic(III) being the more potent form as compared with arsenic(V) (8–10).

In addition, there are several studies indicating that an interaction with DNA repair processes is one predominant mechanism in arsenic-induced genotoxicity. It was first demonstrated in *Escherichia coli* that arsenite, although not mutagenic itself, increased the mutation frequency when combined with UV light (11). Thereafter, arsenic compounds have also been shown to enhance the persistence of DNA damage, cytotoxicity, mutagenicity and clastogenicity in combination with UV light, benzo[*a*]pyrene, X-rays, alkylating agents and DNA crosslinking compounds in cultured mammalian cells (12,13). A link between the enhancing effects and inhibition of DNA repair processes has been documented by Okui and Fujiwara (14): while both As(III) and, at higher concentrations, As(V) increased the sensitivity of normal human fibroblasts to UV light, no effect was seen in the repair-deficient cell line xeroderma pigmentosum group A. Furthermore, arsenic in both oxidation states reduced unscheduled DNA synthesis and excision of cyclobutane pyrimidine dimers after UV irradiation; the latter effect was also confirmed in HeLa cells (15). Regarding possible mechanisms of repair inhibition, an impairment of the ligation step in the presence of arsenite has been proposed by Lee-Chen *et al.* (16), who observed delayed rejoining of repair-mediated DNA strand breaks after UV irradiation in CHO cells. The interaction of arsenic(III) with the removal of DNA damage induced by *N*-methyl-*N*-nitrosourea (MNU*) has been characterized by Li and Rossman (17,18). They observed an accumulation of DNA strand breaks after MNU treatment in the presence of arsenite in permeabilized V79 cells, indicating the inhibition of a later step of base excision repair. When using nuclear extracts from arsenic-treated V79 cells, these authors observed a reduced ligase activity compared with control cell extracts when annealing synthetic oligonucleotides.

The aim of the present study was to elucidate the role of arsenite in more detail with respect to the inhibition of UV-induced DNA repair. Special emphasis was given to the incubation conditions and cytotoxicity, since, with regard to human exposure, it is of major importance to define biological effects which occur at low, non-cytotoxic concentrations of arsenite. The DNA damage induced by UVC light is repaired by nucleotide excision repair (NER), which represents the major pathway for the removal of bulky DNA damage induced by a wide variety of environmental mutagens. Two repair-proficient and one partly repair-deficient human fibroblast cell lines were used to investigate: (i) which step of the repair process is affected most severely by arsenite; (ii) if the global

***Abbreviations:** MNU, *N*-methyl-*N*-nitrosourea; NER, nucleotide excision repair; FdU, fluorodeoxyuridine; BrdU, bromodeoxyuridine; PBS, phosphate-buffered saline; TdR, deoxythymidine; (6–4) photoproducts, pyrimidine-(6–4)-pyrimidone photoproducts.

genome repair pathway and/or transcription-coupled repair are impaired. The results show that two steps of the repair process are affected by arsenite. Most severely, the incision frequency is reduced at very low concentrations of arsenic(III); at higher concentrations, the ligation of repair patches is also impaired. Furthermore, our results obtained with XPC cells indicate that in addition to the global genome repair pathway, the transcription-coupled repair pathway is also inhibited.

Materials and methods

Materials

Ham's F10 medium, fetal calf serum, trypsin and penicillin/streptomycin are products of Gibco (Karlsruhe, Germany). $NaAsO_2$ and bis-benzimidtrihydrochloride (Hoechst 33258) were obtained from Sigma (Munich, Germany). Triton X-100 was bought from Pierce (Oud-Beijerland, The Netherlands) and SDS and hydroxyapatite from Calbiochem (Bad Soden, Germany). All other chemicals were of p.a. grade and were obtained from Merck (Darmstadt, Germany). The culture dishes were supplied by Nunc (Wiesbaden, Germany).

Cell culture

VH16, VH25 and XP21ROC (XPC) cells were grown as monolayers in Ham's F10 medium containing 15% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The cultures were incubated at 37°C with 5% CO_2 in air and 100% humidity.

UV irradiation

UV irradiation of cells was carried out with a General Electric germicidal lamp (Bioblock Scientific) delivering 0.025 J/m^2/s at a wavelength of 254 nm from a distance of 117 cm or 0.125 J/m^2/s from a distance of 52 cm. Cells were rinsed with medium without serum and irradiated in the absence of medium.

Atomic absorption spectrometry

Confluent cells were incubated with $NaAsO_2$ for different times. At the end of treatment, the cells were washed three times with ice-cold Ham's F10, trypsinized, counted and mineralized with 65% (v/v) HNO_3 and 30% (v/v) H_2O_2 (1:1).

Uptake of arsenic was measured by atomic absorption spectrometry in the laboratory of Dr Schiwara and partners (Bremen, Germany), according to the procedure described by Henschler (19).

To calculate the intracellular arsenic concentration, the cell volume was determined using a Coulter Counter to be 1.85×10^{-12} l for VH16 cells, 1.82×10^{-12} l for VH25 cells and 2.57×10^{-12} l for XPC cells as described previously (20).

