Arch. Environ. Contam. Toxicol. 25,525–528 (1993)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Effects of Arsenic on DNA Synthesis in Human Lymphocytes

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Abstract. Effects of arsenic on DNA synthesis in human lymphocytes were biphasic: Either trivalent (arsenic trioxide and sodium arsenite) or pentavalent (sodium arsenate) arsenic compounds at very low concentrations enhanced DNA synthesis in human lymphocytes stimulated by phytohemagglutinin (PHA), whereas higher concentrations inhibited DNA synthesis. There were differences among individual susceptibities to arsenic-induced DNA synthesis. Either stimulating or inhibiting effects of trivalent arsenic on DNA synthesis in PHA-stimulated lymphocytes were always stronger than those of pentavalent arsenic. Both trivalent and pentavalent arsenic could be rapidly taken up into the human lymphocytes and immediately stimulate or inhibit DNA synthesis. A possible dual effect of arsenic at very low concentrations as both comutagen and inhibitor of mutagenesis is discussed.

Arsenic is a metalloid and is widely distributed in nature. Epidemiological studies have shown that arsenic exposure is correlated with increased incidences of human skin, lung, and possibly liver cancers. However, there is no reliable evidence that arsenic induces tumors in experimental animals (Leonard and Lauwerys 1980; Leonard 1984). In some experiments, arsenicals did not produce any tumors (Hueper and Payne 1962), and these compounds were reported to reduce the production of tumors (Boutwell 1963; Milner 1969). Therefore, arsenicals have been considered to be comutagens and cocarcinogens. A possible mechanism of the comutagenic and cocarcinogenic properties of arsenic could be to yield abnormal DNA by processes affecting DNA synthesis or by the inhibition of DNA repair. Trivalent and pentavalent arsenicals are known to inhibit DNA, RNA, and protein synthesis (Sibatani 1959; Petres *et al.* 1977; Nakamuro and Sayato 1981). Recently, arsenic was reported to stimulate the synthesis of eight proteins and inhibit the synthesis of two proteins in rat kidney proximal tubule epithelial cells (Aoki *et al.* 1990). For a better understanding of the mechanisms by which arsenic affects target cell populations, we have studied the effects of arsenic on DNA synthesis in human peripheral blood lymphocytes from adult males or females unexposed to arsenic. The results show that arsenic at very low concentrations enhanced DNA synthesis in human lymphocytes stimulated by phytohemagglutinin (PHA) and at high concentrations inhibited DNA synthesis.

Materials and Methods

Test Substances

Sodium arsenite ($NaAsO_2$), sodium arsenate (Na_2HAsO_4) and arsenic trioxide (As_2O_3) were dissolved in distilled water and added directly to the culture medium. The final concentrations of sodium arsenite in the cultures ranged from 8×10^{-9} to 1×10^{-2} M, of arsenic trioxide from 8×10^{-9} to 1×10^{-5} M, and of sodium arsenate from 1×10^{-7} to 1×10^{-2} M.

Lymphocyte Preparation

Heparinized whole peripheral blood from healthy adult males or females unexposed to arsenic were used, for the study of DNA synthesis in lymphocytes treated with arsenic for 72 h, for the study of DNA synthesis in lymphocytes treated with arsenic for 1 h, peripheral blood lymphocytes were obtained from heparinized blood after separation on a Ficoll-Hypaque density gradient as described by Friedmann and Rogers (1980). Cells harvested from the interface were washed with phosphate-buffered saline (PBS) and then resuspended in culture medium $RPMI_{1640}$.

DNA Synthesis

Heparinized whole blood (0.30 ml) was added to 4.70 ml $RPMI_{1640}$ medium supplemented with 20% heat-inactivated fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and PHA-P (7.5 μg/ml) for 72 h at 37°C in a 5% CO_2 atmosphere. Inorganic arsenic compounds at the indicated concentrations were added at culture initiation. 20 h before harvesting the cultures, 7.4×10^{-4} Bq of ^{3}H-thymidine (^{3}H-TdR) (specific activity: 6.66×10^{11} Bq/mM) in 100 μl saline was added to each tube. The tubes were put in ice water bath, and 10 μ ml of pre-cooled distilled water were added into each tube. The cells were collected onto glass fiber filters (Friedmann and Rogers 1980), and washed with pre-cooled distilled water, 5% trichloroacetic acid (TCA), and absolute ethanol. The filters were dried overnight at room temperature or at 50°C for 2 h. Incorporated radioactivity was counted in a Packard liquid scintillation spectrometer, using a scintillation cocktail of 4 g ppo

