



Genotoxic Effects of Aluminium Acetate by Micronucleus Assay

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Abstract

Aluminium, the mostly used element in the world, and its usage is increasing in many applications. Aluminium acetate (AA) has been used in many therapeutical applications, too. Because of widely usage of AA in medicine, its safety must be clearly defined. This research evaluated the genotoxic effects of AA. It has been known that aluminium had very important toxic effect in long time exposures. It was aimed to determine if AA had same genotoxic effects or not. Genotoxic effects of AA were determined with human lymphocytes MN assay. Control, Negative control (1% DMSO), positive control (MMC, 0.20 µg/ml), 1250, 2500, 5000, 10000, and 15000 ppm doses of AA were used in this assay. MNBC % and NDI values of AA were compared with negative control group. 15000 ppm of AA were observed as EC₅₀ concentration that reduced the mitotic index about 50 %. As a result of MNBC %, there was statistically significant increase in MN frequency at 5000 and 10000 ppm doses. Dose-dependent cytotoxicity were obtained from NDI study. The highest MN frequency and the lowest NDI frequency were obtained from 10000 ppm at 48 h treatment while the lowest MN and the highest NDI frequencies were determined in 1250 ppm at 24 h treatment. This research showed that used concentrations did not produce significant MN frequency but significantly reduced the NDI value when compared to MMC.

Keywords: Aluminium acetate, MN assay, MNBC, NDI, Genotoxicity

1. Introduction

Humans and animals accumulate metals in their tissues, and because of their long half-life periods, metals may cause chronic damages [1]. Metals are known as the oldest toxic materials known by humans [2]. Different types of metals have many side effects such as contamination of foods, water and environment, and may cause some health problems for humans or other living things [3].

The different types of aluminum (Al) have evaluated as systemic toxicants [4], and obtained data about Al indicated that the accumulation of Al in tissues is related with damages of the target organs [5]. Al caused hepatic damages, DNA cross-linking in rat ascites hepatoma cells [6], MN and SCE formations in human peripheral blood lymphocytes [7,8,9].

Al salts may be induced neurodegenerative diseases like Alzheimer [10], Parkinson [11]. DNA, RNA and many mononucleotides [12] and DNA-complexes may have strongly

binded to Al compounds [13].

Daily consumed amount of Al by food and beverages is about 2.7 to 13.4 mg. Drugs can effect the levels Al. Antacid drugs can increased up to 500 mg of Al [14].

Aluminium acetate (AA) is a salt produced by the reaction of aluminium hydroxide and acetic acid. It is a general laboratory reagent used for many qualitative and quantitative chemical experiments. It is widely used as a drug for the treatment of certain diseases of bone, ear (otitis externa), infections in the outer ear canal, as an antiseptic, astringent and as a topical solution to treat severe rashes. It can be used as temporary relief for skin irritations (eczema, diaper rash, acne, poison ivy, insect bites, poison oak, chilblain with detergents, soaps, cosmetics, or other particles). Aluminium acetate can be help to itching and may use for cooling and drying on wet or weeping lesions. [15]. The chemical structure was given in Fig. 1.

AA has induced significant and varied levels of elevation in acetylcholine content and inhibition of acetylcholinesterase activity in all regions of rat brain under both modes of exposure. These findings proved that aluminium might be affecting different phases of metabolic pathway that related with neurotransmitters through endproduct inhibition [16].

Protein metabolism of albino mice has modified with AA [17]. Al decreased the activity of Mg^{2+} dependent to ATPase in cerebrum and cerebellum of rat [18].

Toxicity tests with human peripheral lymphocytes such as chromosomal aberrations (CAs), sister chromatid exchange (SCE) and micronuclei (MN) have been widely used [19,20,21]. MN formation is a biomarker for genotoxicity manifested by two mechanisms, i.e. clastogenic and spindle-damaging actions. Micronuclei (MN) are defined as small, round, formed during cell division by loss of acentric chromatin fragments or whole chromosomes. MN are used as a fast and reliable test for detecting clastogenic or aneugenic activity [22]. In this study, it was aimed to determine if AA had genotoxic effects or not. Genotoxic effects of AA were determined with human lymphocytes MN assay.

