Bull. Inst. Zool., Academia Sinica **29**(2): 121-125 (1990)

SHORT NOTE

INDUCTION OF SISTER CHROMATID EXCHANGES BY LEAD COMPOUNDS IN CHINESE HAMSTER OVARY CELLS

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(Accepted November 10, 1989)

Emily C. H. Tai and Te-Chang Lee (1990) Induction of sister chromatid exchanges by lead compounds in Chinese hamster ovary cells. *Bull. Inst. Zool., Academia Sinica* **29**(2): 121-125. Although an over exposure to lead causes severe human health problems, the carcinogenic potential of lead is still poorly defined. Four lead compounds, including water soluble $Pb(CH_3COO)_2$ and $PbCl_2$ and insoluble $Pb(CH_3COO)_4$ and PbO, were subjected to investigate their activity in inducing sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells. Statistical analysis shows that all four lead compounds significantly induce SCE in a dose-dependent manner. Our results indicate that the activity of lead in inducing SCE in CHO cells is independent on its oxidation status (i. e. divalent and tetravalent) and its water solubility. Therefore, more research on the impact of lead compounds to human health seems necessary.

Key words: Lead, Sister chromatid exchanges.

An over exposure to lead causes severe health problems mainly in the hematopoietic, nervous, gastrointestinal, and renal system. Although exposure to lead induces renal tumors in a variety of animal models has been reported (Stoner *et al.*, 1976; Tsuchiya, 1986), the epidemiological evidence is still inadequate to indicate the carcinogenic potential of lead in humans. Furthermore, contradictory results were obtained the genetic effects of lead in several *in vivo* and *in vitro* systems (Beek and Obe, 1974; Deknudt *et al.*, 1977; Gebhart, 1984; Zelikoff *et al.*, 1988). Since lead is a ubiquitious toxic pollutant in our environment, more concern on its threat to human health is necessary. Therefore, various lead compounds with different valents and salts are subjected to the genotoxicity tests, using sister chromatid exchange (SCE) analysis in Chinese hamster ovary (CHO) cells.

MATERIALS AND METHODS

Chemicals

All lead compounds were obtained from E. Merck (Darmstadt, F. R. G.). Chemicals for cell culture were purchased from GIBCO (Grand Island, NY). Solutions of lead compounds were freshly prepared by dissolving lead (II) acetate and lead (II) chloride in double distilled water, lead (IV) acetate in dimethyl sulfoxide, and lead (II) oxide in 1 N glacial acetic acid, and diluted into medium upon

use. The final concentrations of dimethyl sulfoxide and glacial acetic acid were 0.5% and 0.02%, respectively.

Cell culture

CHO K1 cells, obtained from American Type Culture Collection (Rockville, MD), were grown in McCoy's 5A medium supplemented with antibiotics (penicillin 100 units/ml and streptomycin 100 μg/ml), L-glutamine (0.03%) and heat-inactivated fetal calf serum (10%). The cultures were maintained at 37°C in a humidified incubator with 5% CO_2.

Sister chromatid exchanges

Log phase CHO K1 cells were treated for 4 h with sterilized lead salts in complete medium. The cultures were washed twice with phosphate buffered saline and incubated with bromodeoxyuridine at 20 μM for 22 h in the dark. Colcemid (0.2 μg/ml) was added and after 2 h mitotic cells were collected by shake-off technique. Following centrifugation, metaphase cells were treated with hypotonic 0.05% KCl for 6 min at room temperature, and fixed in freshly prepared

Table 1
Sister chromatid exchanges (SCEs) induced by lead compounds

Treatment	Concentration (μM)	Expt. I SCEs/cell[a]	Expt. I RI[b]	Expt. II SCE/cell	Expt. II RI
Control[d]	—	4.00±0.83	1.81	3.30±0.88	1.70
$Pb(CH_3COO)_2$	50	5.57±1.25	1.65	4.13±1.14	1.62
	100	7.00±1.20	1.68	5.70±1.09	1.66
	200	7.27±1.28	1.58	6.27±1.20	1.60
	400	8.23±1.22	1.61	7.03±1.03	1.64
	800	9.03±1.47	1.53	7.77±1.38	1.55
		$p<0.005$[c]		$p<0.005$	
Control[e]	—	5.20±1.18	1.61	4.40±1.19	1.70
$Pb(CH_3COO)_4$	1	6.43±1.10	1.60	3.90±0.88	1.55
	10	8.13±1.33	1.59	5.40±1.22	1.49
	100	9.50±1.50	1.49	6.77±1.38	1.36
	1,000	10.47±1.75	1.33	9.40±2.31	1.35
		$p<0.005$		$p<0.005$	
Control[d]	—	3.46±0.78	1.76	3.37±0.85	1.84
$PbCl_2$	1	4.43±0.86	1.67	5.03±1.08	1.62
	10	4.77±1.30	1.50	5.12±1.94	1.52
	100	6.80±2.51	1.38	7.25±1.36	1.35
	1,000	7.30±1.51	1.13	8.36±1.92	1.24
		$p<0.005$		$p<0.005$	
Control[f]	—	8.00±2.34	1.71	6.23±1.04	1.70
PbO	1	8.10±1.09	1.50	7.60±1.47	1.65
	10	9.00±1.55	1.61	7.90±1.80	1.67
	100	10.63±1.77	1.44	8.67±1.24	1.67
	1,000	12.53±1.69	1.55	9.87±1.63	1.54
		$p<0.005$		$p<0.005$	