Colony forming ability

For measurement of colony forming ability, confluent cells were treated as described for the respective experiments, trypsinized and 500 cells/dish were seeded together with 2.5×10^4 feeder cells, which had been irradiated previously with 450 J/m^2 UVC. After 14 days incubation with a medium change on day 7, colonies were fixed with ethanol, stained with Giemsa (25% in ethanol), counted and calculated as a percentage of the control.

Alkaline unwinding

DNA strand breaks were determined according to the method of Ahnström and Erixon (21) with modifications as described previously (22). Briefly, logarithmically growing cells were allowed to attach for 24 h, preincubated with arsenic(III), UV irradiated and post-incubated for different times. Afterwards, the medium was removed and an alkaline solution containing 0.03 M NaOH, 0.02 M Na_2HPO_4 and 0.9 M NaCl was added. Separation of single- and double-stranded DNA was performed on 1 ml hydroxyapatite columns (Calbiochem, high resolution) at 60°C, where single- and double-stranded DNA were eluted with each 3 ml of 0.15 and 0.35 M potassium phosphate buffer respectively.

The DNA content of both fractions was determined by adding Hoechst 33258 dye to a final concentration of 7.5×10^{-7} M to 1 ml of each sample and measuring the fluorescence with a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 360 nm and an emission wavelength of 450 nm. The fraction of double-stranded DNA was calculated as described previously (22).

Quantification of DNA strand breaks

To correlate the amount of double-stranded DNA with the frequency of DNA strand breaks, the procedure of alkaline unwinding was calibrated with X-rays. A 200 keV X-ray source with an additional 0.5 mm copper filter (Siemens), adjusted to deliver a dose rate of 4 Gy/min, was used. HeLa cells covered with growth medium were irradiated on ice with different doses of X-rays ranging from 0 to 10 Gy. Subsequently, an alkaline solution was added and DNA strand breaks were determined by the alkaline unwinding assay as described above. The number of DNA strand breaks was calculated according to the formula of Föhe and Dikomey (23)

$$-\ln(F/F_0) = c\times D,$$

where F is the fraction of double-stranded DNA of irradiated cells, F_0 is the fraction of double-stranded DNA of unirradiated cells, c represents the slope of the calibration curve and D is the dose applied in Gy. Based on a number of 10^3 DNA strand breaks per Gy and cell (24–26), c was determined to be 0.1 under the alkaline conditions applied. From this result, the amount of double-stranded DNA was correlated to the actual number of DNA strand breaks per cell by the following equation

$$N = -\ln(F/F_0)\times10^3/c$$

$$= -\ln(F/F_0)\times10^4.$$

Determination of repair replication

The amount of repair replication was quantified essentially as described by Smith *et al.* (27). Briefly, confluent fibroblasts were labeled with 0.06 µCi/ml [^{14}C]thymidine (56 mCi/mmol) for 2–3 days. Afterwards, the cells were trypsinized, pooled, split in a ratio of 1:4 and grown for an additional 2 days in unlabeled medium. Subsequently, the cells were pre-incubated with $NaAsO_2$ for 18 h; 1 µM fluorodeoxyuridine (FdU) and 10 µM bromodeoxyuridine (BrdU) were added for 30 min prior to UV irradiation. After removal of the medium, the cells were washed twice with phosphate-buffered saline (PBS), UV irradiated and incubated with 5 µCi/ml [^{3}H]deoxythymidine (TdR) (82 Ci/mmol), 10 µM FdU and 10 µM BrdU for the respective repair times in the presence of $NaAsO_2$. At the end of the incubation, the cells were washed twice with PBS and lysed by the addition of 0.5% sarcosyl, 0.01 M Tris, pH 8, and 0.01 M EDTA. After 15 min at 37°C, proteinase K was added and the lysate was incubated overnight at 37°C. The molecular weight of the DNA was reduced by two passages through a 25 gauge needle and the DNA was added to CsCl, adjusting the refractive index to $n = 1.4015$ with 0.01 M Tris, pH 8, and 0.01 M EDTA. Subsequently, the samples were centrifuged for at least 36 h at 37 000 r.p.m. in a Beckman ultracentrifuge. The gradients were fractionated and 10 µl of each fraction were analyzed for their ^{14}C and ^{3}H contents. At the position of unreplicated parental DNA, five fractions with the highest ^{3}H content were pooled, mixed with 0.7 ml alkaline solution (1 M KH_2PO_4, pH 12.5), 4 ml CsCl solution (1.76 g/ml) and 1 g solid CsCl and the refractive index was set to 1.4050 with bi-distilled water. The samples were centrifuged again at 37 000 r.p.m. for at least 36 h at room temperature, fractionated and 10 µg calf thymus DNA were added to each sample. The DNA was precipitated by the addition of 10% trichloroacetic acid at 4°C for 30 min, filtered through a 0.45 µM millipore filter, dried and analyzed for its ^{3}H and ^{14}C content. The amount of repair replication was determined by calculating the ratio of ^{3}H repair label to ^{14}C prelabel radioactivity and the values were corrected for the scintillation counter overlap by substracting 20% of the ^{14}C values from the ^{3}H values.

