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Effect of Aluminum Chloride on Mitogenesis, Mitosis, and Cell Cycle in Human Short-Term Whole Blood Cultures: Lower Concentrations Enhance Mitosis

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Abstract Aluminum, the third most common element in the earth's crust (second to oxygen and silicon) and recently suspected by some investigators to be implicated in Alzheimer disease etiology, has been studied in relation to its effect on mitogenesis, mitosis, and cell cycle. We have observed that 2–4 mM concentrations of $AlCl_3$ have decreased the number of cells that undergo mitogenesis (PHA-induced blast transformation) and mitosis in human short term whole blood cultures. We have also shown that the rate of the cell cycle was slowed down, i.e., cell cycle time was increased in the presence of $AlCl_3$. Also, we have demonstrated a reversible effect on aluminum-induced reduced mitotic index in long-term EBV-transformed lymphoblastoid cultures. Although safeguards such as limiting aluminum serum concentrations have been recommended to protect individuals undergoing dialysis, it should be realized that concentration accumulations of aluminum may increase over chronic exposures. Accordingly, if the number of cells stimulated by PHA is reduced in the presence of $AlCl_3$, there may be a reduction of immune competence, since the degree of PHA stimulation has been used as an indicator of immune response. Similar reductions in mitotic index could affect every tissue involved with cell division. Although it may not be the same for higher concentrations, from our results, we have also shown that decreased mitotic rates were reversible in long-term EBV-transformed lymphoblastoid cultures.

Increased numbers of mitoses were observed in human short-term whole blood cultures that were exposed to 2 μM concentrations of aluminum chloride. The concentration is close to those found in normal human serum and within the "safeguard" range recommended for dialysis patients. A similar trend for aluminum sulfate was also observed, while preliminary results for three other aluminum species, lactate, citrate, and maltol, were also reported. Although previous reports have indicated a positive effect of aluminum on mitosis in vitro or in vivo, this is the first such report involving human material.

It is clear that higher concentrations of aluminum chloride at 2.0–4.0 mM reversibly inhibit mitosis while more dilute concentrations of 1–2 μM, closer to those found in normal serum, enhance mitosis. The present results, as well as those in the literature, suggest that aluminum may be an essential element in cellular processes for optimal growth, development, and health maintenance. Future research will further test this hypothesis.

Key words: mitogenesis, mitotic index, recovery, increased mitosis, PHA, reversible mitotic inhibition

INTRODUCTION

Aluminum, the third most common element in the earth's crust (second to oxygen and silicon), has been studied in relation to its effect on cells, chromosomes, and genes and has been suspected by some investigators to be implicated in the etiology of Alzheimer disease. In patients on dialysis, aluminum concentrations have been observed to be 1.6, 4, 15, 35, 440, and 64 times the normal level in lung, brain, serum, bone, liver, and spleen, respectively [Alfrey, 1980; Elinder and Sjogren, 1986]. Some investigators have reported apparently contradictory results in relation to the effects of aluminum on mitogenesis and mitosis. Palekar et al. [1987] have shown that erionite, an aluminosilicate nonasbestos mineral fiber, caused reduced mitotic indices and increased aneuploidy, polyploidy, and chromatid aberrations in V79 Chinese hamster lung cultures. Reduced mitotic index after aluminum (chloride and sulfate) exposure, together with increased chromosome aberrations, have been observed in plants. The effect on mitotic index has been reported to be reversible and

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inversely related to dosage [Sharma and Talukder, 1987]. Onion (*Allium cepa*) meristematic root-tip mitotic activity ceased after a 10 h exposure to 1 mM aluminum chloride [Morimura et al., 1978].

A stimulatory effect of aluminum chloride, sulfate, and potassium sulfate on mitosis was reported by Jones et al. [1986] in Nakano mouse lens epithelial and Swiss 3T3K cell cultures at concentrations of about 1.0–50.0 μM Nashed, [1975] reported mitotic stimulation of mouse, rat, and Chinese hamster peritoneal cells after exposure to 6.4 (5 mg/kg), 25.6 (20 mg/kg), and 51.2 mM (40 mg/kg) aluminum hydroxide, respectively. Quarles et al. [1991] reported aluminum-induced mitogenesis at concentrations of 1.0–50.0 μM $AlCl_3$ in cultured MC3T3-E1 mouse osteoblasts. Finally, a paper was recently presented by Carlisle et al., suggesting that aluminum may be an essential element for normal growth in chicks [Carlisle and Curran, 1993].