Table 1. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenite for 72 h

Donor	$NaAsO_2$ ($\times 10^{-6}$ M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm$ SD)	Increase (%)	Decrease (%)
A	0	29594 ± 946	—	—
	0.1	36688 ± 1111**	23.97	
	0.5	49655 ± 1830**	69.79	
	1.0	56583 ± 1946**	91.20	
	2.0	29754 ± 1002	0.54	
	5.0	5511 ± 340**		81.38
B	0	5863 ± 92	—	—
	0.1	6410 ± 572	9.33	
	0.5	8023 ± 212**	36.84	
	1.0	6753 ± 187**	15.17	
	2.0	6067 ± 188**	3.49	
	5.0	756 ± 59**		87.11*
C	0	70439 ± 1171	—	—
	0.008	72514 ± 1052	2.28	
	0.1	84140 ± 1024**	15.25	
	0.5	91316 ± 1185**	21.70	
	1.0	83093 ± 1081**	13.75	
	2.0	25774 ± 1201**		46.14

**$p < 0.01$ versus controls by t-test.

and 0.4 g popop dissolved in 1 L toluene. The cultures were performed in triplicate.

To determine the effect of 1 h arsenic treatment on DNA synthesis, lymphocytes separated at a density of 5×10^5 cells/ml were cultured in $RPMI_{1640}$ medium supplemented with 20% heat-inactivated fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and PHA-P (7.5 μg/ml) for 48 h at 37°C. The cells were harvested together and resuspended at a density of 2×10^6 cells/ml in $RPMI_{1640}$ medium with the addition of penicillin (100 units/ml) and streptomycin (100 μg/ml), but without fetal calf serum and PHA. One milliliter of the cell suspension was added into each tube. Inorganic arsenic compounds at the indicated concentrations were added into each tube. The cells were incubated for 1 h at 37°C, and then the cells were washed twice with $RPMI_{1640}$ medium without arsenic. 3.70×10^5 Bq of ^{3}H-TdR (specific activity: 6.66×10^{11} Bq/mM) were added to each tube, and then the cells were incubated for 30 min at 37°C. The cells were collected on glass fiber filters, and the filters were washed, dried, and counted as described above. The cultures were performed in triplicate.

Results

The effects of trivalent (As_2O_3 and $NaAsO_2$) and pentavalent (Na_2HAsO_4) arsenic exposure for 72 h on incorporation of ^{3}H-TdR into human lymphocytes stimulated by PHA *in vitro* are shown in Tables 1–3. It can be seen that, in all cases, there was an initial stimulation of incorporation of ^{3}H-TdR into the lymphocytes by arsenic at very low concentrations, followed by an inhibition of incorporation of ^{3}H-TdR into the cells as the concentrations of arsenic were raised. The concentrations of As_2O_3, $NaAsO_2$, and Na_2HAsO_4 at which maximum stimulation on ^{3}H-TdR incorporation was found were 0.1×10^{-6}, 0.5×10^{-6} to 1.0×10^{-6}, and 2×10^{-6} M, respectively.

Tables 1–3 also show that individuals might vary in their susceptibility to arsenic stimulation of DNA synthesis in lymphocytes.

Tables 4–6 summarize effects of a 1-h treatment with arsenic compounds on DNA synthesis in PHA-stimulated human lymphocytes. It was shown that As_2O_3, $NaAsO_2$, and Na_2HAsO_4 at their very low concentrations increased DNA synthesis in the lymphocytes, followed by a decrease of DNA synthesis as the concentrations of these chemicals were raised. The concentrations of As_2O_3, $NaAsO_2$, and Na_2HAsO_4 at which maximum stimulation on DNA synthesis was found were 0.5×10^{-6}, 1×10^{-6}, and 1×10^{-5} M, respectively. The concentrations of As_2O_3, $NaAsO_2$, and Na_2HAsO_4 at which about 50% inhibition of DNA synthesis in respective controls was found were 1×10^{-5}, 1×10^{-4}, and 1×10^{-3} M, respectively. Rates of DNA synthesis in the lymphocytes were changed after the cells were exposed to the arsenicals only for 1 h; this implies that both trivalent and pentavalent arsenic compounds could be rapidly taken up into human lymphocytes and immediately enhance or inhibit DNA synthesis in the cells.

