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The Genotoxicity of Sodium Arsenite in Human Lymphocyte Culture

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خلاصة

تهدف هذه الدراسة إلى معرفة التأثير السمى الوراثى لمركب أرسينات الصوديوم على الخلايا الليمفاوية في الدم باستخدام جرعة واحدة (٥ ملي مول) كذلك دراسة مدى التأثير المشترك مع جرعات مختلفة من أشعة إكس ١٥٠-٣٠٠ جراى مجزأة على دفعتين (١٥٠ + ١٥٠ جراي) واختبار تأثير الفترة الزمنية بين المعاملة بالأرسينات والتعرض للأشعة.

وقد أوضحت النتائج أن مادة الأرسينات تحدث تثبيطاً مؤقتاً بتأخير الدورة الأولى لانقسام الخلايا الليمفاوية المعزولة وأن ٧٥٪ من الخلايا تكون في دورتها الأولى بعد ٦٤ -٦٨ ساعة من بداية المزرعة في حين أن ٩٨٪ من الخلايا الليمفاوية في مزرعة الدم الكامل تكون دخلت انقسامها الأول بعد ٤٨ ساعة فقط من بداية المزرعة.

كذلك دلت النتائج على إحداث كسور وثغرات في الخلايا الليمفاوية المعرضة لمادة الأرسينات ولم يوجد فروق عددية كبيرة بين نوعى المزارع المستخدمة (٤٨-٧٤ ساعة) بينما زادت عدد الخلايا متعددة الكروموسومات (Polyploidy) زيادة إحصائية نتيجة لمعاملتها بمادة الأرسينيت مما يستنتج منه أن مادة الأرسينات تحدث تأثيرا مباشا على جهاز الانقسام الميتوزى مما يؤدى إلى تضاعف عدد الكروموسومات داخل الخلية دون انقسام سيتوبلازمى ولم يستدل على تغير يذكر عند استخدام الجرعات المختلفة من أشعة إكس في نشاط وراثى في المزارع المعالجة لماده الأرسينات مما يوضح أن الماده الأخيرة ليس لها نشاط وراثى مساعد مع أشعة إكس كذلك لم يكن هناك مؤشرات على أن تعرض المزارع لأشعة اكس بعد معالجتها مادة الأرسينات بماشرة أو بعد ثلاثة ساعات من المعالجة الكيميائية قد أحدث تغيرا ملحوظا.

وتوضى هذه الدراسة بضرورة استخدام مزارع الدم الكامل إلى جانب مزارع الخلايا الليمفاوية المعزولة عن اختبار السمية الوراثية للمواد الكيمايئية.

Abstract

Sodium arsenite was tested for its clastogenic effect alone and on isolated lymphocyte culture. The results showed a significant difference in the yield of chromosome aberrations induced with respect to the culture time 48 h. Whole blood culture showed significant increase in gaps and breaks whereas isolated lymphocyte culture showed significant inhibition of cell cycle and 75% of the lymphocytes were in their first cell cycle at 72 hr. Arsenite showed co-mutagenicity with different doses of x-ray delivered immediately or few hours after treatment of the culture with SA. The results suggest that SA is also mutagenic at the dose level used and provide support for the indispensibility of whole blood culture for evaluation of the *in vivo* effect of any suspected mustagen using isolated lymphocytes appear to have problems leading to extensive cell cycle delay.

Introduction

Epidemiological studies have recognized arsenic (SA) compounds or arsenicals as known carcinogens *(Hernberg, 1977; IARC, 1980; Chen C.J. et al., 1988)*. Arsenic contamination of water in different parts of the world has caused symptoms of poisoning which is manifested by skin, gastrointestinal and neurological disorders *(Cebrian et al., 1983)*.

