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Effects of inorganic and organic lead compounds on chromosomal length in human lymphocytes

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In vitro exposure of human lymphocyte cultures to spindle inhibitors reduce the average chromosome length[1,2]. In this report chromosome length measurements were used for indirect but quantitative evaluation of the effects of inorganic and organic lead compounds on spindle function. The data indicate that organic compounds are much more powerful spindle inhibitors than inorganic lead compounds, almost as potent as colchicine. Occupational exposure to organic lead compounds may result in partial spindle inhibition, leading to a high probability of nondisjunction.

Keywords: Organic lead compounds, chromosomal length, spindle function

INTRODUCTION

Human exposures to lead mainly originate from anthropogenic sources,[3] and excess exposures may cause genotoxic effects.[4] In general, inorganic lead compounds constitute the main part of human lead exposures, but organolead compounds also occur, because tetramethyl- and tetrathyllead are widely used as gasoline additives.[5]

In the body, tetraethyllead compounds are dealkylated to the more toxic trialkyllead compounds.[6] Ahlberg et al.[7] demonstrated that triorganolead compounds are strong spindle inhibitors in *Drosophila* and *Allium* cepa. This finding suggests that human exposure to these compounds during production, distribution and handling of leaded gasoline might increase the rate of meiotic and mitotic nondisjunction. Spindle inhibitors, such as parafluorophenylalanin, colchicin, Cd^{++}, Hg^{++} and CH_3Hg^+, reduce the average chromosomal length due to the increased duration of the metaphase stage.[1,2,8] Such induction of 'supercontraction' is indicative of spindle inhibition. The present study compares the effect of inorganic and organic lead compounds on chromosomal length.

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MATERIALS AND METHODS

$(CH_3CH_2)_3PbCl$ and $(CH_3)_3PbCl$ were synthesized by Dr Torben Nielsen, Risø National Laboratory, Denmark, and kindly provided to us. Other chemicals were of analytical purity. Human peripheral blood was obtained from healthy donors. The procedures followed have been previously described.[1,2] In short, lymphocyte cultures (0.5 cm^3 blood in Eagle's MEM with 10% fetal calf serum, 5 i.u. cm^{-3} heparin, 50 $\mu g\ cm^{-3}$ gentamycin and 0.2 cm^3 phytohemagglutinin in a total volume of 9 cm^3) were incubated at 37.5°C for 72 h. One cm^3 of sterile filtered solutions of metal compounds in H_2O were added to the cultures as indicated in the table. Control cultures received 1 cm^3 H_2O. After hypotonic treatment with 0.075 mol dm^{-3} KCl and fixation with acetic acid-methanol (1:3), flame dried and air dried chromosome slides were prepared, stained in Giemsa and mounted with Eukitt. From 100 randomly selected metaphases on flame dried slides the length of one chromosome no. 1 was determined in intervals of 1 μm with a calibrated measuring eye piece. Grouped data from different cultures were compared using Kolmogorov–Smirnov's two-tailed two-sample test.[9] The significance limit chosen was $p = 0.01$. Since $N = 100$ in all samples, this limit equals a D-value of 0.23.

Table 1 Length frequencies (LF), average length ($\bar{x}$) and standard deviations (Sd) for chromosome no. 1 from human lymphoid cells scored as grouped data in intervals of 1 μm. In all samples, the sum of observations $N = 100$. For D values > 0.23 the probability that the sample is drawn from a population with the same length distribution as the control is < 0.01, according to the Kolmogorov–Smirnov two-tailed test for two independent samples. COL = colchicin, Et_3Pb^+ = triethyllead, Me_3Pb^+ = trimethyllead.