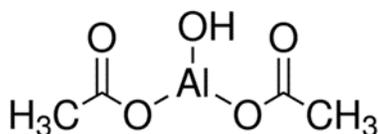


Figure 1 Chemical structure of aluminium acetate basic

2. Materials and Method

2.1 Chemicals

Aluminium acetate (AA) was obtained from Sigma-Aldrich (CAS No. 142-03-0, C₄H₇AlO₅) and dissolved in 1% DMSO. Mitomycin-C, cytochalasin B, chromosome medium B (Biochrom) and Giemsa were obtained from Sigma-Aldrich. The other chemicals were supplied from Merck.

2.2 Lymphocyte Cultures

Whole blood samples were taken from 4 volunteers and were added to heparinized tubes. Samples were obtained from four healthy donors (non-smokers, non-drinkers, not under drug therapy, and aged 22–30 years) under sterile conditions. Voluntary consent form was signed by all donors and the study was performed with the permission of local ethics committee in Üsküdar University Ethics Committee Chairman of Clinical Research (Turkey) with the number 61351342/2013-32 at 23.08.2013.

2.3 Micronucleus Assay

MN analysis was achieved according to [22] Fenech with

some modification. The samples were added to 2.5 ml Chromosome Medium B (including MEM with ascorbic acid, non-essential aminoacids, heparin, fetal bovine serum, glutathione-reduced, penicillin, G-sodium salt, phytohaemagglutinin, and streptomycin sulphate) and incubated at 37°C for 68 h. Cytochalasin B (6 ppm) was put into the medium to block the cytokinesis. Mitomycin-C (MMC, 0.20 ppm) and 1% DMSO was used as positive control, and negative control, respectively. Various concentrations of AA (1250, 2500, 5000, 10000 ppm) were added 24 and 48 h after the incubation. These doses that decreased the mitotic activity more than 50% were used in this study. End of the incubation time, the cells treated with hypotonic solution (0.4% KCl). Cells were centrifuged and fixed with fixative I (methanol:glacial acetic acid, 0.9% NaCl; 5:1:6) about 20 min. Fixation step was repeated twice with fixative II (methanol:glacial acetic acid; 5:1). Microscope slides were setted by dropping cell samples, air drying, and staining with 5% Giemsa solution at pH 6.8 for 14 min. Then slides were washed in dH₂O, and air dried at 25°C.

2.4 Evaluation

Micronuclei were scored from 8000 binucleated cells with well-preserved cytoplasm. Evaluation of the MN protocol was performed according to Fenech [22]. Cell proliferations were analysed, using the nuclear division index (NDI), that determined the average number of cell cycles. Total 2,000 cells were scored to observed the percentage of cells with 1, 2, 3 and 4 nuclei. NDI was calculated using the formula: $[(1 \times M1) + (2 \times M2) + (3 \times M3) + (4 \times M4)] / N$; where M1–M4 represent the number of cells with one to four nuclei and N is the total number of scored cells [22]. The results were statistically evaluated with Dunnett's t-test (two sided).

3. Results

Table 1 presents the data obtained for the MN test. Control, negative control (1% DMSO), positive control (MMC, 0.20 ppm), and different doses of AA (1250, 2500, 5000, 10000, 15000 ppm) were used in MN test system. Before the test, cytotoxic doses were determined, so 15000 ppm of AA were observed as EC₅₀ concentration that reduced the mitotic index about 50%. Doses that were not accepted as cytotoxic, were used in MN tests. In MNBC % determination study, it was observed that there was statistically significant increase in MN frequency at 5000 and 10000 ppm doses in all time intervals, compared with that of the negative control. But these results showed that used concentrations did not produce significant MN frequency like MMC. Binucleated cell photos with micronucleus were given in Fig. 2. Dose-dependent cytotoxicity were obtained from NDI study, so all concentrations of AA were found statistically significant in all treatment periods. As a result of NDI values, 5000 and 10000 ppm concentrations of AA effected

the nuclear division at least as positive control. The highest MN frequency and the lowest NDI frequency were obtained from 10000 ppm at 48 h treatment while the lowest MN and the highest NDI frequencies were determined in 1250 ppm at 24 h treatment. The positive control MMC induced a highly significant increase in MN frequency (7.99 ± 0.09) and a decrease in NDI (1.21 ± 0.06) at 48 h.

