0163-4984/93/3801-0055 $03.00

Cell Division, Chromosomal Aberration, and Micronuclei Formation in Human Peripheral Blood Lymphocytes

Effect of Stannic Chloride on Donor's Age

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Received May 10, 1992; Revised October 30, 1992; Accepted November 3, 1992

ABSTRACT

Age related cytotoxicity of stannic chloride was evaluated in human lymphocytes considering mitotic index (MI), damaged cell (DC), chromosome aberration (CA), and micronuclei formation (MNC) as endpoints. Significant elevation of DCs, CAs, MNCs, and reduction of MI were observed in all classified age groups compared to each control set. The mean frequencies of abnormalities show a statistically significant increase with subject's age. Linearity of the effect of age was common for both untreated and treated lymphocytes of both sexes.

Index Entries: Stannic chloride; aging; human lymphocytes.
Abbreviations: MI = mitotic index; DC = damaged cell; CA = chromosome aberration; MNC = micronuclei formation.

INTRODUCTION

The emergence of the industrial society has led to the use of chemicals of a diverse nature in a wide range of human activities. Heavy metals are an important class of environmental pollutants, and some of these are implicated in the induction of tumors in experimental animals and exposed humans (*1*). Interest in tin compounds has been increased because of their extensive use as additives in PVC products, plating of containers,

general and agricultural biocides, and model compounds in neurobiological research (2). Migration of tin from plated containers into food, beverages, and drinking water, or from plastic medical devices into body fluids or exposure to biocides are the possible sources from which this metal can cause direct toxicological effects in humans.

Age had been shown to significantly affect the frequency of spontaneous micronucleus formation, chromosome aberration, and sister chromatid exchanges, in lymphocyte population of the male and female populations (3,4). Age-related effects of organotin compound have been evaluated recently in detail by Ghosh et al. (5–7) on the frequencies of chromosome aberrations (CAs), micronuclei formation (MNCs), and sister chromatid exchanges (SCEs). Only those with stannic chloride were studied, in three age groups, with a broad classification consisting of 21 individuals. In the present communication the detailed age-related cytotoxicity of stannic chloride was observed in mitotic index (MI), micronuclei formation (MNC), damaged cell (DC), and chromosome aberration (CA) in a large population.

MATERIALS AND METHODS

Human lymphocytes were collected from venous blood of 52 healthy individuals of both sexes who reported that they had not knowingly been exposed to toxic agents. Donors were classified into seven age groups, each group covering a different 10-yr span. Four subjects were considered in each group of both sexes.

From each subject, 5 mL of blood was collected in heparinized vials. Cultures were set up with 0.3 mL of whole blood in RPMI 1640 (GIBCO, Gaithersburg, MD) medium supplemented with heat-inactivated human AB serum and phytohaemagglutinin (GIBCO). An aqueous solution of stannic chloride (4 μg/mL) was added at the time of inoculation. Replicate cultures for control and treated sets were incubated at 37°C for 48 h. Cells were collected following pretreatment with colchicine, hypotonic treatment with 0.09% NaCl, and fixation in 1:3 acetic acid:methanol, and slides were prepared following air drying and Giemsa schedule (*8*).

All slides were coded prior to data analysis. For each subject 200 metaphase plates for DC and CAs, 2000 cells for each of the MI and MNCs were observed for control and treated sets. Observation was taken according to standard procedure (*9,10*). Data were analyzed statistically following Student's *t*-test.

RESULT

Following administration of stannic chloride to the lymphocyte cultures, the alterations in the frequencies of DCs, CAs, and MNCs, and MI in male and female subjects, and are presented in Tables 1 and 2. Stannic

Table 1
Frequency of Chromosome Aberrations in Human Lymphocytes Exposed to Stannic Chloride In Vitro (Male).

