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Modification of the Clastogenic Effects of Cobalt by Calcium in Bone Marrow Cells of Mice *in vivo*

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Cobalt, though an essential element in the living systems, yet induces toxic effects when administered in excess (see review, Domingo 1989, Palit *et al.* 1990) as shown for other essential elements as well (Sharma and Talukder 1987). The known property of cobalt to block the calcium channel in the cell system (Hagiwara *et al.* 1981) has initiated interest in the possibility of modifying the toxicity of cobalt by the use of calcium.

Cobalt interacts with the same membrane sites in isolated nerve cells of *Helix aspora* which bind Ca^{2+} (Yasui *et al.* 1986). A Ca-dependent cobalt-sensitive step is involved in the transfer of membrane proteins to the myelin membrane (Pasquini *et al.* 1987). Addition of Co (2.5 mM) had been seen to reduce the amplitude of both Ca and Ba currents in rat uterine smooth muscle (Jmari *et al.* 1986). Co^{2+} also drastically inhibited rat and human platelet aggregation induced by thrombin, ADP or adrenalin in the presence of 0.32 mM Ca^{2+} (Denis *et al.* 1987). In rats, Ca^{2+} had been shown to inhibit totally the DNA methylase activities, but Co ion activated kidney, spleen and brain DNA-methylase activities (Pfohl-Leszkowicz *et al.* 1987).

No information is yet available about the effect of Ca–Co interaction at the level of cell and chromosome division.

Earlier work in our group has utilised the metal-metal interaction in modifying cytotoxicity in living cellular systems when administered *in vivo*, as for example, the clastogenic action of Pb has been modified by Se (Chakrborty *et al.* 1987), Hg by Se (Das *et al.* 1985), Cd by Se (Mukherjee *et al.* 1988), Al by Ca (Roy *et al.* 1990).

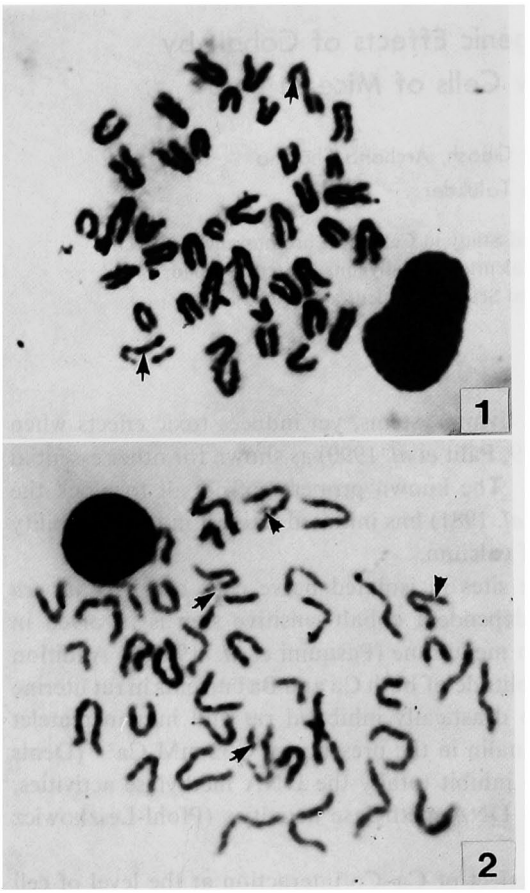
The present work was therfore undertaken to study the level of protection offered by calcium, against the cytotoxic effects of Co, with special reference to divisional frequency and chromosomal aberrations in bone marrow cells of *Mus musculus in vivo*.

Materials and methods

Sixty laboratory bred, Swiss albino male mice, 10–12 weeks old, weighing 30–35 g were maintained on standard pellet diet (Hindusthan Lever, India) and given water *ad libitum*. They were housed five in a cage at a temperature of $20\pm3°C$, relative humidity $50\pm15\%$ and photoperiod of 12 hr.

Two concetrations (40 mg and 20 mg per kg body weight) of cobaltous choride ($CoCl_2 \cdot 6H_2O$; E. Merck/India) and calcium chloride ($CaCl_2 \cdot 2H_2O$). Sarabhai. M. Chemicals, India were prepared in distilled water. The two solutions were administered orally by gavaging to mice *in vivo* accrding to tables 1 and 2. Five mice were used for each set under each concentration.