Exposure to arsenic appears to act at a late stage in the carcinogenic process, i.e. tumour progression *(Brown and Chu, 1983)*. Genetic instability is a characteristic manifestation during tumour progression. The association of cell ploidy with a variety of tumour types in their advanced stages is evident from both human cancers and animal models *(Klein et al., 1982; Tibukait et al., 1982; Frankfurt et al., 1985; Smith et al., 1985)* .

Many human solid tumors, both primary and metastatic have been found to be hyperdiploid, containing more than 46 chromosomes but fewer than 60 chromosomes of hyperdiploid formation remain unclear. Arsenic can induce morphological transformation as well as tetraploidy in Syrian hamster embryo cell over the dose range 100 μM SA, *(Jia-Ran Curr et al., 1993)*. Further, Jha et al., 1992) reported that sodium arsenite (SA) alone and in combination with x-ray in peripheral blood lymphocytes increased the spontaneous incidences of chromosomal aberrations. Also, short wave untraviolet (UV) radiation of primary human fibroblast culture system preincubated with SA Inhibited the cell cycle progression of phytohaemagglutinin (PHA) responsive lymphocytes, and induced chromatid-type aberrations and sister chromatid exchanges. Furthermore

arsenite potentiated the x-ray and UV-induced chromosomal aberrations. The same authors suggested that SA interfers with the DNA repair process presumably by inhibiting the ligasae activity. However. reportes on the clatoganicity of arsenites is scarce and almost non-existing. Further, the contradictory results of whole blood culture and isolated lymphocyte culture justified further experimentation to elucidate the nature of the effect of arsenites in human lymphocyte culture *per si. In* this study (SA) was tested for its mutagenic effect on Go chromosomes. Its comutagenic effect was also studied with x-rays treated lymphocytes in Go phase. The mechanism involved in either arsenite mutagenic or comutagenic effect will be investigated.

Materail and Methods

Venous blood was withdrawn from two healthy donors. In the first set of experiment, whole blood culture technique was used to test for the clastogenic effect of arsenites (SA) and its potentiating effect on x-ray induced chromosomal aberrations. Arsenite was added to the culture for 90 min. at a concentration of 5μM; a dose reported by *Li and Rossman, (1989)* to be non-toxic. Then the culture was washed with serum free medium and cells were transfered to complete medium and allowed to grow for 48 hr. Lymphocytes were harvested with colcimed and subjected then to hypotonic treatment with 0.075 M KCl. After fixation in acetic acid methanol solution, slides were prepared and stained with Giemsa.

Another set of arsenite treated cultures were further x-irradiated with either 1.5 Gy or 3.0 Gy given as one shot or fractionated into 2 equal doses, 1.5 Gy + 1.5 Gy and further incubated as described above. Irradiation took place at zero hour of incubation in one group (A) and 3 hr later in another group (B). Negative non-treated control and positive x-irradiated control culture were set up and cultured for 48 hr. under the same conditions and processed for metaphase preparation.

In a second set of experiment isolated lymphocytes prepared using the Ficoll Histopaque Gradients.. (Bochreniger) were used to test the clastogenicity of arsenite alone and in combination with x-ray.

The PHA proliferation kinetics in the presence of 5-Brdu using the FPG staining technique was studied to allow recognition of cells grown at its first, second or more cell cycle.

Isolated lymphocytes treated with arsenite alone or in combination with x-ray produced very low yield of metaphases which indicated a delaying

effect of arsenite. Therefore, the cells were allowed to remain in the same medium (containing SA) for a total period of 90 min at 37° after which cells were washed twice with serum free medium and transferred to a complete medium for total period of 72 hr. The cells were fixed and metaphase spreads were prepared according to the standard protocol. Irradiation with x-rays was done under the same condition either following immediately SA treatment, or 3 hr. later.