Discussion

In the present study, it was shown that effects of inorganic arsenic compounds tested on DNA synthesis in PHA-stimulated human lymphocytes *in vitro* were biphasic: the chemicals at very low concentrations stimulated DNA synthesis, whereas higher concentrations inhibited DNA synthesis. Further experiments are required in order to interpret these results, although Nordenson and Beckman (1991) have indicated that genotoxic effect of arsenic on cultured human lymphocytes may be mediated by oxygen free radicals. Our study emphasizes the importance of studying toxicological or biological effects of arsenic at very low concentrations in order to obtain as accurate and comprehensive picture as possible.

For the general population, the daily inhalation and intake from ambient air and foodstuffs does not exceed 10 μg because most food contains little arsenic (<0.25 mg/kg) (Leonard 1984). However, the problem is compounded, since arsenic can be accumulated in certain (aquatic) food chains, and is an

Table 2. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenic trioxide for 72 h

Donor	As_2O_3 ($\times 10^{-6}$ M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm SD$)	Increase (%)	Decrease (%)
D	0	13244 ± 1060	—	—
	0.1	18001 ± 1046**	35.92	
	0.5	17514 ± 1012**	32.24	
	1.0	7130 ± 117**		46.16
	2.0	823 ± 22**		93.79
	5.0	159 ± 19**		98.80
E	0	16794 ± 1025	—	—
	0.1	18180 ± 1029	8.25	
	0.5	16522 ± 1287		1.62
	1.0	6623 ± 105**		60.56
	2.0	623 ± 40**		96.29
	5.0	438 ± 39**		97.39
F	0	68753 ± 989	—	—
	0.008	68745 ± 1144	—	—
	0.1	74677 ± 1152**	8.62	
	0.5	79483 ± 1229	2.52	
	1.0	30348 ± 1138**		55.86
	2.0	8168 ± 146**		88.12
G	0	1452 ± 132	—	—
	0.008	1930 ± 123*	32.92	
	0.1	2964 ± 132**	104.13	
	0.5	2342 ± 145**	61.29	
	1.0	1046 ± 124*		27.96
	2.0	321 ± 56**		77.89

*$p < 0.05$, **$p < 0.01$ versus controls by t-test.

Table 3. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenate for 72 h

Donor	Na_2HAsO_4 ($\times 10^{-6}$ M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm SD$)	Increase (%)	Decrease (%)
H	0	58266 ± 1261	—	—
	0.5	59217 ± 1273	1.63	
	1.0	67562 ± 1380**	15.95	
	2.0	78582 ± 1377**	34.87	
	5.0	67167 ± 1106**	15.28	
	10.0	28626 ± 1393**		50.87
	100.0	8980 ± 916**		8459

**$p < 0.01$ versus control by t-test.

Table 4. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenate for 1 h

Concentration of $NaAsO_2$ (M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm SD$)	Enhancement (%)	Inhibition (%)
0	10944 ± 2001	—	—
1×10^{-7}	11797 ± 998	7.79	
1×10^{-6}	15855 ± 1960**	44.86	
1×10^{-5}	13020 ± 985	18.97	
1×10^{-4}	5242 ± 200**		51.20
1×10^{-3}	4195 ± 195**		61.67
1×10^{-2}	6219 ± 198*		43.17

*$p < 0.05$, **$p < 0.01$ versus control by t-test.

accumulative poison in the human body. In these studies, the results show that arsenic at very low concentrations enhanced DNA synthesis in human lymphocytes. Thus, it is possible, for some individuals among the general population, that arsenic in some tissues and cells of their body can be accumulated at sufficient concentrations to affect DNA synthesis or other biochemical processes in human cells. Therefore, studies of the biological effects of arsenic at very low concentrations are very important.