Chromosomes were studied from bone marrow cells after injecting 0.04% colchicine



Figs. 1, 2. Chromatid breaks induced by cobaltous chloride (ca. ×2000).

into each mouse intraperitoneally, 90 min before cervical dislocation. The marrow of femur bones after dissection were aspirated and flushed into 1% sodium citrate, incubated at 37°C for 15–18 min and fixed in 1 : 3 acetic acid-ethanol. Flame dried slides were prepared and finally stained with dilute giemsa solution (see Sharma *et al.* 1980, Preston *et al.* 1987), 250 well spread metaphase plates and 3000 cells were scanned for chromosomal aberrations and mitotic index respectively for each experimental set A to F.

Statistical analysis was carried out following analysis of variance (ANOVA) test followed by Duncan's new multiple range test (Kotz and Johnson 1982) with the help of Harter's table (Harter 1960) for multiple comparison.

Results and discussions

The combined effects of administration of chlorides of calcium and cobalt (two doses in equal proportions) in mice on the frequency of dividing cells, total chromoso mal aberrations as well as aberrant metaphases are shown in tables 3 and 4. Calcium was found to reduce the clastogenicity induced by both concentrations of cobalt as

Table 1.

Treatment chemicals	Experimental set		Concentrations mg/kg body weight	
			$CoCl_2$	$CaCl_2$
Distilled water alone	A		—	—
$CoCl_2 \cdot 6H_2O$ alone	B	B_1	20	—
		B_2	40	—
$CaCl_2 \cdot 2H_2O$ alone	C	C_1	—	20
		C_2	—	40
Simultaneous feeding of $CaCl_2 + CoCl_2$	D	D_1	20	20
		D_2	40	40
$CoCl_2$ followed by $CaCl_2$ after 2 hr	E	E_1	20	20
		E_2	40	40
$CaCl_2$ followed by $CoCl_2$ after 2 hr	F	F_1	20	20
		F_2	40	40

Table 2. Concentration of metals as mg/kg body weight

$CoCl_2 \cdot 6H_2O$	$CaCl_2 \cdot 2H_2O$	Amounts of Co	Amounts of Ca	Co : Ca
40	40	9.907	10.88	1 : 1.1
20	20	4.953	5.44	1 : 1.1

Table 3. Effect of acute exposure to $CoCl_2$ and $CaCl_2$ on mice bone marrow chromosomes[a)]

Experimental set (as per table 1)	Mitotic Index (M. I.) (mean±S. D.)	Aberrant Metaphases (A. M.) (mean±S. D.)	Total chromosomal aberrations (CA)				
			G′	G″	B′	B″	others
A	4.33±2.05*	1.8±0.83*	5	—	1	—	3
B_1	3.19±0.53	6.8±1.30	4	—	10	3	14
C_1	5.00±0.45*	4±1.58*	8	1	6	2	3
D_1	3.33±0.66	4.6±2.70*	14	—	5	1	3
E_1	3.32±1.52	6.6±1.14	16	1	20	1	—
F_1	4.23±0.79*	4.2±1.30*	14	—	3	4	—

a) Dose: Cobaltous chloride—20 mg/kg b. w. (Co content 4.95 mg/kg b. w.)
Calcium choloride—20 mg/kg b. w. (Ca content 5.44 mg/kg b. w.)

* $P \leq 0.05$ as compard with B_1 following ANOVA test and Duncan's multiple range test.

G′, G″=Chromatid and isochromatid gaps; B′, B″=Chromatid and isochromatid breaks; others= polyploids, pulverized cells.

Table 3A. Duncan's moultiple range test[a)]

Mitotic Index	Treatment set	B_1	E_1	D_1	F_1	A	C
	Sample Mean	3.19	3.32	3.33	4.23	4.33	5.00
Chromosomal aberration	Treatment set	A	C_1	F_1	D_1	E_1	B_1
	Sample Mean	1.8	4	4.2	4.6	6.6	6.8

Straight lines denote there is no significant differences between the underlined experimental sets at $P=0.05$ level

Table 4. Effect of acute exposure to $CoCl_2$ and $CaCl_2$ on mice bone marrow chromosomes[b)]

Experimental set	Mitotic Index (M. I.) (mean±S. D.)	Aberrant Metaphases (A. M.) (mean±S. D.)	Total chromosomal aberrations (CA)				
			G′	G″	B′	B″	others
A	4.33±2.05*	1.80±0.83*	5	—	1	—	3
B_2	2.62±0.57	9.8 ±3.76	11	—	14	2	21
C_2	5.23±0.62*	5.60±1.67*	11	1	4	1	12
D_2	2.81±0.75	6.20±2.04*	13	2	11	4	1
E_2	2.08±0.65	8.40±1.52	23	1	16	—	2
F_2	3.94±0.27*	7.20±1.49*	21	—	15	—	5

b) Dose: Cobaltous choloride 40 mg/kg b. w. (Co content 9.907 mg/kg b. w.)
Calcium chloride 40 mg/kg b. w. (Ca content 10.88 mg/kg b. w.)