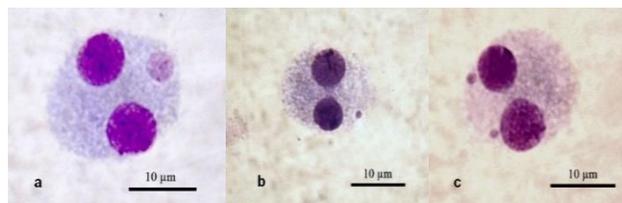


Figure 2 Binucleated cells a) with big micronucleus b) with small micronucleus c) with two micronuclei.

Table 1. The results of the MN assay with aluminium acetate

Test Substance	Time (h)	Dose (ppm)	MNBC (%) \pm SD	NDI \pm SD
Control (-)Control (+) Control A. acetate	24	-	0.40 \pm 0.08	2.07 \pm 0.01
			0.54 \pm 0.05	1.77 \pm 0.05
		0.20	4.10 \pm 0.25*	1.38 \pm 0.13*
		1250	0.51 \pm 0.09	1.60 \pm 0.05*
		2500	0.58 \pm 0.11	1.54 \pm 0.05*
		5000	0.74 \pm 0.11*	1.38 \pm 0.06*
		10000	0.84 \pm 0.12*	1.30 \pm 0.05*
Control (-)Control (+) Control A. acetate	48	-	0.52 \pm 0.06	2.08 \pm 0.03
			0.62 \pm 0.06	1.73 \pm 0.04
		0.20	7.99 \pm 0.09*	1.21 \pm 0.06*
		1250	0.66 \pm 0.09	1.53 \pm 0.04*
		2500	0.81 \pm 0.09	1.50 \pm 0.05*
		5000	0.88 \pm 0.11*	1.33 \pm 0.05*
		10000	0.99 \pm 0.17*	1.27 \pm 0.05*

*The mean difference is significant at the 0.05 level, SD: Standard deviation, MNBC: micronucleated binuclear cells, NDI: nuclear division index, MMC: Mitomycin-C.

4. Discussion

Since AA has many applications, particularly as a therapeutic agent, the evaluation of its genotoxic effect is practically relevant to arrive at a safer mode of exposure. AA can be caused possible irreversible effects by inhalation, swallow, and in contact with skin [23].

The behavioral changes exhibited by the rat exposed to acute and chronic doses of Al coincided with the selected time intervals/days at which the fluctuations in the cholinergic system occurred. These behavioral changes included adipisia (lack of drinking), aphagia (lack of eating), hypokinesia (reduced locomotor activity), fatigue, seizures, lacrymation, salivation, etc. [16]. Also Al has mutagenic activity. Mutagenic effects of waste materials remaining from Al products was evaluated with the Salmonella/microsome assay. All of the waste materials had mutagenic effects, notably in the YG1024 strain [24].

Moreno et al. [25] indicated that a mixture of potassium

aluminum silicates (98%) and sodium dioxide (2%), atmospheric dust from the city of Mexicali, Mexico, induced chromosomal aberrations with the Balb-c 3T3 cell line.

MN frequency was increased slightly in 5000 and 10000 ppm doses of AA. The origin of these micronuclei could be from chromosomes or fragments. In Fig. 2 we showed the binucleated cells with big and small micronuclei and they could be originated from chromosomes or fragments, respectively. Clastogenic and aneugenic effects of toxic metal salts originated from Al was observed with Allium test [26, 27]. Moreover, genotoxic activity of AlCl₃ by Vicia faba cytogenetic assay showed that Al (for 12 h) causes significant increases the MN frequencies and anaphase chromosome aberrations [28]. These researchers supported our results with their demonstration about aneugenic and clastogenic effects of Al. Micronuclei were defined as lose of chromosomes or fragments. So micronuclei can be occur from aneugenic and clastogenic effects of tested materials. But these reserchers were used maximum 270 ppm of Al. But in this study, used concentrations of Al were higher



than these researches. So it can be demonstrated that human lymphocytes are less sensitive to Al and its derivatives than plantal cells.

Al also related with the mitotic components preventing tubulin polymerization, and it was demonstrated that Al had strong cytotoxic effects on microtubules [3]. Lack of chromosomes can be observed as big micronuclei, derived from disturbance of tubulin polymerization. In this research a great number of big micronucleus were observed.

