Biol Metals (1991) 4:197-200

BIOLOGY OF METALS

Comutagenesis of sodium arsenite with ultraviolet radiation in Chinese hamster V79 cells

Jih-Heng Li and Toby G. Rossman

Institute of Environmental Medicine and Kaplan Cancer Center, New York University Medical Center, 550 First Avenue, New York, New York 10016, USA

Received May 20, 1991

Summary. Solar ultraviolet radiation has been associated with the induction of skin cancer. Recent studies have indicated that near-ultraviolet, especially UVB, is mutagenic. Exposure to trivalent inorganic arsenic compounds has also been associated with increased skin cancer prevalence. Trivalent arsenic compounds are not mutagenic *per se*, but are comutagenic with a number of cancer agents. Here, we test the hypothesis that arsenite enhances skin cancer via its comutagenic action with solar ultraviolet radiation. Irradiation of Chinese hamster V79 cells with UVA (360 nm), UVB (310 nm) and UVC (254 nm) caused a fluence-dependent increase in mutations at the *hprt* locus. On an energy basis, UVC was the most mutagenic and UVA the least. However, when expressed as a function of toxicity, UVB was more mutagenic than UVC. Nontoxic concentrations of arsenite increased the toxicity of UVA, UVB and UVC. Arsenite acted as a comutagen at the three wavelengths; however, higher concentrations of arsenite were required to produce a significant ($P<0.05$) comutagenic response with UVB. The increased mutagenicity of UVB and UVA by arsenite may play a role in arsenite-related skin cancers.

Key words: Arsenic - Ultraviolet light - Mutations - Cells - DNA repair

Introduction

Accumulating evidence indicates that solar (near) ultraviolet radiation is responsible for the induction of more than 90% of non-melanoma skin cancers (National Research Council 1982). This is of particular concern in light of the current debate on the causes and consequences of stratospheric ozone depletion. It has been estimated that each 1% depletion of ozone concentration results in a 2% increase in the amount of solar UV reaching the earth (National Research Council, 1982). The action spectrum of solar ultraviolet radiation includes UVB (wavelengths 290-320 nm) and UVA (wavelengths 320-400 nm) with the flux increasing rapidly as the wavelengths increase to UVA (Peak and Peak 1989). Although it has been shown that the most important wavelengths with respect to the carcinogenic potential of sunlight lie in the UVB region of the solar spectrum (Setlow 1974), recent studies have shown that UVA may also be an important factor contributing to human skin cancers (Peak and Peak 1989; Sterenborg and van der Leun 1990). In view of the likely association between mutagenic events and the initiation of oncogenesis, there have been numerous studies demonstrating the mutagenicity of near-ultraviolet radiation in a number of mammalian cell systems (Bradley and Sharkley 1977; Enninga et al. 1986; Hsie et al. 1977; Jones et al. 1977; Zelle et al. 1980).

Ingestion of trivalent inorganic arsenic compounds is also associated with increased prevalence of skin cancers (Tseng 1977; Cuzik et al. 1982). Although trivalent arsenic compounds are non-mutagenic at single gene loci (Lofroth and Ames 1978; Rossman et al. 1980), they have been shown to be comutagenic with UVC (254 nm) (Rossman et al. 1977; Okui and Fujiwara 1986). However, because of atmospheric shielding, there is very little human exposure to UVC irradiation (Parrish et al. 1978; Smith 1981). It was of interest, therefore, to examine the comutagenic effect of arsenite with the more relevant UVA and UVB radiations.

Materials and methods

Cell culture. Chinese hamster V79 cells (strain 743-3-6 originally obtained from Dr Dennis Yep, University of Michigan) were grown in 75-cm^2 flasks at 37° C in an atmosphere of 5% CO_2 in Ham's F12 medium (Gibco, Grand Islands, NY) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 units penicillin and 100 μg streptomycin/ml (Gibco). A clone of V79 cells exhibiting a low spontaneous mutation frequency at the *hprt* locus was isolated, expanded and stored in liquid nitrogen until needed. Cells were thawed and utilized within 4 weeks to ensure a low background mutation frequency in the *hprt* mutation assays.