Manna and Das [1972] reported increased chromatid breakage in mouse bone marrow exposed in vivo to 0.1 M (1 ml/30 kg) $AlCl_3$. Mutagenic and carcinogenic studies involving aluminum have generally been negative, and although there is some evidence for teratogenic effects [Leonard and Gerber, 1988], there were no striking effects noted in a small study of pregnant women exposed to high doses of aluminum sulfate in their drinking water [Golding et al., 1991]. Crapper McLachlan et al. [1989] have suggested that aluminum may be related to a change in chromatin structure that may effect the compaction of the chromatin found in Alzheimer Disease (AD) cerebral cortex. Other investigators [Muma et al., 1988; Parhad et al., 1989] have implicated aluminum in reduced neuronal gene expression.

In order to attempt to explain why aluminum has been reported to be mitogenic in some cases and not in others as reviewed above, we have investigated the in vitro effects of aluminum on phytohemagglutin-induced (PHA) blast cell transformation or mitogenesis, mitosis, recovery, and cell cycle in human lymphocyte cultures.

METHODS

Human short-term whole blood cultures, from anonymous individuals whose blood was obtained as "throw away" specimens, were incubated for 4 days at 37°C. Complete medium for each 4 ml culture was composed of RPMI-1640 (base), 15% fetal bovine serum (GIBCO, Grand Island, NY), 0.2% phytohemagglutinin-P (PHA-P) (DIFCO), 1 mM glutamine and 10,000 U-mcg/ml penicillin-streptomycin (GIBCO, Grand Island, NY). The percentage of PHA-induced blast transformation or mitogenesis, referred to below as BTI (blast transformation index), was determined by ascertaining the number of blast-like cells in 1,000 (unless otherwise specified) mononuclear cells in short-term whole blood cultures. A blast-like cell [Dutkowski et al., 1983] contained one or more large nucleoli as compared to the pycnotic and dense nuclei of non-transformed lymphocytes.

Mitotic index (MI) was the percentage of mitotic cells among 1,000 blast-like mononuclear cells (including the mitotic figures). Sister chromatid staining procedures were carried out according to modifications of those described elsewhere [Verma, 1989], and the distribution of first, second, and third division metaphases [Wolff and Morgan, 1982] was determined to detect cell cycle changes.

Data in Tables I–VI were obtained from short-term whole blood cultures or long-term lymphoblastoid cultures (Table III). EBV-transformed lymphoblastoid cell lines were inoculated and harvested according to a previously described protocol [Krawczun et al., 1986]. All BTI studies were conducted in 4 day short-term whole blood cultures that were continuously exposed to $AlCl_3$ (Sigma Chemical Co., St. Louis, MO; concentrations given in tables), while MI and other studies were exposed to aluminum for the last 24 h (or 48 h for cell cycle studies) of culture. $AlCl_3$ was used in these studies because it has been known to be the most soluble compound of all aluminum species. Aluminum sulfate, lactate, citrate, and maltol were also studied. The latter species was obtained from an organic chemist (see Acknowledgements) who synthesized the compound, while the former were obtained commercially (Sigma Chemical Co., St. Louis, NY, Fisher). Cultures for MI determination were harvested after 2 h exposure to 0.5 μM colchicine (Nutritional Biochemicals).

RESULTS

Table I shows a dose effect of $AlCl_3$ on BTI in two studies of short-term human whole blood cultures. As the aluminum concentration increased the number of cells undergoing blast cell formation under the influence of PHA was reduced [χ^2 (2), (3) for Studies 1 and 2, respectively, $P < .001$]. Cultures with no PHA showed no blastogenesis when exposed continuously to

TABLE I. The Effect of $AlCl_3$ Exposure on PHA-Induced Blast Transformation (BTI)

mM $AlCl_3$	Study 1 % BTI[a]	Study 2[b]	% BTI
0.0	51.9	2,074	69.1
0.5	—	1,996	66.5
2.0	26.7	1,591	53.0
4.0	23.2	685[c]	34.2

[a]% blast-like cells in a total of 1,000 mononuclear cells.
[b]No. blast-like cells in a total of 3,000 mononuclear cells.
[c]No. blast-like cells in 2,000.