5-25mM in lymphocytes, 1 mM in plant cultured cells, and 34 mg/kg mice body weight, are the highest metal toxicity levels to the DNA molecule and the mitotic components [3]. In this study, greatest toxic concentration was found 15000 ppm as EC50 and 5000 and 10000 ppm concentrations of AA induced micronucleus formation.

Despite the failure of AA to induce MN after a single exposure, its genotoxic effect in adult mice is evident from chromosomal and sperm parameters. Induction of MN in fetal erythrocytes suggests that fetal tissues are more sensitive than those of adults to the toxic effects of AA. Under the test conditions of this study, AA at doses ≥ 50 mg/kg was genotoxic in mice. In this research, human blood lymphocytes were used and >10000 ppm dose was accepted as genotoxic with in vitro MN test.

It is well known that cytotoxicity is a direct consequence of DNA/chromosomal damage. Similar to our study, AA-induced toxic effect on cell proliferation in bone marrow was confirmed by the significant reduction in MI and MN tests. This mito-depressive effect was observed only at the higher doses of AA, i.e., 100 and 150 mg/kg bw. At the lower dose (50 mg/kg bw) there was no such effect at any time interval. The major type of AA-induced chromosomal abnormality as noted in the present study is stickiness [29]. Highest genotoxic dose of AA that induced MN frequency was 10000 ppm and the lowest dose used in this study (1250 ppm) had not genotoxic effect at any time.

It has been known that Al has many toxic effects but AA, its derivative form, has been used for many therapeutic applications. This research was aimed to determine if AA had genotoxic activity or not by MN test. It was observed that higher concentrations of AA induced the MN frequency and reduced the NDI. There was statistically significant increase in MN frequency at 5000 and 10000 ppm doses in all time intervals, compared with that of the negative control. But these results showed that used concentrations did not produce significant MN frequency but significantly reduced the NDI value when compared to MMC. These results must be supported by in vivo studies for confirmation of genotoxic effects.

References

1. Zatta, P, Lain, E, Cagnolini, C, Effect of aluminium on activity of kreb's cycle enzymes and glutamate dehydrogenase in rat brain homogenate, *European Journal of Biochemistry*, 2000, 267, 3049–3055.
2. Ferrer, A, Metal poisoning, *Anales del Sistema Sanitario De Navarra*, 2003, 26, 141–153.
3. Lima, P.D.L, Vasconcellos, M.C, Montenegro, R.C, Bahia, M.O, Costa, E.T, Antunes, L.M.G, Burbano, R.R, Genotoxic effects of aluminium, iron and manganese in human cells and experimental systems: A review of the literature, *Human and Experimental Toxicology*, 2011, 30(10), 1435–1444.
4. Exley, C, Burgess, E, Day, J.P, Jeffery, E.H, Melethil, S, Yokel, R.A, Aluminum toxicokinetics, *Journal of Toxicology and Environmental Health*, 1996, 48, 569–584.
5. Becaria, A, Campbell, A, Bondy, S.C, Aluminum as a toxicant, *Toxicology and Industrial Health*, 2002, 18, 309–320.
6. Wedrychowski, A, Schmidt, W.N, Hnilica, L.S, The in-vivo crosslinking of proteins and DNA by heavy metals, *Journal of Biological Chemistry*, 1986, 261, 3370–3376.
7. Migliore, L, Cocchi, L, Nesti, C, Sabbioni, E, Micronuclei assay and FISH analysis in human lymphocytes treated with six metal salts, *Environmental and Molecular Mutagenesis*, 1999, 34, 279–284.
8. Banasik, A, Lankoff, A, Piskulak, A, Adamowska, K, Lisowska, H, Wojcik, A, Aluminum-induced micronuclei and apoptosis in human peripheral-blood lymphocytes treated during different phases of the cell cycle, *Environmental Toxicology*, 2005, 20, 402–406.
9. Geyikoğlu, F, Türkez, H, Keles, M.S, The role of fruit juices in the prevention of aluminum sulphate toxicity in human blood in vitro, *Fresenius Environmental Bulletin*, 2005, 14, 878–883.
10. Ribes, D, Colomina, M.T, Vicens, P, Domingo, J.L, Effects of oral aluminum exposure on behavior and neurogenesis in a transgenic mouse model of Alzheimer's disease, *Experimental Neurology*, 2008, 214(2), 293–300.
11. Hirsch, E.C, Brandel, J.P, Galle, P, Javoyagid, F, Agid, Y, Iron and aluminum increase in the Substantia-Nigra of patients with Parkinson's-Disease An X-raymicroanalysis, *Journal of Neurochemistry*, 1991, 56, 446–451.
12. Ganrot, P.O, Metabolism and possible health effects of aluminium, *Environmental Health Perspectives*, 1986, 65, 363-441.
13. Matsumoto, H, Changes of the structure of pea chromatin by aluminium, *Plant and Cell Physiology*, 1988, 29, 281-287.
14. WHO, Guidelines for drinking-water quality recommendations, Geneva, Italy, WHO, 1996.
15. Wikipedia, Aluminium acetate, http://en.wikipedia.org/wiki/Aluminium_acetate#cite (accessed 17.07.2015).
16. Yellamma, K, Saraswathamma, S, Nirmala Kumari, B, Cholinergic System Under Aluminium Toxicity in Rat Brain, *Toxicology International*, 2010, 17(2), 106–112.