Offprint requests to: T. G. Rossman

Test compounds. Sodium arsenite (Alfa) was stored in a desiccator until needed. A sodium arsenite solution was made fresh by weighing and dissolving this compound in water to prepare a 1 M stock solution that was sterilized using a 0.22-μm syringe filter. The final dilutions were made in serum-free medium immediately prior to use. A 1-mg/ml stock solution of 6-thioguanine (Sigma) was prepared by dissolving it in minimum volume of 0.1 N NaOH, adding adequate distilled H_2O and sterilizing by a 0.22-μm syringe filter.

Mutation assays. The *hprt* mutation assay was performed using a modification of the method described by Chang et al. (1978). V79 cells were seeded for treatment in duplicate at 5×10^4 cells/100-mm dish, and in triplicate for toxicity at 500 cells/100-mm dish. Following a 4-h incubation, attached cells were mutagenized as desired. All ultraviolet mutation assays were performed by exposure of attached cells in dishes with Earle's balanced salt solution (Gibco). Sodium arsenite was added in Earle's balanced salt solution 5 min before irradiation. For UVC (wavelength 254 nm, from a 15-W General Electric germicidal lamp) and UVA (wavelength 300–420 nm, peaking at 360 nm, from a 15-W F15T8BLB integral-filter 'black light' lamp) mutagenesis, the cells were irradiated without lids. For UVB mutagenesis, the cells were irradiated with four 20-W 'sunlamp' fluorescent bulbs (Westinghouse FS 'Sun-Lamp', emitting wavelengths 280–380 nm and peaking at 310 nm) through the plastic lids of tissue-culture dishes. The plastic lids removed wavelengths below 290 nm so that the cells received mostly UVB irradiation with only a small amount of UVA irradiation (Zelle et al. 1980). The fluences were calculated from the fluence rates calibrated with a radiometer.

After irradiation, the cells were washed twice with Earle's balanced salt solution, and sodium arsenite in complete medium, or medium alone, was added for 3 h. The cells were then washed again and refed with F12 medium for a 5-day expression period, replating once to maintain exponential growth. At that time, the survival plates were fixed and stained and the mutagenesis plates trypsinized and reseeded (10 dishes, 10^5 cells/dish) in complete F12 medium containing 10 μg/ml 6TG. For the reseeding survival, five hundred cells were plated concurrently in triplicate into 100 mm dishes containing F12 medium without 6-thioguanine and stained after 7 days. Following a 10-day selection period, the mutagenesis plates were fixed and stained. The mutation frequency/10^6 surviving cells was calculated, using the reseeding survival values.

Results

The effects of a subtoxic dose of sodium arsenite (10 μM for 3 h) on UVC-, UVB-, and UVA-induced cytotoxicity and mutagenicity are shown in Tables 1–3. On the basis of incident energy, the order of potency in causing cell death and *hprt* locus mutation was UVC > UVB > UVA. However, when the data was plotted as a function of cell killing (Fig. 1), UVB was most mutagenic at this locus. Arsenite significantly enhanced UVC-induced cytotoxicity and mutagenicity at the *hprt* locus in V79 cells (Table 1), confirming previous observations that trivalent arsenic compounds are comutagenic with UVC in mammalian cells (Lee et al. 1985; Okui and Fujiwara 1986). Similarly, arsenite also enhanced blacklight (UVA)-induced cytotoxicity and mutagenicity in V79 cells (Table 3). In contrast, UVB-induced mutagenicity was not significantly affected by the nontoxic dose of arsenite, although the UVB-induced cytotoxicity was increased by arsenite (Table 2).

