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Cytologia 55: 197–201, 1990

Antagonism of Calcium to Aluminium-induced Chromosomal Aberrations in Bone Marrow Cells of *Rattus norvegicus in vivo*

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Accepted September 29, 1989

Interaction between Al and Ca is receiving increased attention following the observation that Al interferes with Ca deposition in bone (Lieberrharr *et al.* 1987) and calcification. Al interacts with citrate to form a potent inhibitor of both mineralization and growth of calcium phosphate crystals *in vitro* (Thomas and Meyer 1984). In the bone, Al is reported also to block Ca uptake or to be incorporated instead of Ca (Cannata *et al.* 1983).

In the central nervous system, CaAl silicate deposits in neurons lead to the early appearance of neurofibrillary tangles in amyotrophic lateral sclerosis/Parkinson's dementia (Gajdusek 1986). Al at concentration (75–100 μM), only slightly higher than those found in body fluid competes with Ca and constitutes the first toxic event in the neurodegeneration process (Deleers 1985). Al is also reported to interact with the gastrointestinal Ca-transporting system (Provan and Yokel 1988).

Many reports are available on alleviation of Al toxicity in plants (Horst 1987, Roy *et al.* 1988), but no such reports are found in mammalian system.

Earlier work done in this laboratory has indicated the role of interaction between metals in modifying the clastogenic action, *e. g.* Pb by Se (Chakraborty *et al.* 1987), Hg by Se (Das *et al.* 1985), Cd by Se (Mukherjee *et al.* 1988). Since metals usally occur in combination, these studies are of considerable importance (Sharma and Talukder 1987).

The present work was carried out to study the alterations induced by Ca on the effects of Al in bone marrow chromosomes of *Rattus norvegicus*.

Material and method

Test system: 165 laboratory bred albino male rats, *Rattus norvegicus*, about 8–10 weeks old, weighing 120±15 gms, were maintained under standard laboratory conditions (temperature 20±3°C, relative humidity 50±15% and photoperiod of 12 hours). Standard pellet diet (Hindustan Lever, India) and distilled water were provided *ad libitum*. Five animals were housed in one cage.

Test chemical: Aluminium sulphate [$Al_2(SO_4)_3 \cdot 18H_2O$] and calcium sulphate [$CaSO_4 \cdot 2H_2O$] were obtained from Sarabhai M. Chemicals, India. Two concentrations (53 mg and 35.4 mg/100 gm body weight) were used. The salts were given in equal proportions.

Experimental protocol: The experiment was carried out in six different combinations, fed to the animals:

A. Distilled water (DI)
B. Salts of aluminium (Al)
C. Salts of calcium (Ca)
D. Al and Ca fed simultaneously (Al+Ca)
E. Al followed by Ca after two hours (Al→Ca)
F. Ca followed by Al after two hours (Ca→Al)

Table showing content of different metals in mg/100 gm body weight

Amount of Al salt	Amount of Ca salt	Amount of Al	Amount of Ca	Al: Ca
53	53	4.29	12.33	1:2.88
35.4	35.4	2.87	8.23	1:2.88

Aqueous solutions of the salts were administered orally to the animals daily for 21 consecutive days. In each set, 15 animals per concentration were used. Five animals were sacrificed 24 hours after days 7, 14 and 21.

For chromosome studies, 0.04% colchicine was injected intra-peritoneally to the animal (1 ml per 100 gm body weight). The animal was sacrificed after 90 minutes by cervical dislocation. Marrow of femur bones was flushed out in 0.8% sodium citrate, incubated at 37°C for 25 minutes and fixed in 1: 3 acetic acid: ethanol. The fixative was changed thrice after centrifugation each time and flame dried slides were prepared and stained with diluted Giemsa solution (Sharma and Sharma 1980, Preston *et al.* 1987).

All slides were coded and scored blind by a single person (AKR). Chromosomal aberrations were scored from 60 well-scattered metaphase plates having 42 chromosomes for each animal, making a total of 300 metaphases for each set of experiment.

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

Results and discussion

Ca is found to antagonize the toxic effects of Al in both doses as shown by frequency of abnormal cells (Tables 1 and 2). The frequency of metaphases with chromosomal aberrations was maximum in the animals fed aluminium alone (Set B) taking all the treatments into account. The ANOVA calculation indicates a significant difference among means at 5% level among all groups of treatment. It was most pronounced following administration of both doses of Al alone after 21 days of treatment, where the frequency of aberrant metaphase was significantly higher than that observed in any combination group. It indicates that Ca protection against Al is duration-dependent.

Among the three combinations (Al+Ca, Ca→Al and Al→Ca), Ca→Al (Group F) afforded maximum protection as shown by reduction in the frequency of chromosomal abnormalities.

Since Ca is an essential component of life, the ability of Al^{3+} to replace or displace Ca ions has a major effect in life functions. There are conflicting reports on increase or decrease in tissue Ca concentration following Al administration, the mechanism being not clear (Mayor 1985). Al has a very high affinity for DNA, RNA and mononucleotides (Ganrot 1986) and complexes with DNA (Karlik *et al.* 1980, Dyrssen *et al.* 1987) and interacts with microtubule aggregation (Mac Donald *et al.* 1987). The differences in ionic radii [Al^{3+} (0.54A°), Ca^{2+} (1.00A°)] result in competition between Al and Ca for small molecule ligands and phosphates (Martin 1986).

In the present study, dicentrics and translocations were more frequent in combination treatments, suggesting that the effects extended over more than one cell cycle with Al or Ca alone. However, chromatid breaks and gaps were more frequent indicating renewed activity of the chemical at each cell cycle (Tables 1 and 2).