Results

The proportions of cells at different cell division cycles in lymphocyte cultures harvested at 72 hr after treatment with 5μM of SA for 3 hr. shows a definite delay in cell cycle progression with 70% and 30% of cells in first division and second division, respectively, compared with 2% and 98% for the control at the same fixation time. Table 1 shows the proportion of cells in different cell cycles treated either with SA alone or followed by irradiation with x-rays. It is apparent that irradiation with x-ray does not alter the dynamics of lymphocyte cell cycle up to a dose of 3 Gy. Whereas, SA added separately to a lymphocyte culture and washed after 3 hr or followed by x-ray exposure had a very pronounced delayed effect on cell division progression which result in retardation of 70% of the cells for about 24 hr.

Table (1) : Stage of cell proliferation in 72 hr-culture of isolated lymphocytes

Treatment 72 hr fixation	Progression of cell division	
	First division (%)	Second division (%)
Control	2%	98%
SA-treatment	70%	30%
SA treatment + 1.5 Gy	70%	30%
1.5 Gy	2 %	98%
1.5 Gy + 1.5 Gy	4 %	96%
SA treatment + 3 Gy	72 %	28%
3 Gy	2 %	98%
SA treatment + 3 Gy	75 %	25%

The clastogenic effect of SA was studied in whole blood cultures for 48 hr. Also, the potentiating effect of SA on x-rays induced chromosomal aberrations (CA) was studied in the same system. SA elevated the incidences of aberrant cells and chromatid breaks more than 10-times the

spontaneous level, (Table 2). The induced CA, were of the gap and chromatoid type breaks but with no chromosome breaks or chromosome exchange type of aberrations. SA also, slightly potentiated the clastogenic effect of x-ray doses of the order 1.5 and 3 Gy given as a single or fractionated dose. The frequency of x-ray induced dicentrics + rings have increased in SA pretreated cultures but such effect may not be a significant one. Also, irradiation of SA-pretreated cultures 3 hr later did not result in marked elevation of exchange type aberration and chromatid breaks. This suggest that arsenite treatment did interfere with either repair mechanisms which usually take place between successive irradiation nor the prolongation of arseinte treatment prior to irradiation potentiated CA.

The clastogenic effect of SA was further studied in isolated lymphocytes from 2 donors and the results are given in Tables 3 and 4. The data of each donor show certain differences but in the same direction which justify its presentation, separately. Donor II showed dicentries whereas donor I showed no exchange type CA in SA treated cultures. Both donors showed polyploidy cells in SA and irradiated cultures,whereas non were observed in whole blood cultures. The potentiating effect of SA on radiation induced CA was evident in donor II and also in donor I except for fractionated x-ray dose where dicentrics and rings were higher in cultures exposed to 1.5 Gy. Also, chromosomal aberration in the form of chromatid type breaks increased in some doses 4-6 folds. The incidence of chromosome type breaks was not affected by arsenite in combination with x-ray treatment.

Polypoidy observed increased up to 3 folds in some SA-radiation treated lymphocytes. However, prolonged exposure of lymphocytes to SA prior to irradiation with 1.5 Gy was exceptional and had no effect on the incidence of induced CA.

Table (2) : The frequency of chromosomal aberrations in human lymphocytes treated *in vitro* with x-rays and sodium arsenite (SA) 48 hr culture (whole blood).

Treatment	No. of normal cells	Abnormal cells %	Types of chromosomal aberrations (100 cells)				
			g+cdb	Chsb	Dic	Ring	Dic + Ring
Control	98	2	2	0	0	0	0
SA	75	25	25	0	0	0	0
(A) Irradiation of 0.0 hr. of culture							
SA + 3 Gy	19	81	32	39	55	6	61
3 Gy	22	78	7	37	53	5	58
SA+1.5Gy+1.5Gy	32	68	29	32	46	5	51
1.5 Gy + 1.5 Gy	40	60	9	30	43	4	47
SA + 1.5 Gy	45	55	29	28	30	3	33
1.5 Gy	49	51	3	27	25	4	29
(B) Irradiation at 3 hr-after culture							
SA + 3Gy	15	95	36	38	56	7	63
3 Gy	21	79	9	35	53	6	59
SA + 1.5 Gy	46	54	25	18	29	3	31
1.5 Gy	52	48	0	26	23	2	25

g (gap), cd b (chromatid break), chs b (chromosome break), dic (Dicentrics)