Table 1. Comutagenicity of sodium arsenite with UVC (254 nm) at the *hprt* locus in V79 cells

UVC (J/m^2)	Sodium arsenite (10 μM, 3 h)	Survival (% control)	Mutant frequency (mutants/10^6 survivors)
0	–	(100)	<0.6
0	+	92.4	<0.9
5	–	89.1	68.4
5	+	84.1	83.6*
10	–	75.3	170
10	+	55.5	258*
15	–	67.7	346
15	+	44.0	551*

* Statistically significant ($P<0.01$) compared with UVC alone

Table 2. Comutagenicity of sodium arsenite with UVB (peaking at 310 nm) at the *hprt* locus in V79 cells

UVB (J/m^2)	Sodium arsenite (10 μM, 3 h)	Survival (% control)	Mutant frequency (mutants/10^6 survivors)
0	–	(100)	1.8
0	+	111	1.4
400	–	90.2	101
400	+	81.4	93
600	–	84.2	168
600	+	75.4	159
800	–	62.4	524
800	+	41.7	515

Table 3. Comutagenicity of sodium arsenite with UVA (peaking at 360 nm) at the *hprt* locus in V79 cells

UVA (KJ/m^2)	Sodium arsenite (10 μM, 3 h)	Survival (% control)	Mutant frequency (mutants/10^6 survivors)
0	–	(100)	1.3
0	+	96.5	<1.4
55	–	85.9	7.9
55	+	78.6	16.9*
110	–	81.2	12.7
110	+	73.7	27.0*
220	–	72.1	26.1
220	+	62.7	40.7*

* Statistically significant ($P<0.01$) compared with UVA alone

To determine whether higher doses or longer treatments with arsenite affect the UVB-induced cytotoxicity and mutagenicity, a single low dose of UVB irradiation was followed by a 24-h post-irradiation exposure to various concentrations of arsenite. As shown in Table 4, UVB-induced mutagenicity was significantly increased by higher doses of arsenite (10 μM and 15 μM) in a dose-dependent manner.

Discussion

We had previously observed that arsenite inhibits the repair of 254-nm-induced DNA damage in *Escherichia*

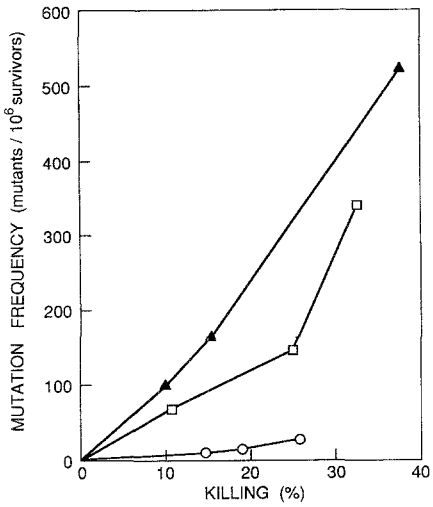

Fig. 1. Induced mutations at the *hprt* locus as a function of toxicity. (○) UVA; (▲) UVB; (□) UVC

Table 4. Effects of varied doses of sodium arsenite on V79 cells treated with a low dose of UVB

UVB (200 J/m^2)	Sodium arsenite (μM, 24 h)	Survival (% control)	Mutant frequency (mutants/10^6 survivors)
−	0	(100)	<1.7
−	5	94.0	1.4
−	10	52.3	<2.5
−	15	18.1	4.2
+	0	99.6	72.8
+	5	79.5	70.9
+	10	42.9	120.8*
+	15	11.7	150.3*

* Statistically significant, $P<0.01$, compared with 200 J/m^2 of UVB alone

coli (Rossman et al. 1977) as well as repair of methylnitrosourea-induced DNA damage in Chinese hamster V79 cells (Li and Rossman 1989a). Based on these findings, we have proposed that interference with DNA repair may be the cause of increased carcinogenesis associated with arsenic exposure. We have also shown that DNA ligase II, the putative ligase involved in DNA excision repair, is uniquely sensitive to arsenite (Li and Rossman 1989b).