The results also indicated large interindividual variations in effects of arsenic on DNA synthesis in human lymphocytes, particularly in its enhancing effects on DNA synthesis of arsenic at very low concentrations. This may be related to differences in metabolic activities and in sensitivities to arsenic.

In general, trivalent arsenic is more lethal and clastogenic than pentavalent (Jacobson-Kram and Montalbano 1985; Aposhian 1989). Our results also show that effects of trivalent arsenic on DNA synthesis are far greater than that of pentavalent, either for enhancing or inhibiting effects. The biological effects of pentavalent arsenic that are weak might be due to a large quantity of pentavalent phosphorus compounds (*e.g.*, phosphate) in cells which are strong competitors against pentavalent arsenic compounds and are not able effectively to contact enzymes and other biological active molecules: The competition-inhibiting effects of pentavalent phosphorus against pentavalent arsenic might interact effectively with enzymes and proteins with an SH group.

The biphasic effects of arsenic on DNA synthesis imply that the toxicological or biological role of arsenic in human health is very complex. Under certain conditions, arsenic at very low concentrations might enhance replication of DNA fragment(s) of mutated gene(s) induced by chemicals or other factors and result in mutagenesis or carcinogenesis of the cells. If so, arsenic as a comutagen or cocarcinogen might enhance cancer development through its enhancing effect of DNA synthesis. Many studies have indicated that arsenic as a comutagen inhib-

Table 5. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenic trioxide for 1 h

Concentration of As_2O_3 (M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm SD$)	Enhancement (%)	Inhibition (%)
0	27647 ± 1131	—	—
0.5×10^{-7}	30912 ± 1084*	11.80	
0.1×10^{-6}	31995 ± 1506**	15.73	
0.5×10^{-6}	33439 ± 1145**	20.95	
1.0×10^{-6}	26722 ± 1264		3.35
2.0×10^{-6}	25561 ± 639*		7.55
1.0×10^{-5}	14428 ± 723**		44.81

*$p < 0.05$, **$p < 0.01$ versus control by t-test.

Table 6. DNA synthesis in PHA-stimulated human lymphocytes exposed to sodium arsenate for 1 h

Concentration of Na_2HAsO_4 (M)	Incorporation of ^{3}H-TdR (cpm) ($\bar{X} \pm SD$)	Enhancement (%)	Inhibition (%)
0	12946 ± 1143	—	—
1×10^{-7}	14107 ± 1146	8.97	
1×10^{-6}	18849 ± 1085**	45.60	
1×10^{-5}	21103 ± 1056**	63.00	
1×10^{-4}	12747 ± 1259		1.54
1×10^{-3}	6760 ± 177**		47.79
1×10^{-2}	5444 ± 145**		57.95

**$p < 0.01$ versus control by t-test.

its DNA repair (Jung *et al.* 1969; Rossman *et al.* 1977; Okui and Fujiwara 1986; Li and Rossman 1989b; Snyder and Lachmann 1989), and potentiates mutagenicity of methyl methanesulfonate (MMS) (Lee *et al.* 1986), *n*-methyl-*N*-nitrosourea (MNU) (Li and Rossman 1989a), *cis*-diamminedichloroplatinum(11) (Lee *et al.* 1986), and UV (Rossman 1981; Lee *et al.* 1985). However, some studies have reported that under certain conditions, arsenic reduces the mutagenicity of MMS (Lee *et al.* 1986), and UV (Rossman *et al.* 1975, 1977; Nunoshiba and Nishioka 1987). In some experiments, animals fed arsenic do not suffer from arsenic carcinogenicity (Hueper and Payne 1962), and arsenic in fact minimizes the induction of cancers (Boutwell 1963; Milner 1969). These observations and the present study raise the question of whether arsenic at very low concentrations might enhance, under certain conditions, replication of gene(s) related to DNA repair and lead to enhancement of DNA repair. If so, arsenic as an inhibitor of mutagenesis or carcinogenesis might reduce the incidence of cancers produced by DNA-damaging agents. If so, arsenic at very low concentrations would have a dual effect in mutagenesis as both a comutagen and inhibitor. This question remains to be confirmed by further studies.

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Manuscript received February 2, 1993 and in revised form May 29, 1993.