Exposure conc, mol dm^{-3}			COL 10^{-5}	COL 10^{-5}	Et_3Pb^+ 10^{-6}	Et_3Pb^+ 10^{-5}	Et_3Pb^+ 10^{-4}	Et_3Pb^+ 10^{-6}	Et_3Pb^+ 10^{-5}	Et_3Pb^+ 10^{-4}	Me_3Pb^+ 10^{-6}	Me_3Pb^+ 10^{-5}	Me_3Pb^+ 10^{-4}	Me_3Pb^+ 10^{-6}	Me_3Pb^+ 10^{-5}	Me_3Pb^+ 10^{-4}
time, h		Control	1	4	1	1	4	4	4	1	1	1	4	4	4	4
Length	2–3														5	
interval,	3–4							1	3			2		6	8	
μm	4–5		1					3	15			5		12	9	
	5–6	3	1	9	6	3		8	33		2	12		10	8	
	6–7	1	7	15	4	10		13	22			18		6	11	
	7–8	3	10	12	13	7		10	8		1	11		10	13	
	8–9	5	10	16	13	22		15	11		3	13		9	19	
	9–10	10	17	9	18	18		11	6		5	11		10	11	
	10 11	6	19	13	17	10		10	2		9	5		5	8	
	11–12	9	11	9	12	8		8			14	4		6	4	
	12–13	5	3	3	3	8		6			7	2		5	1	
	13–14	10	2	4	4	8		2			13	5		4	1	
	14–15	7	5	2	2	4		2			9	6		7	1	
	15–16	11	4	1	2	1		6			9	2		2	1	
	16–17	4		1	3			3			10			2		
	17–18	7	2	1	2			1			7	2		4		
	18–19	6	1			1		1			1					
	19–20	6	2	2							4					
	20–21	6	1								5					
	21–22		2		1									1		
	22–23			1							1	1				
	23–24		2	1												
	24–25	1		1												
	25–26											1		1		
$\bar{x}$		13.83	11.05	9.78	10.12	9.88	[b]	9.60	6.34	[b]	13.89	9.06	[b]	9.36	7.46	[b]
Sd		4.14	3.91	3.85	2.94	2.52		3.32	1.61		3.46	4.10		4.40	2.75	
D			0.39[a]	0.46[a]	0.46[a]	0.44[a]		0.43[a]	0.80[a]		0.11	0.50[a]		0.41[a]	0.64[a]	

[a] Statistically significant difference from control.
[b] No result due to the toxicity of the treatment

RESULTS

Table 1 shows that in vitro exposure of human lymphocyte cultures to organolead compounds for 1 or 4 h induces 'supercontraction' as visualized by reduced chromosomal lengths in treated cultures when compared to untreated control cultures. Positive control cultures exposed to 10^{-5} mol dm^{-3} colchicin also exhibited reduced average chromosomal length, which is in agreement with our earlier results.[1,2]

In Table 2, the time-relationship for induction of supercontraction by $(CH_3CH_2)_3Pb^+$ is shown. The threshold is about 10^{-6} mol dm^{-3} for all culture times between 2 and 44 h. At increasing doses, shorter average chromosomal length is observed and, the chromosomal length is further decreased with increasing exposure time.

These results were further elaborated, and dose-response relationships for chromosome length reduction induced by 2.5 h exposure to Pb^{2+}, $(CH_3CH_2)_3Pb^+$ or colchicin measured in the experiment shown in Table 3. The combined data show that organic lead compounds have a much lower threshold (about 10^{-6} mol dm^{-3}) for induction of supercontraction than the Pb^{2+} ion (about 10^{-3} mol dm^{-3}). Thus, the threshold for triethyllead is almost as low as that for colchicin (about 3×10^{-7} mol dm^{-3}).

Table 2 Effects of $(CH_3CH_2)_3Pb^+$ on chromosome length

Exposure (h)	Concentration (mol dm^{-3})	$\bar{x}$	Sd	D
Control		10.10	2.38	
2	10^{-7}	10.16	2.10	0.09
2	10^{-6}	8.52	2.39	0.32[a]
2	10^{-5}	6.90	1.59	0.63[a]
2	10^{-4}	[b]		
4	10^{-7}	10.19	2.21	0.06
4	10^{-6}	9.18	2.57	0.11
4	10^{-5}	6.09	2.16	0.72[a]
4	10^{-4}	[b]		
6	10^{-7}	9.99	2.27	0.05
6	10^{-6}	8.79	2.86	0.25[a]
6	10^{-5}	5.84	2.32	0.71[a]
6	10^{-4}	[b]		
20	10^{-7}	10.51	2.51	0.15
20	10^{-6}	7.72	2.48	0.37[a]
20	10^{-5}	4.90	2.02	0.84[a]
20	10^{-4}	[b]		
44	10^{-7}	9.90	2.27	0.09
44	10^{-6}	7.91	2.43	0.35[a]
44	10^{-5}	[b]		
44	10^{-4}	[b]		