Results

Uptake and cytotoxicity

To define appropriate incubation conditions for the subsequent repair experiments, the uptake and cytotoxicity of arsenic(III) were investigated in all three cell lines used in this study.

The uptake of arsenic(III) was determined by atomic absorption spectrometry after incubation of VH16, VH25 or XPC human fibroblasts with 10 µM sodium arsenite (Figure 1). The intracellular arsenic content increased in a time-dependent manner, with no further increase after ~16 h incubation. After this time, an intracellular accumulation of arsenic was observed as compared with the extracellular concentration of arsenic(III). The kinetics of arsenic uptake as well as the intracellular arsenic concentrations were quite similar in all three cell lines. To insure the bioavailability of arsenic(III) for the subsequent experiments the cells were preincubated with arsenic for 18 h prior to UV irradiation.

The cytotoxicity of arsenic(III) was determined by means of colony forming ability after 24 h incubation (Figure 2). Both repair-proficient cell lines VH16 and VH25 exhibited

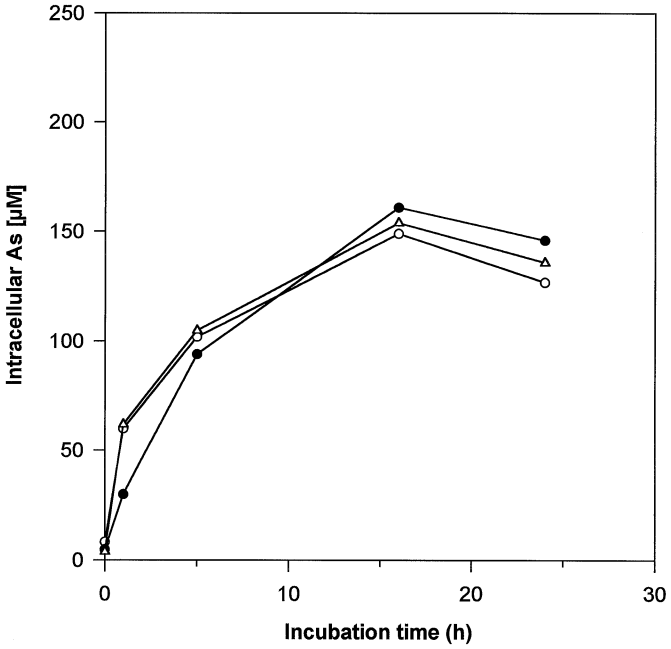

Fig. 1. Uptake of arsenic in human fibroblasts. Confluent cells were treated with 10 µM sodium arsenite for the times indicated. The intracellular content of arsenic was determined by atomic absorption spectrometry as described in Materials and methods. ●, XPC; ○, VH16; Δ, VH25. Shown are mean values of duplicate determinations.

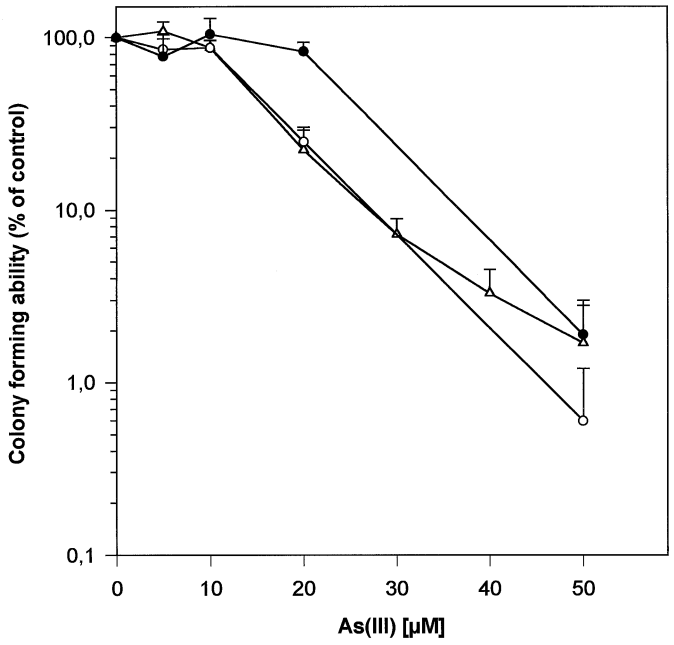

Fig. 2. Cytotoxicity of arsenic(III) in human fibroblasts. Confluent cells were treated with sodium arsenite for 24 h, trypsinized and reseeded for colony forming ability. ●, XPC; ○, VH16; Δ, VH25. The data represent mean ± SD values of six determinations.

similar sensitivities towards sodium arsenite. While concentrations up to 10 µM arsenite led to no reduction in colony forming ability, higher concentrations caused a drop in viability, leaving ~1% of cells viable at 50 µM arsenite. The repair-deficient cell line XPC was somewhat more resistant, showing no decrease in colony forming ability after treatment with concentrations up to 20 µM arsenite.

