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Interaction between two group IV metals—lead and zirconium—in bone marrow cells of *Mus musculus in vivo*

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The interaction between two group IV metals, the highly toxic lead and the relatively inactive and low toxic zirconium, was studied in the bone marrow chromosomes of *Mus musculus in vivo*. Low and high doses of zirconium oxychloride were fed orally to the experimental mice (i) 2 h before, (ii) 2 h after or (iii) together with different doses of lead nitrate. Protection against lead-induced clastogenicity was observed only when the lower dose of zirconium was administered prior to lead. All other combinations gave an additive or synergistic effect as was seen by significant increases in the frequencies of chromosomal aberrations.

Keywords: lead, zirconium, clastogenicity, interaction, mouse bone marrow cells

Introduction

Interactions between metals may greatly influence their biological effects and are currently receiving increasing attention while assessing their effects on living systems (Sharma & Talukder 1987). The majority of these studies have, however, been restricted to biochemical and histochemical aspects, and information on the interaction between metals in modifying clastogenic effects is very scant. The present work was carried out to assess any modifications in clastogenicity following interaction between two group IV metals of the periodic table, i.e. zirconium and lead.

The toxicity of lead as a clinical entity in man and in animals has been known for centuries. The clastogenic effects of this non-essential heavy metal has been well established (Dhir *et al.* 1985a, Omoto *et al.* 1988, Chakraborty *et al.* 1989, Rasheva 1990). It is known to react readily with organic and inorganic molecules, and several metals have been shown to ameliorate the toxicity of lead either by precipitating the metal or by releasing bound lead from tissues (Dhir *et al.* 1985b).

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However, the relatively inactive zirconium shows low biological toxicity with the capacity to form stable complexes (Browning 1969, De Longeas *et al.* 1983). It is ubiquitous in the biosphere and is present in living organisms, often in appreciable quantities. This non-essential, non-toxic element is known to behave as an essential trace element and is considered a natural contaminant with no considerable physiological effects (Schroeder & Balassa 1966). However, with increasing exposure to this element following radioactive fallout (Cuddihy *et al.* 1989) and the increasing application of zirconium compounds in several industries such as electronics, textiles, ceramics, tanneries, glass industry—many of which it shares with lead—has enhanced interest in the study of its effects on living organisms (Venugopal & Luckey 1978). Cytotoxicity and mitogenicity of zirconium oxychloride has been reported following oral exposure in mice (Ghosh *et al.* 1991), while fish and algal assays conferred low but genuine zirconium toxicity (Couture *et al.* 1989). On the other hand, zirconium salts also have pharmaceutical and protective uses and have been reported to induce immunologic tolerance against metals such as aluminium (Epstein 1971).

Thus, in the present investigation, the interaction between zirconium and lead was studied in mouse bone marrow chromosomes *in vivo*.

Materials and methods

Laboratory bred Swiss albino mice *Mus musculus* 7–8 weeks old, weighing 25 ± 3 g and maintained under standard laboratory conditions at temperature 22 ± 2 °C, relative humidity 50 ± 10% and a 12 h photoperiod were housed in groups of six in polycarbonated cages. Commercial pellet diet (Lipton, India) and deionized water were provided *ad libitum*.

Lead nitrate [$Pb(NO_3)_2$, molecular weight 331.20, E. Merck, India] and zirconium oxychloride [$ZrOCl_2$, molecular weight 322.25, Loba Chemicals, India], the two metal salts used, were dissolved in deinoized water to make concentrations of 5 and 10 mg kg^{-1} body weight of $Pb(NO_3)_2$, and 110 and 220 mg kg^{-1} body weight of $ZrOCl_2$. A low and a high dose of each metal salt, based as proportions of LD_{50}, i.e. 1/80 and 1/40 LD_{50} of $Pb(NO_3)_2$ and 1/40 and 1/20 LD_{50} of $ZrOCl_2$, were selected (Dhir *et al.* 1990, Ghosh *et al.* 1990).

Different concentrations of zirconium salt were administered (i) 2 h before, (ii) 2 h after or (iii) simultaneously with different concentrations of lead nitrate. Both metal salts were administered by oral gavage. The treatment schedule is presented in Table 1.