* $P \leq 0.5$ as compared with B_2 following ANOVA test and Duncan's multiple range test.

Table 4A. Duncan's multiple range test[b)]

Mitotic Index	Experimental set	E_2	B_2	D_2	F_2	A	C_2
	Sample Mean	2.008	2.618	2.807	3.935	4.33	5.233
Chromosomal aberration	Experimental set	A	C_2	D_2	F_2	E_2	B_2
	Sample Mean	1.80	5.60	6.20	7.20	8.40	9.80

Straight line denotes there is no significant differences between the underlined experimental sets.

was shown by a decrease in the number of abnormal cells.

The frequency of aberrant metaphases was maximum in the animals fed both doses of $CoCl_2$ alone taking all the treatments into consideration (Tables 3, 4).

Of the three combinations (D. F. E.) the first two at both lower and higher doses reduced significantly the number of aberrant cells as compared to the same concentration of $CoCl_2$ given singly (Tables 3, 4). Higher concentrations of $CaCl_2$ gave maximum protection when administered simultaneously (D_2) with $CoCl_2$, while lower concentrations gave similar portection when given simultaneously (D_1) as well as *before* (F_1) $CoCl_2$.

When calcium chloride was fed two hours after cobalt chloride in both protocols E_1 and E_2, there was no reduction in the percentage of abnormalities induced by $CoCl_2$. Therefore, post-administration of calcium failed to reduce the clastogenic effect of cobalt on chromosomes.

Comparing the experimental protocols, the frequency of dividing cells was highest in the animals fed $CaCl_2$ alone (Tables 3 and 4). Both lower and higher doses of $CaCl_2$, when administered two hours before $CoCl_2$, reduced significantly the mitostatic effect of $CoCl_2$ but not when given simultaneously. The observation that $CaCl_2$ could enhance the divisional frequency can be attribted from the property of Ca^{2+} to stimulate G_0–S transition, activating to the G_1 and G_2 phases and mitosis (Whitfield *et al.* 1966, Whitfield 1982, Izant 1983, Hepler 1985). Though the exact mechanism of action of Ca in stimulating DNA synthesis is not known, yet Ca^{2+} ion might share the role of natural condensing agents, initiating cell division by promoting coiling and aggregation of chromosomes, possibly by combining with the phosphate group of the DNA molecule (Fujita and Nakao 1988). Calcium is also suggested to be necessary to mintain the activity of DNA polymerase (Zwierchowski *et al.* 1984).

The fact that post-administration of calcium failed to reduce both mitostatic and clastogenic action of $CoCl_2$ may be due to the competition of cobalt with calcium efflux and influx (Lando *et al.*, 1986, Boissonneault 1985). The action of divalent cations, such as Co^{2+} as inhibitors of Na^+/Ca^{2+} exchanger, in cultured arterial smooth muscle was correlated with the closeness of the crystal ionic radius of that of Ca^{2+} (Smith *et al.* 1987). Therefore, the antagonism of Ca to cobalt clastogenicity may depend on the competition between the two ions and the success of the former in overcoming the barrier afforded by Co on Ca channel.

Summary

The interaction between cobaltous chloride and calcium chloride was observed using as endpoints mitotic index and frequency of chromosomal abonrmalities. The two salts were administered orally to laboratory-bred male mice *in vivo* singly or one followed by the other or both simultaneously. Chromosomes were studied from bone marrow preparations after 24 hr. In all cases, the administration of $CaCl_2$ two hours before $CoCl_2$ protected against the clastogenic effects of the latter to a significant extent. Simultaneous feeding of the two salts reduced the damage by $CoCl_2$ only when $CaCl_2$ was given in a higher concentration. The reduction of clastogenicity of cationic cobalt by calcium has been attribited to the competition between the two ions. This report of the use of Ca^{2+} in reducing the clastogenicity of Co^{2+} is a new one.

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