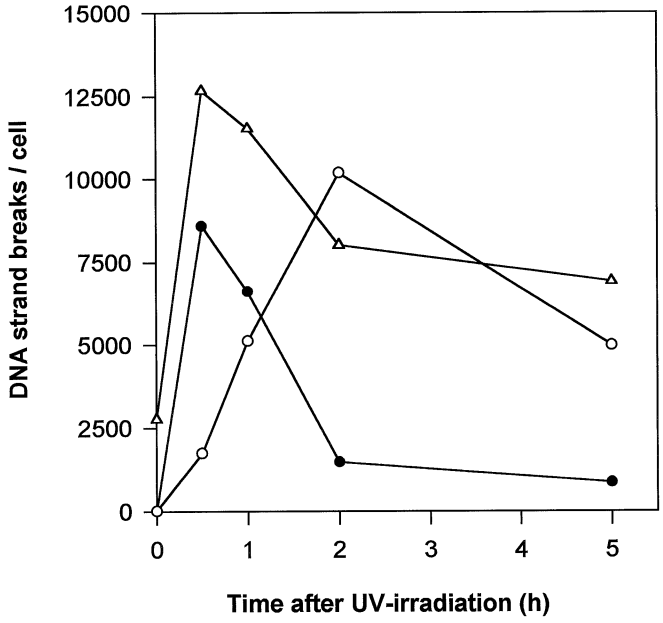

Fig. 3. Effect of arsenic on the transient generation and ligation of DNA strand breaks after UV irradiation in human fibroblasts. VH16 cells were pre-incubated with 10 mM hydroxyurea for 1 h, UV irradiated with 2 J/m^2 and allowed to repair for different time periods in the presence of hydroxyurea. When investigating the effect of arsenic(III), the cells were preincubated with sodium arsenite for 18 h before the addition of hydroxyurea and all subsequent steps were carried out in the presence of arsenic(III). DNA strand breaks were analyzed by the alkaline unwinding technique. ●, UV; ○, UV + 10 µM As(III); Δ, UV + 20 µM As(III). The data represent mean values of three determinations.

Interaction with UV-induced DNA repair

As described above, arsenite has been previously shown by other authors to interact with DNA repair processes, presumably by disturbing the ligation step. However, in most studies high, cytotoxic concentrations were applied or, according to our experience, the pre-incubation periods were too short to reach a steady level of intracellular arsenic. Therefore, we investigated the effect of arsenic on NER under conditions which are non-cytotoxic and which result in maximal uptake of arsenic to find out which step of the repair process is affected most severely. In a first approach, VH16 human fibroblasts were irradiated with UVC light (2 J/m^2, 254 nm) and the transient generation and ligation of DNA strand breaks in the absence and presence of sodium arsenite was analyzed by the alkaline unwinding technique (Figure 3). Since the number of DNA strand breaks is usually low due to rapid ligation of repair patches, we measured the repair kinetics in the presence of 10 mM hydroxyurea; under these conditions, repair events still take place, but repair patches stay open for a prolonged period of time, thereby increasing the sensitivity of the test system (28). Regarding UVC alone, the maximum number of DNA strand breaks occurred 30 min after irradiation, while after 1 h the frequency of repair events dropped and almost no strand breaks could be detected after 2 h. As compared with the UV control at 30 min, there was a marked reduction in the initial frequency of DNA breaks at UV + 10 µM arsenic(III), indicating impairment of the incision process. However, once incisions were made, ligation of repair patches was delayed as well. In the case of UV + 20 µM arsenic(III), we observed an increase in breaks at all time points investigated, suggesting that inhibition of the ligation

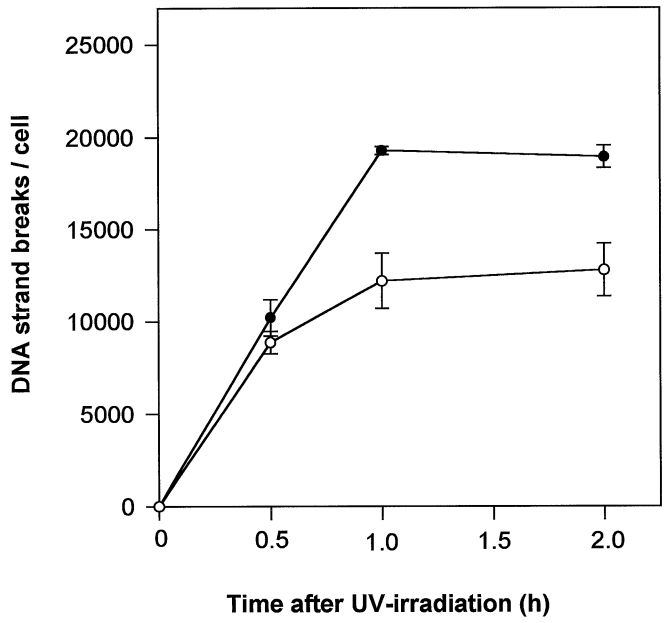

Fig. 4. Effect of arsenite on the kinetics of incisions after UV irradiation in human fibroblasts. VH16 cells were pre-incubated with 15 μM aphidicolin and 10 mM hydroxyurea for 1 h, UV irradiated with 0.2 J/m^2 and allowed to repair for different time periods in the presence of the inhibitors. When investigating the effect of arsenic(III), the cells were pre-incubated with sodium arsenite for 18 h before the addition of the inhibitors and all subsequent steps were carried out in the presence of arsenic(III). DNA strand breaks were analyzed by the alkaline unwinding technique. ●, UV; ○, UV + 10 μM As(III). The data represent mean ± SD values of three determinations.