Lead and zirconium control groups were maintained by single oral administrations of lead nitratge or zirconium oxychloride solutions, respectively. Deionized water was fed to maintain negative (vehicle) control and mitomycin C (3.5 mg kg^{-1} body weight) (Sigma, St Louis, MO, USA) was administered intraperitoneally as positive (reference) control.

Six animals were used per set of treatment/control. The mice were killed 24 h following the last treatment dose. At 2 h prior to sacrifice, each animal was injected intraperitoneally with 0.04% colchicine (0.01 ml kg^{-1} body weight) (Sisco Laboratories, India). The animals were killed by cervical dislocation and femoral bone marrow was flushed in 0.075 M potassium chloride, incubated at 37 °C for 20 min, repelleted and fixed in cold 1:3 glacial acetic acid:ethanol. Slides were prepared by flame drying and stained in Giemsa (E. Merck, India) (Preston *et al.* 1987).

Slides were coded and scored blind for chromosomal abnormalities to eliminate observer bias. Fifty well-scattered and uniformly stained metaphase plates were scanned from each animal, making a set of 300 metaphases studied per set. For computing breaks per cell (CA/cell), chromatid and chromosome breaks and chromosomal rearrangements were counted as one break irrespective of

Table 1. Experimental protocol

Experimental sets	Treatment chemicals	Concentrations			
		mg kg^{-1} body weight		Fractions of LD_{50}	
		$Pb(NO_3)_2$	$ZrOCl_2$	$Pb(NO_3)_2$	$ZrOCl_2$
Negative control	distilled water	–	–	–	–
Positive control	mitomycin C (3.5 mg kg^{-1} body weight)	–	–	–	–
PbL	$Pb(NO_3)$Control	5	–	1/80	–
PbH		10	–	1/40	–
ZrL	$ZrOCl_2$ Control	–	110	–	1/40
ZrH		–	220	–	1/20
PbL + ZrL	$ZrOCl_2$ + $Pb(NO_3)_2$	5	110	1/80	1/40
PbL + ZrH	given together	5	220	1/80	1/20
PbH + ZrL		10	110	1/40	1/40
PbH + ZrH		10	220	1/40	1/20
ZrL → PbL	$ZrOCl_2$ followed by	5	110	1/80	1/40
ZrH → PbL	$Pb(NO_3)_2$ after 2 h	5	220	1/80	1/20
ZrL → PbH		10	110	1/40	1/40
ZrH → PbH		10	220	1/40	1/20
PbL → ZrL	$Pb(NO_3)_2$ followed by	5	110	1/80	1/40
PbL → ZrH	$ZrOCl_2$ after 2 h	5	110	1/80	1/40
PbH → ZrL		10	110	1/40	1/40
PbH → ZrH		10	220	1/40	1/20

Six mice were used per each set.

the number of breakage events involved. Gaps were not included. In the count of damaged cells (DC), all cells with at least one aberration (excluding gaps) were included, see Table 2 (Tice *et al.* 1987).

Statistical analysis was carried out following the one-tailed trend test to determine if a dose-related response for chromosomal aberrations was obtained (Margolin *et al.* 1986). The level of significance was established at $P < 0.01$ to $P < 0.001$. Further analysis was carried out following one-way ANOVA (with replication) (Sokal & Rohlf 1973) and Duncan's multiple range test (Kotz & Johnson 1982) to compare the significances of difference, if any, amongst different experimental sets. The level of significance was established at an α of 0.05.

Results and discussion

Lead and zirconium induced a dose-dependent increase in chromosomal aberrations as was observed by significant P values ($P < 0.001$) following the one-tailed trend test. Treatment with the lower dose of zirconium (ZrL 110 mg kg^{-1} body weight) alone, showed no significant ($P < 0.05$) changes in the frequency of breaks per cell as well as the percentage of damaged cells when compared with the negative control values (Tables 3 & 4). Low toxicity of oral administration of zirconium compounds in animals is possibly due to their relatively low affinity for biological macromolecules, low solubility in the intestine or to the low pH of the intestine which favors olation of zirconium salts (Schroeder & Balassa 1966). The higher dose of zirconium (ZrH 220 mg kg^{-1} body weight), however, showed a significant ($P < 0.05$) increase in the frequency of chromosomal aberrations when compared with the negative control values and showed no significant difference with the lower dose (5 mg kg^{-1} body weight) of single lead treatment (PbL) (Tables 3 & 4).