Age groups	Doses	Total chromosome aberrations (CA) G′	G″	B′	B″	DIC	RR	CA/cell (mean ± SD) + Gaps	− Gaps	Damaged cell (DC %) (mean ± SD)	Micronucleous counts (%) (mean ± SD)	MI (mean ± SD)
I	Control	2	—	10	2	—	—	0.035 ± 0.013	0.030 ± 0.008	3.50 ± 1.29	0.49 ± 0.16	4.56 ± 0.87
	Treated	2	—	17	7	—	—	0.065 ± 0.013	0.060 ± 0.014	5.75 ± 0.96	1.16 ± 0.32	2.82 ± 0.30
	t-test *p*-value								$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.01$
II	Control	8	—	18	1	—	—	0.068 ± 0.009	0.047 ± 0.005	6.25 ± 1.71	0.50 ± 0.18	4.33 ± 0.93
	Treated	6	5	41	9	4	2	0.167 ± 0.022	0.135 ± 0.024	14.00 ± 2.94	1.41 ± 0.26	2.22 ± 0.72
	t-test *p*-value								$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.05$
III	Control	4	1	20	2	1	—	0.070 ± 0.008	0.057 ± 0.010	6.25 ± 0.50	0.85 ± 0.19	3.75 ± 0.89
	Treated	13	2	39	10	4	3	0.177 ± 0.013	0.140 ± 0.014	15.00 ± 2.16	1.66 ± 0.16	1.65 ± 0.55
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$
IV	Control	6	1	17	3	2	—	0.072 ± 0.012	0.055 ± 0.058	6.25 ± 1.26	0.93 ± 0.10	3.06 ± 0.71
	Treated	13	6	43	14	10	6	0.230 ± 0.043	0.182 ± 0.027	19.50 ± 4.51	1.92 ± 0.31	1.29 ± 0.34
	t-test *p*-value								$p < 0.01$	$p < 0.01$	$p < 0.001$	$p < 0.01$
V	Control	10	—	20	2	2	1	0.087 ± 0.009	0.062 ± 0.013	6.75 ± 2.50	0.98 ± 0.15	2.36 ± 0.79
	Treated	9	6	41	17	4	9	0.215 ± 0.024	0.177 ± 0.005	17.00 ± 2.16	2.20 ± 0.90	1.30 ± 0.34
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.05$
VI	Control	1	1	25	7	3	—	0.092 ± 0.025	0.087 ± 0.021	9.00 ± 2.16	1.03 ± 0.22	1.74 ± 0.31
	Treated	1	2	60	15	6	5	0.222 ± 0.038	0.215 ± 0.037	19.50 ± 4.65	2.40 ± 0.50	0.93 ± 0.35
	t-test *p*-value								$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.05$
VII	Control	3	—	29	4	3	1	0.100 ± 0.014	0.092 ± 0.005	9.00 ± 0.82	1.17 ± 0.18	1.15 ± 0.15
	Treated	7	6	48	23	7	4	0.237 ± 0.025	0.205 ± 0.024	21.00 ± 2.94	2.68 ± 0.59	0.66 ± 0.15
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.01$
	Total control								0.062 ± 0.023	6.71 ± 2.27	0.85 ± 0.29	2.99 ± 1.38
	Total treated								0.159 ± 0.054	15.96 ± 5.61	1.92 ± 0.67	1.55 ± 0.80
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

G′ = chromatid gap, G″ = chromosome gap, B′ = chromatid break, B″ = chromosome break, DIC = dicentrics, RR = rearrangements, CA = chromosome aberrations, +gaps = including gaps, −Gaps = excluding gaps.

Table 2
Frequency of Chromosome Aberrations in Human Lymphocytes Exposed to Stannic Chloride In Vitro (Female).