[a]Statistically significant difference from control
[b]No result due to the toxicity of the treatment

Table 3 Effects of inorganic and organic lead compounds and colchicin on average chromosome length. Exposure was 2.5 h

Treatment	Concentration (mol dm^{-3})	$\bar{x}$	Sd	D
Control	—	11.71	2.48	—
Pb^{++}	3×10^{-6}	11.37	3.05	0.07
	10^{-5}	11.14	2.63	0.12
	3×10^{-5}	10.36	2.06	0.26[a]
	10^{-4}	11.50	2.97	0.10
	3×10^{-4}	11.99	2.98	0.12
	10^{-3}	8.93	2.65	0.39[a]
$(CH_3CH_2)_3Pb^+$	10^{-7}	11.09	2.71	0.10
	3×10^{-7}	11.25	2.78	0.07
	10^{-6}	10.34	3.09	0.20
	3×10^{-6}	7.48	2.00	0.65[a]
	10^{-5}	6.55	1.62	0.78[a]
	3×10^{-5}	[b]		
Colchicin	10^{-7}	11.68	2.75	0.04
	3×10^{-7}	9.21	2.70	0.43[a]
	10^{-6}	10.03	3.04	0.30[a]
	3×10^{-6}	8.56	2.97	0.51[a]
	10^{-5}	9.13	2.79	0.41[a]
	3×10^{-5}	10.26	3.07	0.45[a]
	10^{-4}	9.09	2.64	0.45[a]

[a]Statistically significant difference from control
[b]No result due to the toxicity of the treatment

Microscopic examination of air dried slides showed that at concentrations of $(CH_3CH_2)_3Pb^+$ well below the threshold value the spindle was still present, while typical C-mitoses were observed at concentrations at 10^{-6} mol dm^{-3} or higher concentrations. Figure 1 shows partial spindle destruction after 2.5 h exposure to 10^{-6} mol dm^{-3} $(CH_3CH_2)_3Pb^+$ and a typical C-mitosis after 2.5 h exposure to 3×10^{-6} mol dm^{-3} $(CH_3CH_2)_3Pb^+$.

DISCUSSION

Collectively the data presented here are strongly indicative of organic lead compounds being much more powerful spindle inhibitors than inorganic lead compounds, almost as potent as colchicin.

The kinetics of spindle inhibition induced by $(CH_3CH_2)_3Pb^+$ differs from that of colchicin induced spindle inhibition. While colchicin has a very sharp threshold, below which no effect is