TABLE II. Effect of $AlCl_3$ Exposure on Mitotic Index (MI)

mM $AlCl_3$	Study 1[a]	% MI	Study 2	% MI
0.0	77	2.6	175	5.8
0.5	—		139	4.6
2.0	61	2.0	—	—
4.0	56	1.9	131	4.4

[a]No. of mitotic figures in 3,000 blast-like cells.

TABLE III. Mitotic Index After 24 h Recovery From $AlCl_3$ in Lymphoblastoid Cultures

mM $AlCl_3$	Mit[a]	% MI	Recov.	% MI
0.0	80	2.7	97	3.2
2.0	74	2.5	108	3.6
4.0	47	1.6	99	3.3

[a]No. of mitoses in 3,000 blast-like cells.

aluminum chloride. These data are not shown in Table I.

When short-term whole blood cultures were exposed to 0.5 to 4.0 mM $AlCl_3$ during the last 24 h of culture, the MI also decreased as shown in three separate studies (Tables II, III). Study 2 in Table II showed statistically significant decreases in MI, while Study 1 showed the same trend but was borderline in significance: $P < .05$, $P < .1$, respectively. Exposure of long-term lymphoblastoid cell cultures to $AlCl_3$ concentrations of 2 and 4 mM of aluminum chloride decreased mitotic indices at the higher concentration ($P < .01$). The effect was eliminated, however, when the cultures were washed free of exogenous Al and allowed to recover for 24 h (Table III). This indicates that the effect of $AlCl_3$ on MI is reversible. A χ^2 comparison of the degree of increased MI in the cultures with no $AlCl_3$ vs. those with 4.0 mM, after recovery, showed that the increased MI in the cultures that were washed free of 4.0 mM $AlCl_3$ was due to recovery and not just an effect of fresh medium ($P < .05$).

TABLE IV. $AlCl_3$ Exposure to Short-Term Human Whole Blood Cultures Reduced the Rate of the Cell Cycle

Study	mM $AlCl_3$	1st Div.	2nd Div.	3rd Div.
1_{24}[a]	0	91	207	2
		(30.3)[b]	(69.0)	(0.7)
	2.0	132	167	1
		(44.0)	(55.7)	(0.3)
2_{24}	0	131	169	0
		(43.7)	(56.3)	(0.0)
	2.0	155	145	0
		(51.7)	(48.3)	(0.0)
2_{48}	0	10	70	220
		(3.3)	(23.3)	(73.3)
	2.0	13	176	111
		(4.3)	(58.7)	(37.0)
3_{48}[c]	0	125	231	44
		(31.2)	(57.8)	(11.0)
	2.0	185	196	19
		(46.2)	(49.0)	(4.8)
4_{48}	0	35	54	311
		(8.8)	(13.5)	(77.8)
	2.0	23	161	216
		(5.8)	(40.2)	(54.0)

[a]300 cells per variable exposed to $AlCl_3$ during the last 24 h of culture.
[b]%.
[c]400 cells exposed to $AlCl_3$ for the last 48 hours of culture.

From our observations above, it appears that BTI and MI were reduced by Al concentrations of 2.0–4.0 mM. Table IV shows that the cell cycle time was increased when cultures were exposed to $AlCl_3$ for either 24 or 48 h at concentrations of 2.0 mM, and comparisons of first, second, and third divisions were made. χ^2 analyses indicated that the observed shifts in cell cycle with aluminum exposure would occur by chance less than one time in a hundred for Study 1_{24} and less than one time in a thousand for studies 2_{48}, 3_{48}, and 4_{48} while an insignificant trend in the same direction was observed for Study 2_{24}. This shows that $AlCl_3$ at a concentration of 2.0 mM increased cell cycle times in human short-term whole blood cultures.

When lower concentrations of $AlCl_3$ were studied, there was an increase in MI. Table V summarizes three studies all of which exhibited statistically significant increases in mitotic index when cultures were exposed to 2 μM $AlCl_3$ ($P < .001$). When another species of aluminum, $Al_2(SO_4)_3$, was studied, a similar trend was observed among three studies totalling analysis of 88,000 cells,

TABLE V. Increased Mitotic Indices in Cultures Exposed to Lower Concentrations

μM $AlCl_3$	No. mit.[a] Study 1	% MI	Study 2[b]	% MI	Study 3[b]	% MI
0.0	548	4.57	308	3.85	342	4.28
0.2	—	—	—	—	392	4.90
1.0	—	—	384	4.80	—	—
2.0	692	5.77	350[c]	5.00	434	5.92
20.0	670	5.58	390	4.88	—	—
200.0	666	5.55	339	4.24	—	—
2,000.0	542	4.52	341	4.26	—	—
4,000.0	—	—	—	—	311	3.89

[a]*12,000* cells per variable.
[b]8,000 cells per variable except where specified.
[c]7,000 cells read here.