Age groups	Doses	Total chromosome aberrations (CA) G′	G″	B′	B″	DIC	RR	CA/cell (mean ± SD) + Gaps	− Gaps	Damaged cell (DC %) (mean ± SD)	Micronucleous counts (%) (mean ± SD)	MI (mean ± SD)
I	Control	2	—	18	2	—	—	0.055 ± 0.013	0.050 ± 0.008	5.25 ± 0.96	0.51 ± 0.11	4.77 ± 0.73
	Treated	12	1	36	4	1	1	0.138 ± 0.009	0.105 ± 0.013	11.50 ± 1.73	0.91 ± 0.18	3.00 ± 0.56
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.01$
II	Control	3	1	15	1	1	—	0.053 ± 0.019	0.043 ± 0.005	4.75 ± 1.71	0.78 ± 0.17	4.56 ± 1.04
	Treated	4	3	34	6	3	2	0.130 ± 0.018	0.113 ± 0.010	12.00 ± 0.82	1.62 ± 0.50	2.70 ± 0.90
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.05$
III	Control	4	2	17	2	1	—	0.065 ± 0.006	0.050 ± 0.016	4.25 ± 0.96	0.86 ± 0.08	3.98 ± 0.88
	Treated	7	3	31	7	4	2	0.135 ± 0.013	0.110 ± 0.008	10.00 ± 1.15	1.67 ± 0.26	2.48 ± 0.36
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$
IV	Control	2	—	16	2	—	—	0.050 ± 0.014	0.045 ± 0.006	4.50 ± 0.58	0.95 ± 0.12	3.52 ± 0.37
	Treated	3	5	29	7	2	1	0.117 ± 0.017	0.097 ± 0.013	9.25 ± 1.71	1.81 ± 0.11	2.16 ± 0.47
	t-test *p*-value								$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.01$
V	Control	1	1	24	3	1	—	0.075 ± 0.013	0.070 ± 0.014	7.50 ± 1.29	0.94 ± 0.13	2.63 ± 0.63
	Treated	5	2	50	11	4	3	0.187 ± 0.010	0.170 ± 0.008	17.75 ± 1.26	1.72 ± 0.37	2.03 ± 0.39
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.01$	NS
VI	Control	4	1	31	3	2	—	0.102 ± 0.026	0.090 ± 0.026	8.25 ± 1.26	1.05 ± 0.16	2.60 ± 0.53
	Treated	15	6	44	12	8	5	0.225 ± 0.010	0.172 ± 0.022	19.50 ± 1.29	2.48 ± 0.33	1.81 ± 0.16
	t-test *p*-value								$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.05$
	Total control								0.058 ± 0.021	5.75 ± 1.89	0.85 ± 0.21	3.68 ± 1.08
	Total treated								0.128 ± 0.034	13.33 ± 4.14	1.70 ± 0.54	2.36 ± 0.62
	t-test *p*-value								$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

G′ = chromatid gap, G″ = chromosome gap, B′ = chromatid break, B″ = chromosome break, DIC = dicentrics, RR = rearrangements, CA = chromosome aberrations, +gaps = including gaps, −gaps = excluding gaps, NS = not significant.

chloride resulted in elevation of DCs, CAs, and MNCs, and in depression of MI in lymphocytes of both sexes. The elevation of abnormalities and reduction of dividing cells were linearly related to the donor's age, which was more pronounced in male than in female subjects. The differences in control and treated sets were statistically significant in all age groups. The types of aberrations observed in lymphocytes induced by stannic chloride were chromatid and chromosome breaks, gaps, dicentrics, ring, and rearrangements (Fig. 1); when aberration frequency was compared with control sets, gaps were excluded from total aberrations.

DISCUSSION

The aging of a cell population that leads to senescence was defined as a group of harmful changes (*11*) and may also be accelerated by environmental and industrial toxicants. Steenland et al. (*12*) have demonstrated a marked influence of age on the enhanced frequency of spontaneous chromosomal aberrations. Organotin compound, namely trimethyltin chloride, enhanced the frequencies of CA, DC, SCE, and MNC in aged lymphocytes compared to younger ones, although there was no linear correlation with the donor's age (*5,7,13*), and the interaction of the donor's age and chemical concentrations was statistically significant in almost all endpoints. In another report it was found that stannic chloride resulted in severe damage in lymphocytes of elderly individuals (*14*).

In the present report aging of male and female individuals was found to influence the clastogenic and mitostatic activity of stannic chloride in lymphocytes in vitro. Elevated frequencies of mean CAs, DCs, and MNCs were estimated in all classified age groups of both sexes and compared to control within each age group. The dose-related clastogenicity was observed in a previous report with the significant increase of CAs, DCs, and SCEs (*15*). Similar enhancement of abnormalities were observed in the treated lymphocytes of individual age groups.