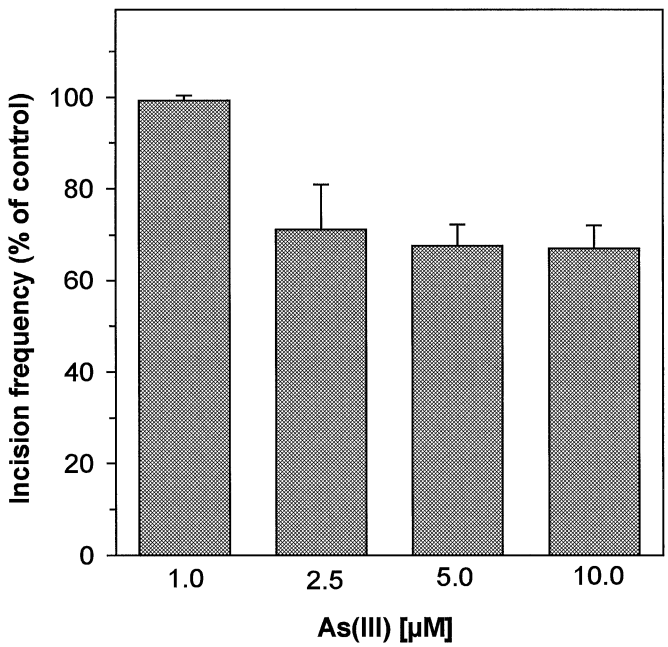

Fig. 5. Dose-dependent inhibition of the incision frequency after UV irradiation by arsenite in human fibroblasts. VH16 cells were pre-incubated with 15 μM aphidicolin and 10 mM hydroxyurea for 1 h, UV irradiated with 0.2 J/m^2 and allowed to repair for 1 h in the presence of the inhibitors. When investigating the effect of arsenic(III), the cells were pre-incubated with sodium arsenite for 18 h before the addition of the inhibitors and all subsequent steps were carried out in the presence of arsenic(III). DNA strand breaks were analyzed by the alkaline unwinding technique. The data represent mean ± SD values of three determinations.

step is more pronounced at 20 μM arsenic(III) as compared with 10 μM.

While inhibition of the ligation step of UV-induced DNA repair confirms the results reported earlier, the finding that the incision step might be affected at even lower concentrations of arsenic(III) is novel. Therefore, we investigated this effect more closely by quantifying the kinetics of incisions occurring after UV irradiation. VH16 human fibroblasts were UV irradiated with 0.2 J/m^2 and DNA strand breaks that accumulated in the presence of 15 μM aphidicolin and 10 mM hydroxyurea were determined up to 2 h after irradiation (Figure 4). In the absence of arsenite, the maximum number of ~19 000 incisions/cell was observed 1 h after UV irradiation, with no further increase at 2 h; in the presence of 10 μM arsenite, the incision frequency was reduced to a maximum of ~12 000, amounting to ~65% of the control value.

In a next approach, the dependency of this inhibitory effect with respect to the arsenic dose was investigated. VH16 human fibroblasts were irradiated with 0.2 J/m^2 UV and DNA strand breaks accumulated in the presence of 15 μM aphidicolin and 10 mM hydroxyurea were determined 1 h after irradiation (Figure 5). While 1 μM arsenite had no effect on the incision frequency, a maximal inhibition is seen at concentrations as low as 2.5 μM sodium arsenite, with no further increase up to 10 μM, reducing the frequency of incisions to ~65% compared with UV alone.

Since the data presented in Figure 3 also indicate an interference of arsenite with the ligation of repair patches, this effect was investigated in more detail. To quantitate the ligation frequency, DNA strand breaks were accumulated in the presence of aphidicolin and hydroxyurea for 2 h, after which all four nucleosides were added to the cell culture medium for 15 min, thereby allowing open repair patches to be ligated (29). By applying these conditions, nearly all DNA strand breaks were resealed in the absence of arsenite (data not shown) and the ligation process was not markedly affected at concentrations up to 10 μM arsenite (Figure 6). At 20 μM sodium arsenite, however, the ligation frequency was reduced to ~50% of the control value, and to ~20% at 50 μM arsenite. Similar effects were observed in VH25 cells (data not shown). Therefore, the ligation step of NER is inhibited by arsenic(III), but only at relatively high, cytotoxic concentrations.