In the combination sets, when the lower doses of both metals were administered (sets PbL and ZrL), a decrease in the frequency of chromosomal aberrations was observed when zirconium preceded lead treatment or vice versa compared with single lead treatment (sets ZrL → PbL and PbL → ZrL) (Table 2). This decrease however was not at a significant ($P < 0.05$) level (Tables 3 & 4). When the two metals were administered together (set PbL + ZrL), the toxic effect of lead was further enhanced (Table

Table 2. Effect of $Pb(NO_3)_2$ and $ZrOCl_2$ singly and in combination on bone marrow chromosomes of *M. musculus*

Experimental sets	CA/cell (excluding gaps)	DC (%) (excluding gaps)
Distilled water (Negative control)	0.02 ± 0.01	1.67 ± 0.61
Mitomycin C (Positive control)	0.24 ± 0.01	23.33 ± 0.94
PbL	0.06 ± 0.12	5.67 ± 1.20
PbH	0.09 ± 0.01	7.67 ± 0.95
Trend test P value	*** $P \leq 0.001$	*** $P \leq 0.001$
ZrL	0.04 ± 0.01	2.67 ± 0.67
ZrH	0.06 ± 0.01	5.00 ± 0.45
Trend test P value	** $P \leq 0.01$	*** $P \leq 0.001$
PbL + ZrL	0.09 ± 0.01	7.00 ± 0.86
PbL + ZrH	0.09 ± 0.01	7.33 ± 0.67
PbH + ZrL	0.12 ± 0.01	11.00 ± 0.86
PbH + ZrH	0.17 ± 0.02	14.00 ± 1.15
ZrL + PbL	0.05 ± 0.01	3.67 ± 0.33
ZrH + PbL	0.07 ± 0.01	5.67 ± 0.61
ZrL + PbH	0.07 ± 0.01	6.67 ± 0.67
ZrH + PbH	0.13 ± 0.01	9.67 ± 0.61
PbL + ZrL	0.06 ± 0.12	4.33 ± 0.61
PbL + ZrH	0.08 ± 0.01	6.67 ± 0.67
PbH + ZrL	0.13 ± 0.02	12.00 ± 1.63
PbH + ZrH	0.18 ± 0.01	11.00 ± 0.86

The values are expressed as mean ± SEM of six animals per set of treatment.

Table 3. Multiple comparison for breaks per cell following one-way ANOVA

Ordering the sample means:

Dose: 5 mg kg^{-1} body weight $Pb(NO_3)_2$ and 110 mg kg^{-1} body weight $ZrOCl_2$ (PbL and ZrL)

Neg C	Zr	Zr → Pb	Pb → Zr	Pb	Zr + Pb
0.02	0.04	0.05	0.06	0.06	0.09

Dose: 10 mg kg^{-1} body weight $Pb(NO_3)_2$ and 110 mg kg^{-1} body weight $ZrOCl_2$ (PbH and ZrL)

Neg C	Zr	Zr → Pb	Pb	Zr + Pb	Pb → Zr
0.02	0.04	0.07	0.09	0.12	0.13

Dose: 5 mg kg^{-1} body weight $Pb(NO_3)_2$ and 220 mg kg^{-1} body weight $ZrOCl_2$ (PbL and ZrH)

Neg C	Zr	Pb	Zr → Pb	Pb → Zr	Pb + Zr
0.02	0.06	0.06	0.07	0.08	0.09

Dose: 10 mg kg^{-1} body weight. $Pb(NO_3)_2$ and 220 mg kg^{-1} body weight $ZrOCl_2$ (PbH and ZrH)

Neg C	Zr	Pb	Zr → Pb	Zr + P	Pb → Zr
0.02	0.06	0.09	0.13	0.17	0.18

The blocks which are underlined together do not differ significantly (at level 5%) from each other.
Neg C, deionized water; Zr → Pb, $ZrOCl_2$ followed by $Pb(NO_3)_2$; Pb → Zr, $Pb(NO_3)_2$ followed by $ZrOCl_2$; Zr + Pb, $ZrOCl_2$ and $Pb(NO_3)_2$ administered together; Zr, $ZrOCl_2$ alone; Pb, $Pb(NO_3)_2$ alone.