17. Sushma, N.J, Sivaiah, U, Suraj, N.J, Kishore, S, Javantha Rao, K, Aluminium acetate induced oxidative stress in brain of albino mice, *Journal of Pharmacological Toxicology*, 2006, 1(6), 579–584.
18. Nehru, B, Bhalla, P, Garg, A, Evidence for centrophenoxine as a protective drug in aluminium induced behavioural and biochemical alterations in rat, *Molecular and Cellular Biochemistry*, 2006, 290(1-2), 33–42.
19. Çelik, M, Ünal, F, Yüzbaşıoğlu, D, Ergun, M.A, Arslan, O, Kasap, R, In vitro effect of karathane LC (dinocap) on human lymphocytes, *Mutagenesis*, 2004, 20, 101–104.
20. Rencüzoğulları, E, İla, H.B, Kayraldız, A, Arslan, M, Budak Diler, S, Topaktas, M, The genotoxic effect of the new acaricide etoxazole, *Russian Journal of Genetics*, 2004, 40(11), 1300–1304.
21. Gomez-Arroyo, S, Diaz-Sanchez, Y, Meneses-Perez, M.A, Villalobos-Pietrini, R, De Leon-Rodriguez, J, Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides, *Mutation Research*, 2000, 466, 117–124.
22. Fenech, M, The in vitro micronucleus technique, *Mutation Research*, 2000, 455, 81–95.
23. Sushma, N.J, Sivaiah, U, Suraj, N.J, Kishore, S, Javantha Rao, K, Lipid peroxidation and some oxidative stress enzymes in aluminium acetate intoxicated albino mice, *Toxicology International*, 2009, 16(1), 1–4.
24. Varella, S.D, Pozetti, G.L, Vilegas, W, Varanda, E.A, Mutagenic activity in waste from an aluminum products factory in Salmonella/microsome assay, *Toxicology In Vitro*, 2004, 18, 895–900.
25. Moreno, E.A, Rojas, G.F, Frenk, F.H, De La Huerta, A.O, Belmares, R.Q, Vargas, A.R.O, In vitro induction of abnormal anaphases by contaminating atmospheric dust from the City of Mexicali, Baja California, Mexico, *Archives of Medical Research*, 1997, 28, 549–553.
26. Dovgaliuk, A.I, Kaliniak, T.B, Blium, I.B, Assessment of phytoand cytotoxic effects of heavy metals and aluminum compounds using onion apical root meristem, *Cytology and Genetics*, 2001a, 35, 3–9.
27. Dovgaliuk, A.I, Kaliniak, T.B, Blium, I.B, Cytogenetic effects of toxic metal salts on apical meristem cells of *Allium cepa* L. seed roots, *Cytology and Genetics*, 2001b, 35, 3–10.
28. Yi, M, Yi, H, Li, H, Wu, L, Aluminum induces chromosome aberrations, micronuclei, and cell cycle dysfunction in root cells of *Vicia faba*, *Environmental Toxicology*, 2010, 25, 124–129.
29. D'Souza, S, Vijayalaxmi, K.K, Naik, P, Assessment of genotoxicity of aluminium acetate in bone marrow, male germ cells and fetal liver cells of Swiss albino mice, *Mutation Research*, 2014, 766, 16–22.