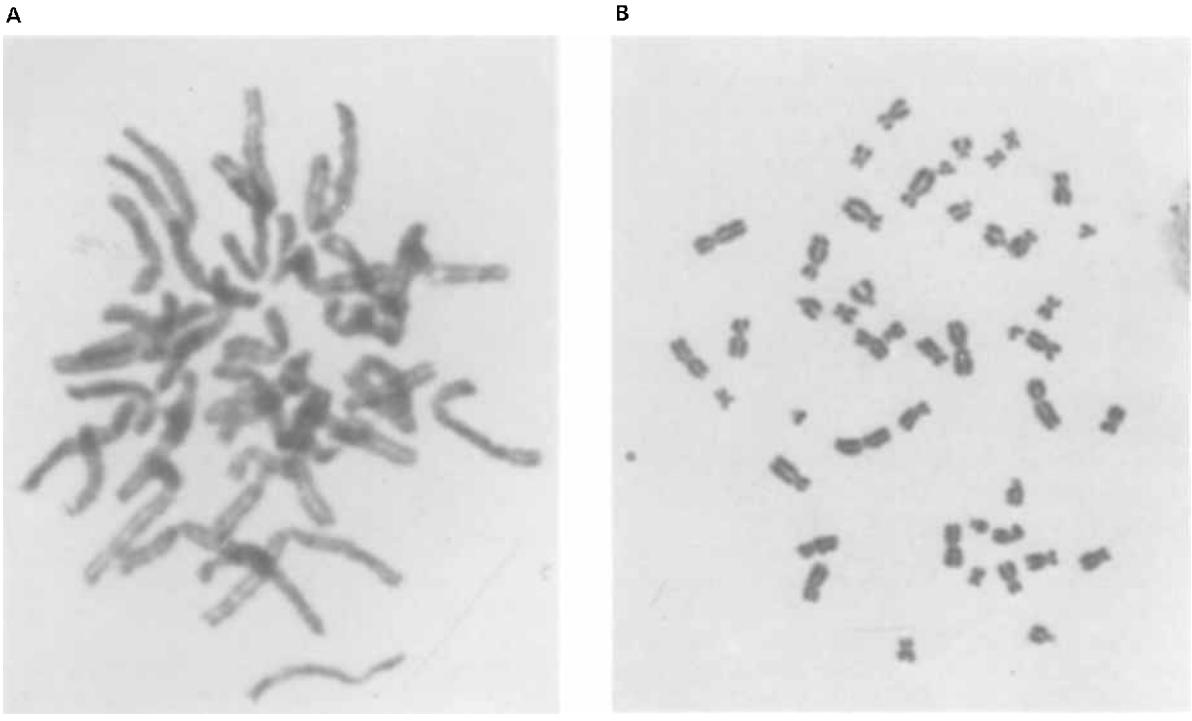

Figure 1 A: Partial destruction of the spindle after 2.5 h exposure to 10^{-6} md dm^{-3} $(CH_3CH_2)_3Pb^+$. B: C-mitosis. 2.5 h exposure to 3×10^{-6} mol dm^{-3} $(CH_3CH_2)_3Pb^+$.

observed and above which complete spindle inhibition or supercontraction is rapidly achieved (cf. Table 3),[10] $(CH_3CH_2)_3Pb^+$ induces a gradual reduction in average chromosomal length at increasing concentration. This observation suggests, that at concentrations around the threshold, partial spindle inhibition may take place in some mitotic or meiotic cells, leading to a high probability of nondisjunction.

In vitro studies have shown that trialkyllead compounds may inhibit cell division and result in the formation of multinuclear giant cells.[11] Spindle inhibition is likely to be involved. Thus, in vitro assembly of cerebral microtubules was gradually inhibited by $(CH_3CH_2)_3Pb^+$ concentrations from 10^{-6} mol dm^{-3} and upwards,[12] while inhibition was detected with 10^{-4} mol dm^{-3} $(CH_3)_3Pb^+$ but not with Pb^{2+} concentrations up to 6.5×10^{-4} mol dm^{-3}.[12]

Chemically, triakyllead compounds would be expected to show some similarities to monoalkylmercury compounds, such as CH_3Hg^+.[6] With regard to induction of chromosomal supercontraction, CH_3Hg^+ is also a potent spindle inhibitor and induces chromosomal supercontraction, with thresholds of action close to that of colchicin.[2,10,14] In the case of organic mercury compounds, the possibility that low level exposure might induce chromosomal anomalies led to cytogenetic investigations of people who were exposed either dietary[15,16] or occupationally[17] to different mercury compounds. These investigations clearly demonstrated that organic mercury compounds can induce aneuploidy and chromosome aberrations in the peripheral lymphocytes of exposed individuals.

To our knowledge, cytogenetic investigations of organolead exposed individuals have not been performed. Although the use of tetraalkyllead as octane booster is being phased out in several countries, large population groups are still exposed to organolead compounds. Cytogenetic investigations (SCE-test, analysis of ploidy and structural chromosome aberrations) should therefore be initiated with workers exposed to organic lead compounds. The fact that laboratory experiments indicate that organolead compounds are far more potent genotoxic agents than are inorganic lead compounds would suggest that this possibility be assessed.

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