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CHROMOSOME STUDIES IN HUMAN LYMPHOCYTES AFTER IN VITRO EXPOSURE TO METAL SALTS

Gh. DEKNUDT* and M. DEMINATTI**

* *Mammalian Genetics Laboratory, Department of Radiobiology, S.C.K.-C.E.N., B-2400 Mol (Belgium) and* ** *Laboratoire de Cytogénétique, Faculté de Médecine, 1 Place de Verdun, 59045 Lille Cédex (France)*

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SUMMARY

The toxic concentration of different heavy metal salts was determined in normal stimulated human lymphocyte cultures and was found to be 3×10^{-3}, 1×10^{-2} and 5×10^{-4} for zinc chloride, lead acetate and cadmium chloride respectively. Furthermore 3 subtoxic doses of each salt (2, 10 and 100 times less than the toxic dose) were added to 48- and 72-h cultures at 0 h and 24 h after initiation. Chromosome preparations were made and 100 well spread metaphases from each culture were analysed for the presence of numerical and structural aberrations. The most common aberration found for all tested metal salts was the occurrence of chromosome fragments. Dicentric chromosomes were only recorded in lymphocyte cultures treated with the lowest concentration of zinc chloride (3×10^{-5} M) added at time 0, regardless whether the cultures were fixed after 48 or 72 h.

INTRODUCTION

Investigations on workers in the zinc industry, who had been exposed simultaneously to several metals, have shown the occurrence of severe chromosome aberrations in lymphocytes of peripheral blood [3,7,8]. The principal metal components of zinc ores are, in decreasing order of importance, zinc, lead and cadmium mostly in their form of sulphides known as blende or sphalerite, galene and greenockite respectively. Since individuals, who are exposed exclusively to one of these metals, can not be found, it is impossible to decide which element was responsible for the abnormalities found in the lymphocytes of the workers of the zinc industry. Therefore, we have investigated the action on chromosomes of the 3 metals added separately to cultures of human lymphocytes.

MATERIAL AND METHODS

Human lymphocytes from the same healthy donor were cultured for 48 (first cellular division) or 72 h (second cellular division) in Ham's F 10 medium [14] to which different concentrations of zinc chloride, lead acetate or cadmium chloride had been added. The concentration inhibiting mitotic activity was found to be 3×10^{-3}, 1×10^{-2} and 5×10^{-4} M for zinc chloride, lead acetate and cadmium chloride respectively and 3 subtoxic doses of each salt (2, 10 and 100 times less than the toxic dose), as shown in Table I, were added to 48 and 72-h cultures at 0 h and 24 h after initiation. Chromosome preparations were made according to a method described previously [7] and 100 well spread metaphases from each culture were analysed for the presence of numerical and structural aberrations.

RESULTS

The mitotic index decreases for each salt tested with increasing salt concentration. The cultures treated with the highest subtoxic concentration of each salt were unsuitable for an adequate chromosome analysis because the mitotic index was too small and/or the chromosomes were too contracted. Tables II, III and IV display, for the different salt concentrations, duration of culture and time of application with the results of cytological analysis. Only gaps with can not be considered as true discontinuities of the chromosome structure [5] were recorded in the control cultures. The incidence of gaps as well as the yield of aneuploid cells was apparently not affected by the treatment with the metal salts. There were more chromosome fragments in all treated groups compared to controls especially in the cultures harvested after 48 h, but this difference was not significant. Severe aberrations such as dicentric chromosomes were recorded only in lymphocyte cultures treated with the lowest concentration of zinc chloride ($3 \times$

TABLE I

EFFECTS AND DOSES OF METAL SALTS ADMINISTERED TO HUMAN LYMPHOCYTE CULTURES

Observations	Effects observed	Doses of salts studied (molar)		
		Zinc chloride	Lead acetate	Cadmium chloride
Capacity of cell division	No cell division	3×10^{-3}	1×10^{-2}	5×10^{-4}
	Sufficient mitotic index	3×10^{-4}	1×10^{-3}	5×10^{-5}
Aspect and number of mitoses	Cells not suitable for analysis	1.5×10^{-3}	0.5×10^{-2}	2.5×10^{-4}
	Cells suitable for analysis	3×10^{-4}	1×10^{-3}	5×10^{-5}
		3×10^{-5}	1×10^{-4}	5×10^{-6}