Table (3) : The frequency of chromosomal aberrations in human lymphocytes treated *in vitro* with X-rays and sodium arsenite (SA) 72 hr. culture (donor I)

Treatment	No. of normal cells	Abnormal cells %	Types of shromosomal aberrations (100 cells)					Remarks
			G+cd	Chs h	Dic	Ring	Dic + Ring	
Control	100	0	0	0	0	0	0	0
SA	67	33	35	2	0	0	0	3 polyploids
(A) Irradiation at 0.0hr of culture								
SA + 3Gy	23	77	25	13	50	12	62	5 poly + 1 trirodial
3 Gy	56	44	4	16	45	8	53	3 poly + 1 tetraploid
SA+1.5Gy+1.5Gy	28	72	33	5	50	4	54	4 poly + 1 tetraploid
1.5 Gy + 1.5 Gy	46	54	8	14	32	4	36	1 polypoloid
SA + 1.5 Gy	45	55	25	11	23	1	24	2 poly + 1 tetraploid
1.5 Gy	64	36	26	12	26	2	28	1 polyploid
(B) Irradiation at 3 hr after culture								
SA + 3Gy	29	71	38	12	51	13	64	3 poly + 2 tetraploid
3 Gy	47	53	3	13	53	9	62	2 poly + 1 tetraploid
SA + 1.5 Gy	37	63	38	8	24	1	25	2 poly + 1 tetraploid
1.5 Gy	62	38	2	9	21	2	23	2 poly + 1 tetraploid

Poly (polyploid)

g (gap), cd b (chromatid break), chs b (chromosome break), dic (Dicentrics)

Table (4) : The frequency of chromosomal aberrations in human lymphocytes treated *in vitro* with X-rays and sodium arsenite (SA) 72 hr. culture (donor II)

Treatment	No. of normal cells	Abnormal cells %	Types of shromosomal aberrations (100 cells)					Remarks
			G+cd	Chs h	Dic	Ring	Dic + Ring	
Control	94	6	6	2	0	0	0	0
SA	69	31	29	2	0	0	0	2 polyploids
(A) Irradiation at 0.0hr of culture								
SA + 3Gy	21	79	26	16	40	6	55	9 poly + 1 trirodial
3 Gy	42	58	6	18	43	5	48	7 poly + 1 tetraploid
SA+1.5Gy+1.5Gy	27	73	21	17	46	5	51	4 poly + 1 tetraploid
1.5 Gy + 1.5 Gy	43	57	4	16	43	3	46	1 polypoloid
SA + 1.5 Gy	48	52	32	19	22	2	24	2 poly + 1 tetraploid
1.5 Gy	63	37	5	18	19	2	21	1 polyploid
(B) Irradiation at 3 hr after culture								
SA + 3Gy	4	76	30	19	49	4	53	9 poly + 2 tetraploid
3 Gy	39	61	7	18	47	4	51	2 poly + 1 tetraploid
SA + 1.5 Gy	49	51	29	1	24	2	26	4 poly + 1 tetraploid
1.5 Gy	51	49	27	13	23	2	24	3 poly + 1 tetraploid

Poly (polyploid)

g (gap), cd b (chromatid break), chs b (chromosome break), dic (Dicentrics)

Discussion

The earlier sudies of *Petres et al., 1977; Wen et al., 1981; Mc Cobe et al., 1983; Jha et al., 1992* , suggested that arsenicals inhibit the cell cycle progression of cultured human and bovine lymphocytes under *in vitro* conditions. This inhibitory effect of arsenicals was not observed in this study where whole blood was used for the culture exposed to either SA alone or in line with x-irradiation. However, the results of cultures of isolated human lymphocytes are in agreement with the findings of other authors which showed profound delay of the proliferation of lymphocytes.