The results show that Ca, when given in combination with Al, counteracts the clastogenic effects of the latter. Since Ca administered before Al affords the maximum protection, it is possible that Ca^{2+} ions present in excess in the cell are able to prevent the action of Al^{3+}

Table 1. Effect of chronic treatment of aluminium sulphate and calcium sulphate on rat bone marrow chromosomes**

Period of treatment (in days)	Experimental set	Percentage of abnormalities						
		Gaps	Breaks	Dicentrics	Translocations	Pulverized cells	Polyploid cells	Aberrant metaphase (Mean±SEM)
7	A	2.00	1.33	0.33	0.33	0.33	0.33	4.67±0.82*
	B	3.33	9.67	—	—	2.00	1.00	14.67±0.34
	C	2.67	5.00	—	1.00	—	0.67	8.67±1.00*
	D	2.00	6.67	1.00	2.00	0.67	1.33	13.67±1.87
	E	3.67	7.00	0.67	0.33	3.67	0.67	15.00±1.58
	F	3.67	4.33	2.00	0.67	1.67	0.33	11.67±2.11
14	A	2.33	2.33	0.67	0.67	1.00	0.67	6.33±1.61*
	B	4.00	10.00	0.33	1.00	2.00	2.00	17.33±2.21
	C	1.67	5.00	0.33	1.00	0.67	1.33	10.00±0.79*
	D	4.33	7.33	1.33	1.33	2.00	4.00	20.00±3.31
	E	0.67	7.67	0.33	2.00	0.67	3.00	13.67±1.87
	F	3.00	7.00	2.00	1.00	1.00	—	12.67±2.09*
21	A	2.33	2.00	1.00	0.33	1.00	1.00	6.67±0.75*
	B	4.00	2.00	2.00	0.67	3.33	3.00	22.00±1.61
	C	1.67	7.00	0.67	—	—	2.33	10.67±1.46*
	D	4.00	4.67	1.33	3.67	0.67	1.00	15.00±1.77*
	E	1.67	4.67	1.67	—	3.00	2.33	13.00±2.00*
	F	2.00	6.00	2.67	1.00	3.67	0.33	15.67±0.94*

* $P \leq 0.05$ as compared with Group B following ANOVA test and Duncan's multiple range test.

** Dose: Aluminium sulphate 33 mg/100 gm body wt. (Al content 4.29 mg/100 gm body wt.). Calcium sulphate 53 mg/100 gm body wt. (Ca content 12.33 mg/100 gm body wt.).

Table 2. Effect of chronic treatment of aluminium sulphate and calcium sulphate on rat bone marrow chromosomes**

Period of treatment (in days)	Experimental set	Percentage of abnormalities						
		Gaps	Breaks	Dicentrics	Translocations	Pulverized cells	Polyploid cells	Aberrant metaphase (Mean±SEM)
7	A	2.00	1.33	0.33	0.33	0.33	0.33	4.67±0.82*
	B	4.33	8.33	0.67	0.33	1.00	1.00	14.67±1.33
	C	1.00	5.67	—	0.33	—	1.67	8.67±1.00*
	D	2.67	4.00	2.00	0.67	1.67	—	10.67±0.94
	E	1.67	3.67	2.67	1.00	—	1.67	10.00±0.79
	F	1.00	7.00	2.00	2.00	1.00	1.00	10.67±3.40
14	A	2.33	2.33	0.67	0.67	1.00	0.67	6.33±1.61*
	B	4.00	4.67	0.67	1.00	2.67	5.33	17.33±1.94
	C	2.00	2.00	1.00	—	1.00	2.33	8.33±1.27*
	D	2.67	6.67	1.33	1.67	0.67	4.00	15.67±3.10
	E	5.67	9.33	0.67	1.00	0.67	0.33	16.00±1.00
	F	3.33	10.33	0.67	2.67	2.67	0.67	16.67±0.91
21	A	2.33	2.00	1.00	0.33	1.00	1.00	6.67±0.75*
	B	2.33	6.67	2.33	1.33	2.00	3.33	17.67±1.00
	C	1.00	7.67	1.00	0.33	1.00	2.33	13.00±0.92*
	D	4.33	6.67	1.67	2.00	1.00	—	14.00±0.61*
	E	2.00	4.33	—	3.33	0.67	1.67	12.00±1.61*
	F	1.00	7.00	2.00	2.00	1.00	1.00	14.00±1.70*

* $P \leq 0.05$ as compared with Group B following ANOVA test and Duncan's multiple range test.

** Dose: Aluminium sulphate 35.4 mg/100 gm body wt. (Al content 2.87 mg/100 gm body wt.). Calcium sulphate 35.4 mg/100 gm body wt. (Ca content 8.23 mg/100 gm body wt.).

ions on chromosomal proteins and spindle microtubules. This point is further strengthened by the observation that after 21 days of treatment, Ca in all combination significantly reduced the percentage of aberrant metaphases (Tables 1 and 2).

Summary

The interaction of Al with Ca in six different combinations was studied in bone marrow chromosomes following daily oral administration for prolonged periods. Cytotoxic effects of Al, measured by the induction of chromosomal aberrations, were countered by treatment with Ca. The protection afforded was maximum when Ca was fed at a ratio of 3: 1, as related to Al, 2 hours before the administration of Al.

Acknowledgments

The authors are grateful to Professor A. K. Sharma, Programme Co-ordinator, Centre for Advanced Studies, for the laboratory facilities provided and the University Grants Commission, New Delhi and Council for Scientific and Industrial Research, New Delhi for financial assistance.

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