The finding that a nontoxic dose of arsenite was comutagenic with UVC and UVA, but not with UVB, was surprising (Tables 1-3). The DNA damage caused by UVB is often viewed as a mixture of UVC and (the X-ray like) UVA effects. It has been reported that at 254 nm (UVC) and 313 nm (UVB), mutation to 6-thioguanine resistance and survival are consistent with the relative levels of cyclobutane-type pyrimidine dimers induced (Tyrrell 1984). However, at 313 nm (UVB), the ratio of ouabain-resistant/6-thioguanine-resistant mutants is 10 times higher than at 254 nm (UVC). Since ouabain resistance results from mutation in the essential gene encoding Na^+/K^+ ATPase, mutations at this locus are thought to be base-pair substitutions leading to a Na^+/K^+ ATPase enzyme which can function in the presence of ouabain (Arlett et al. 1975). This suggests that a unique type of premutagenic lesion, capable of leading to base-pair-substitution mutations, is induced by UVB.

The putative UVB-induced lesion may be repaired via a mechanism other than excision repair, and such a repair mechanism may be less susceptible to arsenite inhibition than is excision repair or repair of strand breaks. An alternative hypothesis might be that the putative UVB-induced premutational lesion is not repairable at all. Tyrrell (1984) has suggested that a UVB-induced lesion leading to mutations at the Na^+/K^+ ATPase locus is not toxic; our results showing a high mutation frequency per lethal dose (Fig. 1) with UVB support this hypothesis. If such a lesion does not interfere with transcription or replication of DNA, it may indeed not be repaired.

When photoactive cellular components such as riboflavin and porphyrins are exposed to black ultraviolet light (UVA), H_2O_2 is produced, which can then produce hydroxyl radicals (·OH) via metal-catalyzed Fenton reactions (Coohill et al. 1987). Hydroxyl radical and other free radicals cause DNA strand breaks and other DNA damage in cells (Coohill et al. 1987). Thus, unlike UVC (and to some extent UVB) which damages DNA directly, UVA induces DNA damage via an indirect route. The repair of UVA-induced DNA damage is not dependent upon DNA polymerase α, but may depend upon DNA polymerase β (Tyrrell and Amaudruz 1984). We have found that, whereas DNA polymerase α is inhibited by arsenite, DNA polymerase β is stimulated by low concentrations of arsenite in vitro (Li 1989). DNA polymerase β has been shown to be highly error-prone, compared with DNA polymerase α (Roberts and Kunkel 1986). If the UVA-induced DNA damage is repaired by DNA polymerase β, the stimulatory effect of arsenite on this error-prone repair enzyme could account for part of its comutagenicity. Inhibition of DNA ligase by arsenite would also be expected to play a role in arsenite's comutagenic effect on UVA.

Although action spectra show that UVA imposes DNA damage less efficiently than does an equivalent fluence of UVB, the greater fluxes of UVA in the environment and the greater penetration of longer wavelengths of radiation into biological organisms would tend to increase the significance of UVA-induced mutagenesis. Epidemiological studies reveal that the prevalence for skin cancer increases with the arsenic content of water intake (Tseng 1977). Although UVC is highly mutagenic and is comutagenic with arsenite (Table 1), it may be less important in terms of natural ultraviolet irradiation and human exposure (Parrish et al. 1978; Smith 1981). The comutagenicity of arsenite with UVA and (at higher doses) UVB deserves our attention because it may explain why excess arsenic intake is associated with a high prevalence of skin cancers.

Acknowledgements. This work was supported by National Cancer Institute grant CA29258 and is part of New York University Institute of Environmental Medicine Center programs supported by grant CA13343 from the National Cancer Institute, grant ES00260 from the National Institute of Environmental Health Sciences and by American Cancer Society grant SIG-9. We thank Stephen Hosselet for his technical assistance with the ultraviolet exposures; Ms Christine Winslow for her expert help in document preparation; Elizabeth T. Snow, Nirmal K. Roy and Catherine B. Klein for their helpful discussions.