The presene of red blood cells and the protein rich plasma of the whole blood apparently, managed to suppress an inhibitory role of arsenicals on the proliferating lymphocytes. The inhibitory effect of arsenicals on cell cycle progression has been attributed to its affinity for proteins containing sulphydryl group *(Leonard & Lauwerys, 1980).* It is probale that under *in vivo* conditions the proteins rich in sulphydryl groups are in abundance and buffers the inhibitory effect of arsenicals making, therefore, any inhibitory effects on cell kinetics. That whole blood and essentially *in vivo* conditions produce different end result than isolated lymphocytes when exposed to arsenicals, is an assumption provided by *Larramendy et al., (1981)* and *ha et al., (1992)* as for the induction of SCEs.

The experiments using isolated lymphocytes seems to have problems leading to an extensive cell cycle delay which in turn causes variable outcome. This leads to an important conclusion that using whole blood *in vitro* studies of mutagencity and clastogenicity is indispensible and can not be replaced or substituted for the isolated lymphocyte system unless it is proved that the two systems produce similar end results.

The induction of chromosomal aberrations by SA has been demonstrated by several authors. The postulated mechanism involed interference with the DNA repair process namely the ligation process *(Huang et al., 1991; Jha et al., 1992).* In the present study, a significant elevation in the frequency of chromatid type of aberration was noted in both 48-hr whole blood culture, and in 72 hr lymphocyte culture. However, dicentrics and polyploidy were observed only in the 72 hr cultures. This suggests further impairmant of lymphocyte proliferation kinetics involving delay of cytokinesis and endoreduplication of the genome set. The incidence of polyploidy, in particular, underestimates the actual values as 25 - 30% of the cells scored were in 2nd cell cycle and the 70 - 75% were in the lst cell cycle as revealed by the FPG technique.

The data obtained in the present study illustrated the fact that SA is similar to other chemicals is inducing specifically chromatid type of aberration as compared with radiation which induces chromosome type aberration. *Larramendy et al., (1981)* found similar results and concluded that SA can induce DNA synthesis dependent effect such as chromatid aberration and SCEs; if it interferes with the lighation process.

Lee et al., (1986 a, b) classifed arsenite as S-dependent clastogen when used at a low dose range. Whether arsenite can induce chromatid aberrations by directly breaking DNA strands is an interesting question to be answered, if we use the smaller dose of arsenite 5 μ M. It has been reported that arsenite by itself is unable to induce gene mutation *(Rossman et al., 1980; Lee et al., 1985 a, b).* A recent study failed to demonstrate the induction of DNA strand breaks by using the techniques of nucleoid sedimentation, alkaline sucrose centrifugation and alkaline elution *(Lee - Chen et al., 1993).* However, *Yamanka et al., (1989)* have shown that dimethyl arsenic acid, a major metabolite of inorganic arsenic, can indue DNA strand breaks in lungs of mice via the production of active oxygen.

Inhibitors of DNA repair process have been reported to potentiate the yield of CA induced by various clastogens *(Natarajan, and Obe, 1982; and preston, 1982).*

The design of the experiments permits evaluation of the interactions of SA in repair of radiation induced aberrations particularly chromosome types. SA, by itself did not induce chromosome breaks, dicentrics or rings. However, SA plus irradiation resulted in higher frquencies of CA in nearly all tested doses. The data, therefore, suggest that SA is comutagenic in combination with x-ray irradiation. These results are in good agreement with those obtained by *Jha et al., (1992),* who attributed this effect of SA on the final step in the repair of DNA lesions. *Huang et al., (1991),* also suggested similar effect of arsenicals to interfere with DNA ligase activity resulting in delay of DNA repair after irradiation.

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