TABLE II

CHROMOSOME ANALYSIS OF LEUCOCYTE CULTURES CONTAMINATED BY LEAD ACETATE

Duration of the culture (h)	Dose of salt administered (M)	Interval of time between initiation of the culture and salt administration (h)	Number of cells analysed	Aneuploid cells	Structural aberrations	Type and number of structural aberrations			
						Chromatid aberrations		Chromosome aberrations	
						Gap	Break	Gap	Fragment
48	0	—	100	2	2	1		1	
	1×10^{-3}	0	100	3	3		1		2
		24	100	4	4	2		1	1
	1×10^{-4}	0	100	3	0				
		24	100	0	1			1	
72	0	—	100	1	1	1			
	1×10^{-3}	0	100	2	0				
		24	100	4	0				
	1×10^{-4}	0	100	4	1	1			
		24	100	5	0				

TABLE III

CHROMOSOME ANALYSIS OF LEUCOCYTE CULTURES CONTAMINATED BY CADMIUM CHLORIDE

Duration of the culture (h)	Dose of salt administered (M)	Interval of time between initiation of the culture and salt administration (h)	Number of cells analysed	Aneuploid cells	Structural aberrations	Type and number of structural aberrations	
						Chromatid aberrations Gap	Chromosome aberrations Fragment
48	0	—	100	3	2	2	
	5×10^{-5}	0	100	1	2	2	
		24	100	1	4	4	
	5×10^{-6}	0	100	5	2	2	
		24	100	0	2	1	1
72	0	—	100	1	1	1	
	5×10^{-5}	0	100	3	1	1	
		24	100	2	4	3	1
	5×10^{-6}	0	100	3	0		
		24	100	1	3	2	1

TABLE IV

CHROMOSOME ANALYSIS OF LEUCOCYTE CULTURES CONTAMINATED BY ZINC CHLORIDE

Duration of the culture (h)	Dose of salt administered (M)	Interval of time between initiation of the culture and salt administration (h)	Number of cells analysed	Numerical aberrations		Structural aberrations	Type and number of structural aberrations			
				Aneuploid cells	Cells in endoreduplication		Chromatid aberrations	Chromosome aberrations		
							Gap	Gap	Fragment	Dicentric
48	0	—	100	3	0	1	1			
	3×10^{-4}	0	100	1	0	2	2			
		24	100	4	0	4	2		2	
	3×10^{-5}	0	100	4	0	3	3			1
		24	100	4	0	4	2		2	
72	0	—	100	2	0	3	3			
	3×10^{-4}	0	100	3	0	0				
		24	100	6	0	4	3		1	
	3×10^{-5}	0	100	4	0	5	3			2
		24	100	2	1	3	2	1		