In general, the organotin compounds are highly toxic to living organisms because of their lipid solubility and retention at tissue PH. However, in one experiment the relative higher dose of stannic chloride (20 μg/culture) revealed more clastogenicity and that may be because a greater amount of the chemical was present in the medium. The elevation of abnormalities in organotin treated lymphocytes were not linear to the increase of the donor's age (*7*).

In the present study the enhancement of CAs and MNCs were directly related to the increase of the donor's age. This is consistent with the earlier findings of Galloway et al. (*16*) and Soper et al. (*4*). Chromatid type aberrations were most commonly observed, which are distinct features of damaged induced by heavy metals, and which are independent of the phase of cell cycle at which damage occurs (*17*).

The literature contains conflicting reports regarding the effect of aging on the variation of CAs, MNCs, and SCE frequencies. Galloway et

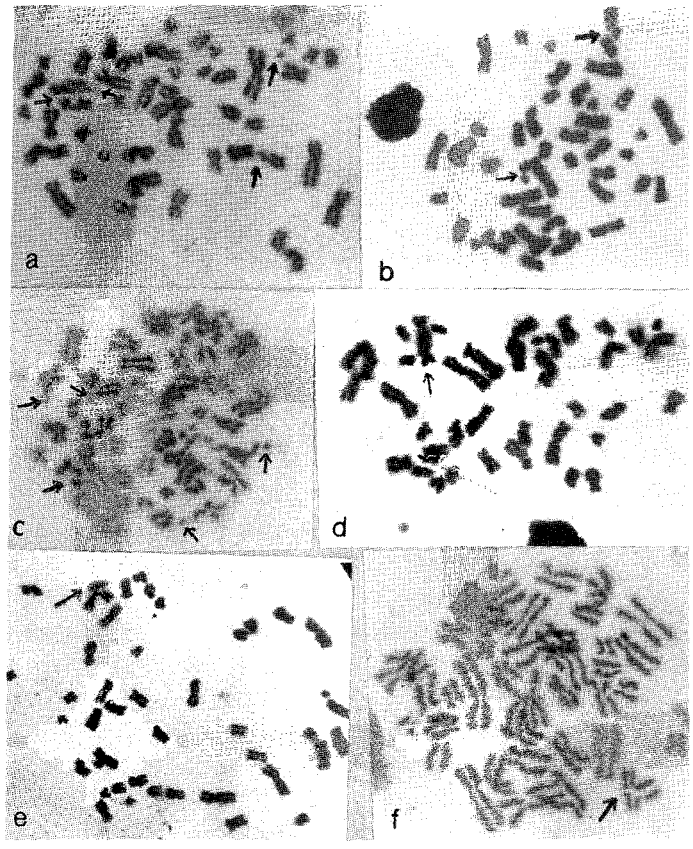

Fig. 1. (a,b,c) Chromatid gaps and breaks; (d) dicentric; (e) quadriradial; and (f) triradial rearrangements in lymphocytes treated with stannic chloride.

al. (*16*) and Ghosh et al. (*18*) all reported a significant increase in aberrations and SCE frequencies with increasing age, whereas no age effect was seen for SCEs and chromosome aberrations in other reports (*19*). Yew and Johnson (*20*) have shown that UV-induced excision repair decreased with age in T and B lymphocytes. In the present study structural aberrations in control as well as in treated sets were more frequent in older individuals. This is similar with the previous report of Martin and Rademaker (*21*). Interindividual variability was frequently observed in both untreated and treated lymphocytes of both sexes. This is consistent with the earlier findings of Gebhart (22) in chemical induced chromosome aberrations.

The mode of action of stannic chloride is yet unclear. Tin (Sn_4) compounds readily combine with dithiol groups of protein and can form stable complexes with SH compounds (23). Again tin (Sn_4) can be bio-

methylated in the environment and possibly in the cell where it can cause toxicity to mammalian systems (*24*).

ACKNOWLEDGMENTS

The author wishes to thank A. K. Sharma and A. Sharma, at the Centre of Advanced Studies in Cell and Chromosome Research, Department of Botany, University of Calcutta, for valuable suggestions and the facilities provided, and also the Council of Scientific and Industrial Research, Government of India, for financial assistance.

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