Inhibition of incision is expected to have a direct effect on DNA repair synthesis and therefore the effect of arsenite on UV-induced repair replication was studied (Figure 7). Confluent VH16 cells exposed to 10 μM sodium arsenite for 18 h prior to UV irradiation (10 J/m^2) were labeled with [^{3}H]TdR in the presence of FdU/BrdU for periods of up to 24 h. DNA was isolated and purified by neutral and alkaline CsCl density gradient centrifugation and the relative amount of repair synthesis (compared with ^{14}C uniformly labeled DNA) was determined. After 2, 4, 8 and 24 h post-UV incubation, repair synthesis was reduced by 20–30% in the presence of 10 μM sodium arsenite. Similar experiments performed with VH25 cells revealed a more pronounced inhibitory effect, yielding 48 and 56% reductions of repair synthesis after 8 and 24 h post-UV incubation respectively. These data confirm that the number of repair events in the presence of sodium arsenite is reduced. Furthermore, the amount of reduction correlates well with the reduction in incisions in the presence of arsenite shown in Figures 4 and 5. Since the frequency of repair events is reduced, but not completely abolished, comparative studies were conducted with XPC cells to find out whether transcription-coupled repair in actively transcribed DNA is also affected.

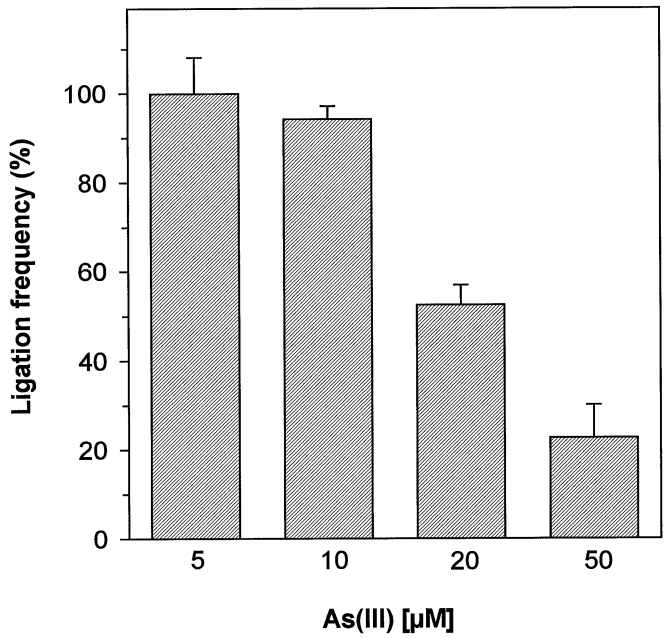

Fig. 6. Effect of arsenite on the ligation frequency after UV irradiation. VH16 cells were pre-incubated with 15 μM aphidicolin and 10 mM hydroxyurea for 1 h, UV irradiated with 0.2 J/m^2 and were to repair for 2 h in the presence of the inhibitors. Afterwards, 100 μM of all four nucleosides were added to the cell culture medium for 15 min to allow ligation of the repair patches. When investigating the effect of arsenic(III), the cells were pre-incubated with sodium arsenite for 18 h before the addition of the inhibitors and all subsequent steps were carried out in the presence of arsenic(III). DNA strand breaks were analyzed by the alkaline unwinding technique. The ligation efficiency refers to the frequency of accumulated DNA strand breaks measured at each concentration of arsenic(III) before addition of the nucleosides. The data represent mean ± SD values of three determinations.

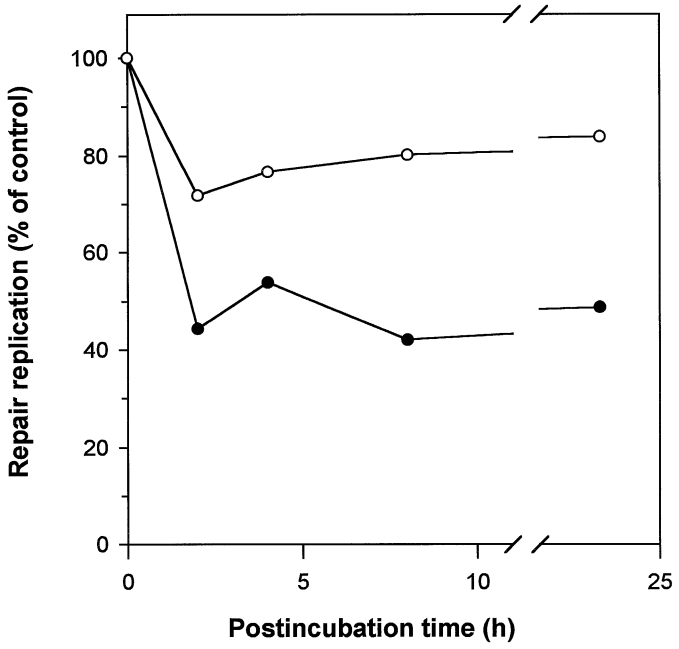

Fig. 7. Effect of arsenite on UV-induced repair replication. Repair-proficient VH16 and repair-deficient XPC cells were pretreated with sodium arsenite for 18 h, UV irradiated and post-incubated in the presence of [^{3}H]TdR, FdU and BrdU and sodium arsenite as described in Materials and methods. ●, XPC; ○, VH16.