a. No. SCEs per cell ± standard deviation.
b. Replication index calculated according to Schneider *et al.* (1981).
c. The probability of trend analysis.
d. Double distilled water.
e. 0.5% dimethyl sulfoxide.
f. 0.02% glacial acetic acid.

methanol/acetic acid (3:1) as described previously (Lee *et al.*, 1985a). Cells were dropped on clean slides, air dried and stained using a modified fluorescence plus Giemsa techniques (Jan *et al.*, 1982). At least 30 second-division (M2) cells were randomly sampled from each treatment to score SCE frequency. The significance of SCE induction was statistically analyzed by the methods described by Galloway *et al.* (1985) and Margolin *et al.* (1986).

RESULTS AND DISCUSSION

Lead compounds are not highly cytotoxic in CHO cells, since our preliminary result indicated that the survival rates were higher than 65% in cells treated with the highest dose of each compound used in this study. The activity of lead in inducing SCE was summarized in Table 1. The values of replication index indicate that cell-cycle kinetics was slightly retarded by lead compounds. These results are in agreement to the low cytotoxicity of lead compounds. However, all 4 lead compounds significantly induced SCE in CHO cells (Table 1). According to trend analysis, the induction of SCE by lead followed a dose-dependent manner (p values<0.005).

Among the 4 lead compounds studied, lead oxide and lead (IV) acetate are water insoluble and lead chloride and lead (II) acetate are moderately soluble. These results suggest that the induction of SCE by lead is not dependent on its water solubility, whereas the toxic and carcinogenic effects of metal compounds are frequently associated with their solubilities in water (Leonard and Lauwerys, 1980; Hansen and Stern, 1983). In contrast to our observation, Zelikoff *et al.* (1988) reported that both lead sulfide (insoluble) and lead nitrate (moderately soluble) failed to induce SCE in V79 cells (another Chinese hamster cell line). The ambiguous results in SCE induction by lead were also found in *in vivo* studies (Willems *et al.*, 1982; Grandjean *et al.*, 1983). Therefore, the activity of lead in inducing SCE may depend on cell type and conditions of treatment.

The oxidation status is another important factor determining the toxicity and carcinogenicity of metals, such as chromium (Sunderman, 1979) and arsenic (Lee *et al.*, 1985b). However, our data showed that divalent and tetravalent lead compounds have similar activity of inducing SCE in CHO cells.

Although controversial results were frequently reported from the studies on the induction of SCE and chromosomal aberrations by lead (Sharma and Talukder, 1987), lead compounds were found to be mutagenic at the hypoxanthine phosphoribosyltransferase locus in V79 cells (Zelikoff *et al.*, 1988) and also induce transformation in mouse embryo cells (Patierno *et al.*, 1988). These observation together with the present results strongly suggest that lead compounds may be classified as environmental contaminants with carcinogenic potential. Since lead is still used as anti-knock agents in internal combustion fuels in Taiwan and in many parts of the world, more work should be done to elucidate the impact of lead compounds on human health.

Acknowledgements: We thank Dr. T.C. Wang for his help on statistical analysis, and Dr. K.Y. Jan for his carefully reading this manuscript. Emily C.H. Tai (from Taipei First Girl High School) wishes to thank the National Science Council, Republic of China, for supporting the outstanding high school student training program.

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鉛化物誘引中國倉鼠卵巢細胞姊妹染色分體互換

戴欽賢　李德章

雖然過度接觸鉛化物對人類健康影響甚鉅，但是鉛化物是否會誘發癌症尚未定論。本研究乃利用中國倉鼠卵巢細胞探討四種鉛化物，包括水溶性的二價醋酸鉛及氯化鉛與不溶於水的四價醋酸鉛及氧化鉛，誘引姊妹染色分體互換的能力。統計分析顯示，這四種鉛化物均可顯著增加姊妹染色分體互換率並與劑量呈正相關。本結果亦顯示鉛化物之氧化狀態（即二價或四價）與對水之溶解度並不影響其對姊妹染色分體之誘引。因此鉛化物是否具致癌性急須更進一步的探討。