References

Arlett CF, Turnbull D, Harcourt SA, Lehmann AR, Colella CM (1975) A comparison of the 8-azaguanine and ouabain-resistance systems for the selection of induced mutant Chinese hamster cells. Mutat Res 33:261–278

Bradley MO, Sharkley NA (1977) Mutagenicity and toxicity of visible fluorescent light to cultured mammalian cells. Nature 266:724–726

Chang CC, Castellazzi M, Glover TW, Trosko JE (1978) Effects of harmon and nonharmon on spontaneous and ultraviolet light-induced mutagenesis in cultured Chinese hamster cells. Cancer Res 38:4527–4533

Coohill TP, Peak MJ, Peak JG (1987) The effects of the ultraviolet wavelengths of radiation present in sunlight on human cells in vitro. Photochem Photobiol 46:1043–1050

Cuzik J, Evans S, Gillman M, Price-Evans DA (1982) Medicinal arsenic and internal malignancies. Br J Cancer 45:904–911

Enninga IC, Groenendijk RTL, Filon AR, van Zeeland AA, Simmons JWIM (1986) The wavelength dependence of u.v.-induced pyrimidine dimer formation, cell killing and mutation induction in human diploid skin fibroblasts. Carcinogenesis 7:1829–1836

Hsie AW, Li AP, Machanoff R (1977) A fluence response study of lethality and mutagenicity of white, black and blue fluorescent light, sunlamp and sunlight irradiation in Chinese hamster ovary cells. Mutat Res 45:333–342

Jones CA, Huberman E, Cunningham ML, Peak MJ (1987) Mutagenesis and cytotoxicity in human epithelial cells by far- and near-ultraviolet radiations: action spectra. Radiat Res 110:244–254

Lee TC, Huang RY, Jan KY (1985) Sodium arsenite enhances the cytotoxicity, clastogenicity, and 6-thioguanine-resistant mutagenicity of ultraviolet light in Chinese hamster ovary cells. Mutat Res 148:83–89

Li J-H (1989) PhD Thesis. New York University

Li J-H, Rossman TG (1989a) Mechanism of comutagenesis of sodium arsenite with *N*-methyl-*N*-nitrosourea. Biol Trace Element Res 21:373–381

Li J-H, Rossman TG (1989b) Inhibition of DNA ligase activity by arsenite: a possible mechanism of its comutagenesis. Mol Toxicol 2:1–9

Lofroth G, Ames BN (1978) Mutagenicity of inorganic compounds in *Salmonella typhimurium*: Arsenic, chromium, and selenium. Mutat Res 53:65–66

National Research Council (1982) Report on causes and effects of stratospheric ozone reduction: an update. National Academy Press, Washington DC

Okui T, Fujiwara Y (1986) Inhibition of human excision DNA repair by inorganic arsenic and the comutagenic effect in V79 Chinese hamster cells. Mutat Res 172:69–76

Parrish JA, Anderson RR, Urbach F, Pitts D (1978) Biological effects of ultraviolet radiation with emphasis on human responses to long-wave ultraviolet. Plenum Press, New York

Peak MJ, Peak JG (1989) Solar-ultraviolet-induced damage to DNA. Photodermatology 6:1–15

Roberts JD, Kunkel TA (1986) Mutational specificity of animal cell polymerases. Environ Mutagen 8:769–789

Rossman TG, Meyn MS, Troll W (1977) Effects of arsenite on DNA repair in *Escherichia coli*. Environ Health Perspect 19:229–233

Rossman TG, Stone D, Molina M, Troll W (1980) Absence of arsenite mutagenicity in *E. coli* and Chinese hamster cells. Environ Mutagen 2:371–379

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Natl Acad Sci USA 71:3363–3366

Smith KC (1981) Photobiology and photomedicine: the future is bright. J Invest Dermatol 77:2–7

Sterenborg HJCM, van der Leun JC (1990) Tumorigenesis by a long-wavelength UVA source. Photochem Photobiol 51:325–330

Tseng WP (1977) Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19:109–119

Tyrrell RM (1984) Mutagenic action of monochromatic UV radiation in the solar range on human cells. Mutat Res 129:103–110

Tyrrell RM, Amaudruz F (1984) Alpha polymerase involvement in excision repair of damage induced by solar radiation at defined wavelengths in human fibroblasts. Photochem Photobiol 40:449–451

Zelle B, Reynolds RJ, Kettenhagen MJ, Schuite A, Johman PHM (1980) The influence of the wavelengths of ultraviolet radiation on survival, mutation induction and DNA repair in irradiated Chinese hamster cells. Mutat. Res. 72:491–509