Table 4. Multiple comparison for percentage of damaged cells followed one-way ANOVA

Ordering the sample means:

Dose: 5 mg kg^{-1} body weight. $Pb(NO_3)_2$ and 110 mg kg^{-1} body weight $ZrOCl_2$ (PbL and ZrL)

Neg C	Zr	Zr → Pb	Pb → Zr	Pb	Zr + Pb
1.67	2.67	3.67	4.33	5.67	7.00

Dose: 10 mg kg^{-1} body weight $Pb(NO_3)_2$ and 110 mg kg^{-1} body weight $ZrOCl_2$ (PbH and ZrL)

Neg C	Zr	Zr → Pb	Pb	Zr + Pb	Pb → Zr
1.67	2.67	6.67	7.67	11.00	12.00

Dose: 5 mg kg^{-1} body weight. $Pb(NO_3)_2$ and 220 mg kg^{-1} body weight $ZrOCl_2$ (PhH and ZrH)

Neg C	Zr	Zr → Pb	Pb	Pb → Zr	Zr + Pb
1.67	5.00	5.67	5.67	6.67	7.33

Dose: 10 mg kg^{-1} body weight. $Pb(NO_3)_2$ and 220 mg kg^{-1} body weight $ZrOCl_2$ (Pbh and ZrH)

Neg C	Zr	Pb	Zr → Pb	Zr + Pb	Pb → Zr
1.67	5.00	7.67	9.67	14.00	15.67

See footnotes to Table 3.

2) and the frequency of breaks per cell showed a synergistic effect (Table 3).

On increasing the concentration of lead to 10 mg kg^{-1} body weight (PbH), zirconium could reduce lead-induced clastogenicity when administered 2 h prior to lead (ZrL → PbH) (Table 2), but not to a significant level ($P < 0.05$) when compared with the single lead treatment (PbH) (Tables 3 & 4). When lead preceded zirconium treatment (PbH → ZrL) or when the two salts were administered together (PbH + ZrL), the frequency of chromosomal aberrations showed a marked increase when compared with the single metal treatments, indicating a synergistic effect (Tables 3 & 4).

When the higher dose of zirconium (ZrH 220 mg kg^{-1} body weight) was administered in combination with the lower dose of lead (PbL 5 mg kg^{-1} body weight), none of the combination sets showed significant changes from the single metal treatments in their frequencies of chromosomal aberrations (Table 4). The effect was, however, additive for breaks per cell when the two metals were administered together (Table 3).

The frequency of damaged cells as well as breaks per cell showed a sharp increase in all three combination sets compared with the single metal treatments, when the higher doses of both metals were administered to the mice (PbH and ZrH) (Table 2). Following statistical analysis, a significant synergistic effect was observed in all the three combination sets (Tables 3 & 4).

The results of this experiment thus showed that both the doses of zirconium, when fed 2 h prior to lead, could prevent enhancement in clastogenicity induced by a less toxic dose of lead. This protective effect was lost when the two metals were administered at the higher doses. Similar results were obtained with lead–selenium interactions in rat bone marrow chromosomes where it was suggested that preferential binding of selenium with the sites at which lead reacts possibly counteracted lead toxicity in chromosomes, when selenium was administered prior to lead (Chakraborty *et al.* 1987). Zirconium has also been reported to have a high affinity for free phosphate ions, forming insoluble precipitates and is thus known to hamper the co-precipitation of metals such as lead (Osanai *et al.* 1989).

When lead followed zirconium treatment or the two were fed simultaneously, the effect was additive or synergistic. Administration of lead nitrate in rats has been shown to increase glutathione content and the latter is known to contain some potential ligands for complex formation with metal ions (Kosower & Kosower 1976). The increased glutathione content induced by lead nitrate could facilitate the formation of stable complexes of zirconium with thiols and thereby storage of zirconium, as was observed in interactions between lead and mercury (Congiu *et al.* 1979).

The clastogenic effects of lead have been reported to depend on synergistic factors and is unlikely to result only from a greater availability of lead and is most likely due to an interaction at the level of the chromosome (Hickey *et al.* 1974).

As exposure to zirconium is increasing through its greater demand in various industries, and since both lead and zirconium are capable of crossing the blood–brain and placental barriers, the results of this present investigation necessitates further research on the interaction between these two elements.

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References

Browning E. 1969 In: *Toxicity of Industrial Metals*, 2nd edn. London: Butterworths.