TABLE VI. Increased Mitotic Indices in Cultures Exposed to Lower Concentrations of Aluminum Sulfate

μM Al[a]	1[b]	%	2[c]	%	3[d]	%
0	554	6.93	245	6.13	252	6.30
0.1	544	6.8	222	5.55	246	6.15
1	596	7.45	276	6.90	332	8.30
10	579	7.24	229	5.58	285	7.13
100	581	7.26	265	6.63	270	6.75
500	524	6.55	246	6.15	245	6.13

[a]$Al_2(SO_4)_3$
[b]Studies 1: cells in mitosis, 3,000 cells per variable.
[c]8,000 cells.
[d]4,000 cells.

as shown in Table VI, but a statistically significant increase was observed in only Study 3 where the highest mitotic index was 8.3% at a concentration of 1 μM ($P < .001$). This same trend can be seen in Studies 1 and 2 where the highest increase in the numbers of cells in mitosis occurred in cultures at the same concentration of 1 μM.

Other species of aluminum were examined in a preliminary way. Aluminum lactate showed a statistically significant increase in MI at 2 μM in one of two studies. Two studies with aluminum citrate showed numerical, but not statistically significant, increases in mitotic index at 2 and 20 μM concentrations. Finally, in one of three studies, aluminum maltol showed a decrease ($P < .01$) in MI at 0.1 μM.

DISCUSSION

It is clear from these studies that $AlCl_3$ concentrations of 2–4 mM affect blastogenesis, mitotic index, and cell cycle time in human short-term whole blood cultures by increasing the latter and decreasing BTI and MI. Although safeguards such as limiting serum concentrations have been recommended to protect uremic individuals undergoing dialysis [Alfrey, 1984], it should be realized that concentration accumulations may increase over chronic exposures [Tsou et al., 1991; Priest, 1993]. At such concentrations, it is possible that BTI may decrease. Since BTI is an indicator of immune response [Hoffmann et al., 1989], it is possible that reduced BTI caused by high levels of aluminum exposure could in turn be associated with reduced immune competence. Similar reductions in MI would affect every tissue that is involved with cell division. Although it may not be the same for higher concentrations [Sharma and Talukder, 1987], from our results, it should be remembered that we have shown that the effect of reduced MI is reversible.

It is also clear that exposure to very low concentrations of aluminum chloride and sulfate consistently exhibited increased mitotic indices in human short-term whole blood cultures, and this trend, although less evident in preliminary studies, was also observed in the other aluminum species studied. Since only a relatively small number of studies were conducted on five species, additional work should be done to confirm the effects, to determine whether the strongly significant increase in mitotic index is unique to aluminum chloride, i.e., a species effect [van der Voet, 1992], and to replicate findings in blood samples from other individuals.

To our knowledge, this is the first time that a positive effect for aluminum chloride has been observed in human material, although similar stimulatory effects of aluminum chloride, sulfate, and hydroxide have been reported in nonhuman cells [Jones et al., 1986; Nashed, 1975; Quarles et al., 1991]. Our optimal concentrations appeared to be about 2 μM for the alumi-

num ion. These concentrations are slightly lower than the range of the serum concentration safeguards recommended for dialysis patients [Elinder and Sjogren, 1986; Tsou et al., 1991] (3.7 to 7.4 μM) but are of the same order of magnitude. (Normal serum concentrations are about 0.037 to 0.37 μM.) On the other hand, we did not see detrimental effects of aluminum exposure until concentrations were well above the recommended safeguards.

Our results as well as the reports from the literature [Nashed, 1975; Jones et al., 1986; Quarles et al., 1991; Carlisle and Curran, 1993] suggest that aluminum may be an essential element for the promotion of optimal growth, development, and maintenance of cells. Further research will confirm this hypothesis.

Our present findings clarify the apparent contradictions in the literature relative to the inhibitory or stimulatory effects of aluminum on mitogenesis and mitosis. Based on our results and a careful review of previous studies, we may conclude that there is a nonlinear relationship between exposure level and effect, and that no contradiction is seen when both the concentration and the species of aluminum are considered.

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