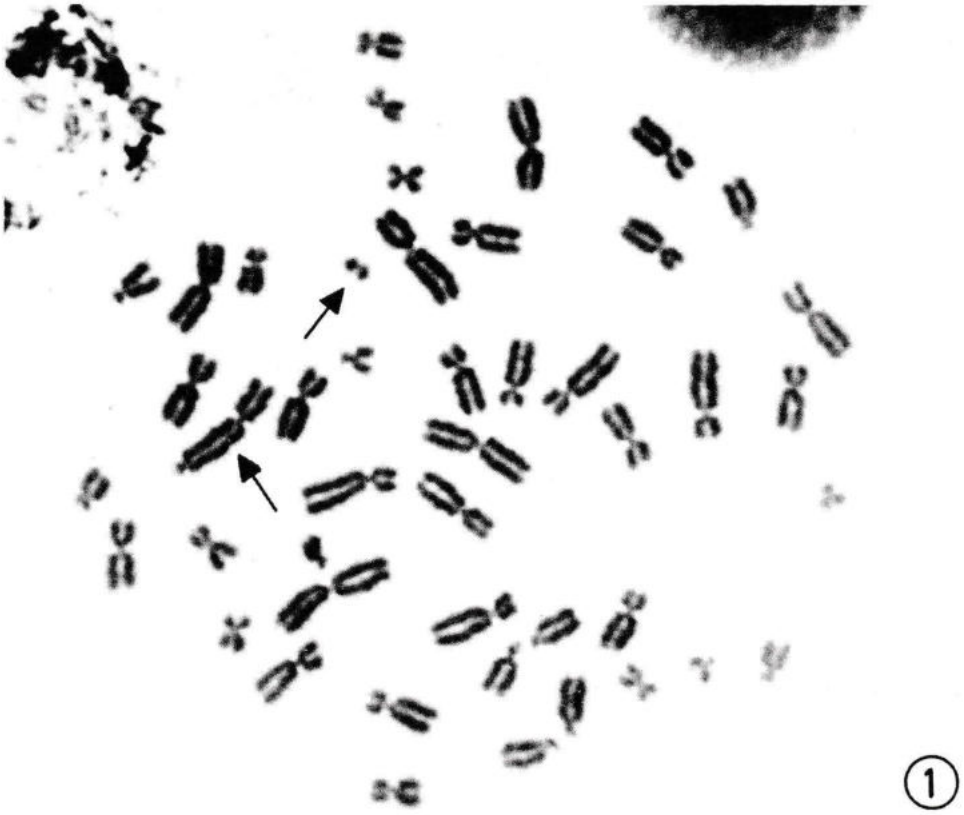

Fig. 1. Cell with dicentric and fragment (arrows).

10^{-5} M) added at time 0, regardless whether the cultures were fixed after 48 or 72 h. As expected [5] the dicentric chromsome observed in a 48-h culture was associated with an acentric fragment (Fig. 1). Karyotyping the abnormal metaphase demonstrated that 1 chromosome from the A group and 1 of the G group were involved in the dicentric structure. Since both anomalies result from the same event the fragment was not taken in consideration in Table IV. The lack of fragment in the cells showing dicentric chromosomes after a 72-h culture time confirms that the aberrant cells had proceeded through at least 1 mitotic division after treatment [5]. Analysis of the karyotype of the abnormal cells showed that one dicentric resulted from the association of 2 chromosomes from the B group (Fig. 2) and the second one from the association of a chromosome from the F and G groups. The chi-square analysis did not show that the incidence of dicentrics in the cells treated with zinc were significant when tested against the controls only. If, however, controls combined with the cadmium and lead exposed were tested against all zinc treated lymphocytes the difference was highly significant ($P = 0.004$).

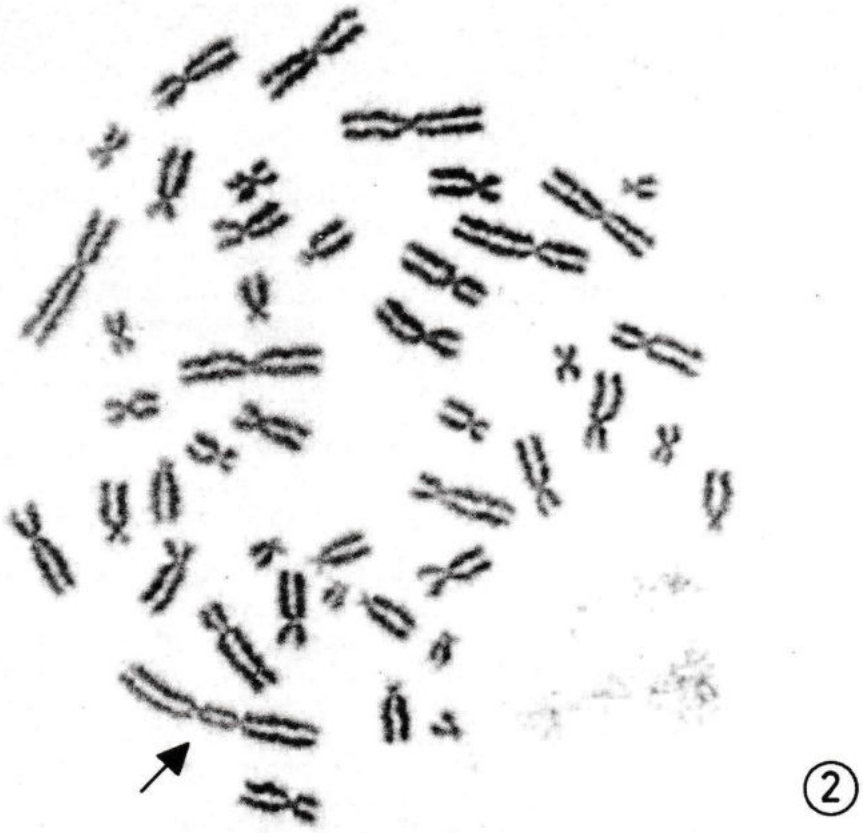

Fig. 2. Cell with dicentric without fragment (arrow).