XPC cells have been shown not to be able to perform NER in the overall genome after UV irradiation, however, they possess a residual repair capacity, which is restricted to repair of the transcribed strand of active genes (30). Regarding the effect of arsenite on repair replication after UV irradiation in this cell line, inhibition at 10 μM is even more pronounced as compared with VH16 cells, yielding a 50–60% reduction in repair replication at all time points investigated. Nevertheless, this inhibition is of the same order of magnitude when compared with VH25 cells. These data indicate that transcription-coupled repair is also inhibited by arsenite, in addition to repair in the overall genome.

Finally, the effect of arsenite on UV-induced cytotoxicity was determined (Figure 8). Confluent cells were pretreated with arsenic(III) for 18 h, UV irradiated and post-incubated with arsenic(III) for 5 h before reseeding for colony forming ability. Regarding repair-proficient VH16 cells, the viability was nearly unaffected after UV doses up to 10 J/m^2 under the conditions applied. However, in the presence of the non-cytotoxic concentrations of 5 and 10 μM arsenic(III), colony forming ability after UV irradiation was reduced in a dose-dependent manner (Figure 8A). The partly repair-deficient XPC cells were much more sensitive to UVC irradiation, however, UV-induced cytotoxicity was not further increased by sodium arsenite (Figure 8B).

Discussion

The data presented in this paper show that concentrations as low as 2.5 μM arsenic(III) interfere with NER after UV irradiation. The incision frequency is affected most severely, whereas the ligation step is inhibited only at comparatively high, cytotoxic concentrations of 20 and 50 μM sodium arsenite. These results somewhat contradict observations made by Lee-Chen *et al.* (16) and Li and Rossman (17,18), who suggested that the ligation step is the major target of arsenite-induced repair inhibition after UV irradiation or MNU treatment respectively. Regarding the data presented by Lee-Chen *et al.* (16), this discrepancy is most likely due to differences in the incubation conditions. Since chronic exposure to low concentrations of arsenite resembles more closely the situation of exposed people, in the present study much care was taken in selection of the incubation conditions applied. As shown in Figure 1, uptake of arsenic(III) in mammalian cells occurs comparatively slowly, reaching a maximum after 18 h incubation. Therefore, the intracellular concentration and presumably distribution of arsenic are likely to be quite different in our study as compared with that by Lee-Chen *et al.* (16), in which either no or only short pre-incubation periods with high concentrations of arsenite (50–200 μM) were used prior to UV irradiation. The studies of Li and Rossman (17,18) focused on a different repair system. While UV-induced DNA damage is removed by the NER system, DNA damage generated by alkylating agents is removed via base excision repair. These repair systems differ most strikingly in the damage recognition/incision step. With regard to NER, this process is mediated by at least 15–18 proteins involved in damage recognition and the actual incision event (31). In contrast, the recognition and removal of alkylated DNA bases repaired by base excision repair is mediated by a single glycosylase (for a review see 32). Furthermore, post-incision events differ as well between the two repair pathways. Regarding the ligation step in base excision repair, the repair-deficient CHO mutant