Chakraborty I, Talukder G, Sharma A. 1987 Antagonistic and synergistic effects of lead and selenium in *Rattus norvegicus*. *Toxicol Lett* **37**, 21–26.

Chakraborty I, Sharma A, Talukder G. 1989 Genotoxic effects of lead in mammals. *The Nucleus* **32**, 12–22.

Congiu L, Corongiu FP, Dore M, *et al.* 1979 The effect of lead nitrate on the tissue distribution of mercury in rats treated with methyl-mercury chloride. *Toxicol Appl Pharmacol* **51** 363–366.

Couture P, Blaise C, Cluis D, Bastien C. 1989 Zirconium toxicity assessment using bacteria, algae and fish assays. *Water Air Soil Pollut* **47**, 87–100.

Cuddihy RG, Finch GL, Newton GL *et al.* 1989 Characteristics of radioactive particles released from the Chernobyl nuclear reactor. *Environ Sci Technol* **23**, 89–95.

De Longeas JL, Burnel D, Netler P, *et al.* 1983 Toxicity and pharmacokinetics of zirconium oxychloride in the mouse and in the rats. *Lab Pharmacol* **14**, 437–447.

Dhir H, Talukder G, Sharma A. 1985a Comparison of cytotoxic effects of lead following acute and chronic treatments in mammalian system. *Proc Natl Acad Sci (India)* **55B III**, 209–215.

Dhir H, Sharma A, Talukder G. 1985b Alteration of cytotoxic effects of lead through interaction with other heavy metals. *The Nucleus* **28** 68–89.

Dhir H, Roy AK, Sharma A, Talukder G. 1990 Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* fruit extract. *Mutat Res* **241**, 305–312.

Epstein WL, 1971 Metal induced granulomatous hypersensitivity in man. *Adv Biol Skin* **11**, 313–335.

Ghosh S, Sharma A, Talukder G. 1990 Cytotoxic effects of zirconium oxychloride on bone marrow cells of mice. *Mutat Res* **243**, 29–33.

Ghosh S, Sharma A, Talukder G. 1991 Relationship of clastogenic effects of zirconium oxychloride to dose and duration of exposure in bone marrow cells of mice *in vivo*. *Toxicol Lett* **55**, 195–201.

Hickey RJ, Clelland RC, Boyer DE, Bowers EJ. 1974 Atmospheric sulphur dioxide, nitrogen and lead as

mutagenic hazards to human health. *Mutat Res* **26**, 445–446.

Kosower NS, Kosower EM. 1976 The glutathione-glutathione disulphide system. In: *Free Radicals in Biology*. New York: Academic Press.

Kotz S, Johnson NL. 1982 In: *Encyclopedia of Statistical Sciences*, Vol. 2. New York: Wiley; 424–425.

Margolin BH, Resnick MA, Rimpo LY, *et al.* 1986 Statistical analysis of *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environ Mutagen* **8**, 183–204.

Omoto M, Imai T, Seki K, Nomura R, Hirai M. 1988 Effects of ethanol and lead on mouse chromosomes. *Jpn J Alcohol Stud Drug Depend* **23**, 137–146.

Osanai T, Hayakawa K, Kikuchi N, *et al.* 1989 Investigation of interference by inorganic phosphate on co-precipitation of cadmium, copper, manganese and lead in water with zirconium hydroxide. *Eisei Kagaku* **35**, 297–300.

Preston RJ, Dean BJ, Galloway S, *et al.* 1987. Mammalian *in vivo* cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mutat Res* **189**, 157–165.

Rasheva V. 1990 Investigation of the clastogenic effect of lead nitrate on mice. *Genet Sel* **23**, 170–177.

Schroeder HA, Balassa JJ. 1966 Abnormal trace metals in man: zirconium. *J Chron Dis* **19**, 573–586.

Sharma A, Talukder G. 1987 Effects of metals on chromosomes of higher organisms. *Environ Mutagen* **9**, 191–226.

Sokal RR, Rohlf FJ. 1973 Introduction to analysis of variance. In: *Introduction to Biostatistics*. San Francisco, Freeman; 134–160.

Tice RR, Boucher R, Luke CA, Shelby MD. 1987 Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F mice by multiple exposure to gaseous, 1,3-butadiene. *Environ Mutagen* **9**, 235–250.

Venugopal B, Luckey TD, eds. 1978 *Metal toxicity in Mammals*, Vol. 2. New York: Plenum Press.