DISCUSSION

The negative observations on the ability of lead to induce 'in vitro' chromosome aberrations confirm previous reports on chinese hamster cells [1] or on human leucocytes [4,23]. The increase in the yield of 'light' aberrations such as gaps and fragments reported by Beek and Obe [4] and Obe and Sperling [18] may have been due to their longer culture time of much more than 48 h.

The present results on cadmium are in agreement with the observations of Paton and Allison [21] on human lymphocytes, the dose of cadmium chloride found to be toxic in their experiments (5×10^{-8} M) being, however, much lower than the present one (5×10^{-4} M). The high incidence of dicentric chromosomes reported by Shiraishi et al. [24] in human leucocyte cultures contaminated with cadmium sulfide, is unexplained since the anion composition should have no influence [12]. It may have resulted from a misinterpretation of the cytological figures due to the poor quality of the material as is suggested by the illustrations in their paper. 'Light' chromo-

some anomalies were, however, observed in root tips of Vicia faba grown in a cadmium nitrate solution [19], in a Chinese hamster cell line treated with cadmium sulphate [2] and in Chinese hamster ovary cells treated with cadmium chloride [6]. Some undefined cytological changes were found by Jamagami et al. [27] in fibroblast cultures treated with cadmium chloride but not by Paton and Allison [21].

In contrast to lead and cadmium, zinc is essential for the function and/or structure of several enzymes and is present in DNA bound to the phosphate groups [25]. Furthermore, zinc compounds can cause lymphocyte transformation in vitro [17] similar to PHA stimulation and also is involved in different stages of cell division [11]. Our results suggest that high levels of zinc (3×10^{-3} M) are cytotoxic, a value somewhat higher than that reported by Kirchner and Ruhl [17] (3×10^{-4} M) and that lower concentration (3×10^{-5} M) can cause severe chromosome aberrations (dicentrics). It should be noted that this type of aberration is different from the disturbance in differentiation of chromosomal matrix and in spiralisation of chromosomes found in Vicia faba after zinc treatment [15,16] but chromosomal despiralisation and dicentric chromosomes were seen in the cultured lymphocytes of workers of the zinc industry [7,8]. As Evans [10] has stated, small lymphocytes from peripheral blood are a most useful system to investigate human mutagenesis after prolonged 'in vivo' exposure as well as a model for 'in vitro' studies. 'In vitro' studies in contrast to the 'in vivo' ones permit one to choose proper controls and to test each potential factor separately although they can of course not simulate long term exposure or possible intervention of metabolism in other tissues. Our experiments suggest that, at least at the concentrations tested, only zinc but not lead or cadmium 'per se' may be mutagenic. Although the difference was not statistically significant when all zinc treated groups were compared with their respective controls, it was so when the lead and cadmium treated cultures were included with the controls. This procedure may be accepted when it is remembered that all cultures came from the blood of the same person. Zinc could thus be the agent responsible for the chromosome damage observed earlier in workers professionally exposed from the zinc industry. It must be pointed out, however, that the concentration of zinc, lead and cadmium used in the present experiments are extremely high representing up to 1000, 3300 and 2700 times the respective concentrations reported in the blood of people professionally contaminated by heavy metals and which have been shown to be 22.4 μg% for zinc [22]; 68.4 μg% for lead [7,8] and 2.3 μg% for cadmium [8].

Other possibilities in heavy metal mutagenicity in particular nutritional or synergistic factors should, however, also be considered. Earlier [9], we found calcium deficiency in monkeys to be an important co-factor for the induction of serious abnormalities by lead, while with sufficient calcium available only 'light' aberrations were produced. It should be mentioned that zinc may protect against the toxic effects of cadmium [20,26] and that lead and zinc can interact at porphyrin synthesis, where the zinc containing enzyme [13] (aminolevulinic acid dehydratase) is inhibited by lead. Thus, it

can not be decided at the present time whether zinc 'per se' or nutrition synergistic factors are responsible for the severe aberrations found in workers from the zinc industry.

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