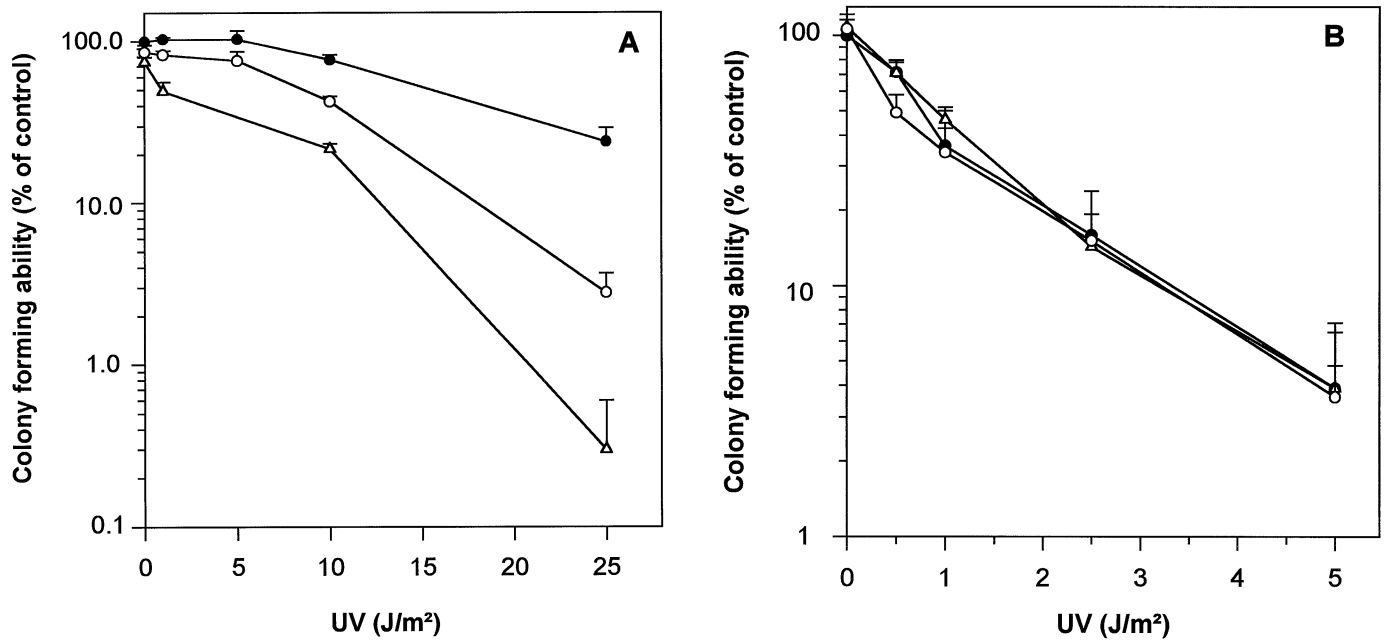

Fig. 8. Effect of arsenite on UV-induced cytotoxicity in repair-proficient VH16 (**A**) and repair-deficient XPC (**B**) human fibroblasts. Confluent cells were pre-incubated with sodium arsenite for 18 h, UV irradiated as indicated and post-incubated with sodium arsenite for 5 h. Afterwards, the cells were trypsinized and 500 cells were reseeded for colony forming ability. ●, UV; ○, UV + 5 μM As(III); Δ, UV + 10 μM As(III). The data represent mean ± SD values of six determinations.

EM9, which is sensitive to DNA alkylating agents but not to UV irradiation, has been shown to be defective in ligation of repair patches due to a mutation in XRCC1, which forms a complex with DNA ligase III (33). In contrast, DNA ligase I seems to be involved in NER. The cell line designated 46BR is sensitive to UV irradiation as well as other DNA damaging agents (34) and exhibits a defect in DNA ligase I activity (35). Therefore, current evidence suggests the involvement of different DNA ligases in the two repair pathways.

In the present study, the incision step of the repair process is not impaired completely, since the frequency of incisions made by cellular repair enzymes is only reduced by ~30–40% in the presence of arsenic(III). This is also confirmed by the measurement of repair replication, whose extent was reduced by the same order of magnitude in the presence of 10 μM arsenic(III) in repair-proficient VH16 fibroblasts. The total incision frequency reflects incisions at cyclobutane pyrimidine dimers as well as at pyrimidine-(6–4)-pyrimidone photoproducts [(6–4) photoproducts]. However, the latter type of lesion is removed more efficiently from the genome as compared with cyclobutane pyrimidine dimers. While human cells remove ~50% of the (6–4) photoproducts within 1 h after UV irradiation, only 50% of cyclobutane pyrimidine dimers are repaired in ~8 h (36,37). Incisions generated within the first few hours after irradiation occur predominantly at sites of (6–4) photoproducts. Our results showing comparable levels of inhibition at early and late times after UV irradiation suggest that repair of (6–4) photoproducts as well as cyclobutane pyrimidine dimers is inhibited by arsenite. Basically, two NER subpathways can act on DNA photolesions, i.e. the global genome repair pathway and the transcription-coupled repair pathway. The latter is confined to lesions in the transcribed strand of active genes and is dependent on transcription. Arsenic could affect either global genome repair or transcription-coupled repair or both pathways. The extent of repair inhibition in normal cells suggests that arsenic must inhibit the global genome repair pathway. The results of the experiments with XPC cells demonstrate that arsenic also affects the repair of DNA photolesions by transcription-coupled repair.

Regarding the effect of arsenic(III) on UV-induced cytotoxicity, the increased sensitivity in repair-proficient VH16 cells confirms results presented previously by Okui and Fujiwara (14). However, like results obtained for completely repair-deficient XPA cells (14), no additional enhancement of cytotoxicity was seen in XPC cells. This provides further evidence that the increase in UV-induced cytotoxicity seen in normal human fibroblasts is caused by inhibition of NER. Moreover, the results show that the effect is primarily due to inhibition of the global genome repair pathway; the additional inhibition of transcription-coupled repair does not contribute further to this effect.

Taken together, the results presented in this study add further evidence that the inhibition of DNA repair processes is a relevant mechanism in arsenic-induced genotoxicity. For bulky DNA lesions, which are repaired via NER, arsenic(III) creates partially repair-deficient conditions, which may well explain the co-mutagenic effect of arsenic(III) observed by Okui and Fujiwara (14) and the increased frequency of chromosomal aberrations in UV-irradiated cells (9). Repair inhibition may further account for the increased incidences of skin cancer observed in areas with high arsenic levels in the drinking water (1). Since DNA damage is continuously induced by exogeneous and endogeneous mutagens, inhibition of NER may also be of general relevance for the carcinogenic potential of arsenic compounds.

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