The addition of acetic acid or ethanol to orally taken aluminum salt solution enhanced its availability and then induced toxic effects

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Fac Med Baghdad 2010; Vol. 52, No. 3
Received Sept.2009
Accepted Nov. 2009

Summary

Background:Aluminum (Al) intoxication was initially reported in patients undergoing hemodialysis and then was linked to Alzheimer's disease. Man usually is exposed to Al containing antacids, vaccines or foods cooked in Al utensils. The assumption of ingesting sour juices as acetic acid or ethanol may affect favorably the degree of Al absorption is justified when taken in conjunction with the above mentioned appetizers.

Materials and methods: Four groups of 10 mature male rats each were used. The drinking water (DW) containing 5mM of $Al2(SO_4)_3$ with 1% glacial acetic acid and/or 10% ethanol made available ad-libidum. Weekly body weight and each other day drinking water volume were measured. Brain and plasma analysis at and Ca were determined from blood obtained by cardiac puncture, in addition to automated complete blood count (CBC), after 10 wks of treatments.

Results: The results of plasma Al levels indicated that, the addition of acetic acid or ethanol to Al containing drinking water enhanced significantly its absorption and even more when combined relative to control. This enhancement was evident as well in increased Al and Ca deposited in the brain while no change in plasma Ca. The CBC results showed highly significant thombocytopnia for the 1st time, in addition to microcytosis and hypochromia. Evidently, thrombocytopoietin synthesis, and/or action is blocked by Al from acting on the cellularity of bone marrow.

Conclusion: The addition of acetic acid and/ or 10% ethanol to Al containing drinking solution enhanced Al absorption when acid is used and an addative effect when both are used. The toxic plasma level caused hypochromia, microcytosis and severe thrombocytopenia in addition to brain atrophy.

Key words: Aluminum toxicty, Acetic acid, Ethanol, Microcytosis, Thrombocytopenia

Introduction:

Aluminum (Al) salts present abundantly in earth crest where human beings are inevatibly ingesting Al in foods and drinking water especially in acid rains areas(1), foods cooked in Al containing utensils(2), besides the commonly taken tea, bakeries processed with baking powder and foods ingested with lemonjuice(3), (table 1).Nasal lining is another route of contamination is air-born particles of Al salt or the metal itself inspired with air and carried by olfactory neurons to the brain(4). The use of Al containing antacid or vaccine is still another form of Al exposure although limited (5).The initial reports on Al intoxication was in patient under going hemodialysis and children on infusion nutrition(6).Based on the associated symptoms it was linked to Alzheimers disease(7). Despite the alarming amount of ingested Al salts, the bioavailability of the element is only 0.3% of the ingested Al(8) and the absorbed fraction is mostly excreted with bile and urine(9).The objective of this project was to test the assumption that drinking Al containing solution acidified with acetic acid as a weak organic acid and/or with ethanol could affect its bioavailability and may induce toxic effects in various sites in rats.

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Materials and methods:

Mature litter-mate male rats (Sprague Dawley) weighing 85.3±2.95 grams, were used to investigate the effects of ingesting 5mM/L of $Al_2 (SO_4)_3. 18H_2O$ in drinking water ad-libidum alone as control or with 1% of glacial acetic acid and/or 10% ethanol with ample supply of rats pellets. The drinking water with Al salt made palatable by adding 5.0% of glucose presented to all animals.

The animals were grouped into 10 animals in each designed to include a control ingesting the usually tap water without Al (C+O), group Al+O received the Al salt solution, group Al+A received the salt solution with the acetic acid, group Al+E received the salt solution with ethanol and finally group Al+A+E received Al salt with both the acid and ethanol.

The project last for 10 weeks. Throughout the project, body weight and volume of fluid drank were measured weekly and each other-day respectively. Initially, 2-25 ml of blood was obtained from all animals under ether anesthesia by cardiac puncture in heparinized syringes as controls. On the scheduled day of termination, again 3.0-3.5 ml of blood sample was withdrawn from each animal again by cardiac puncture for complete blood counts (CBC) by an automated digital Cole counter and the levels of Al and Ca were determined in plasma and brain after ashing using atomic absorption spectrophotometer (SP90). The collected data was tabulated and analyzed statistically and express in terms of means ± standard deviation, (X ±SD) and the differences were evaluated using student t-test of paired variance.

Results:

All aluminum ingesting animals were appearently normal showing no ill signs throughout the project. The volume of daily fluid drank decreased in groups Al+O and Al+A relative to control (C+D) but increased in groups taking the ethanol (Al+E and Al+A+E). similarly, the mass of the left hemisphere and body gain decreased progressively with the addition of the acid and then the ethanol (fig 1). The aluminum levels in plasma showed progressive rise in all groups indicating that acidification or the additional of ethanol enhandec its bioavailability with further enhancement when both were added (group Al+E and Al+A+E), on the other hand, plasma Ca levels showed no significant differences. The Al and Ca contents of the brain showed progressive and significant increased deposition in all groups, (Table 1). This is in addition to significant decreases in RBC counts, Hb conents and PCV percentages which induced significant drops in MCV and MCH. ($P<0.01$). and there were drastic drops in platelets counts and its MPV ($P<0.001$), (Table 2).

Table1a: Aluminium concentration in some food, mg/100gm

Cheese	29.7	Cinnamon	40.4
Fish	0.04	Black pepper	14.3
Cream	13.9	Thyme	75
spinach	2.5	Basil	30.8
Tea-tea bag	128	Backing powder	2300

Table 1b: Concentrations of Aluminium in foods before and after cooking in aluminium and stainless steel cookware- ppm wet wt

Food product	Uncooked	Cooked in Al utensils	Cooked in stainless steal utensils
Apple sauce	0.13	7.1	0.12
Cabbage	0.13	3.6	0.2
Beef	0.19	0.85	0.16
Chicken	0.47	1.0	0.66
Tomato sauce	0.1	57.16	0.16

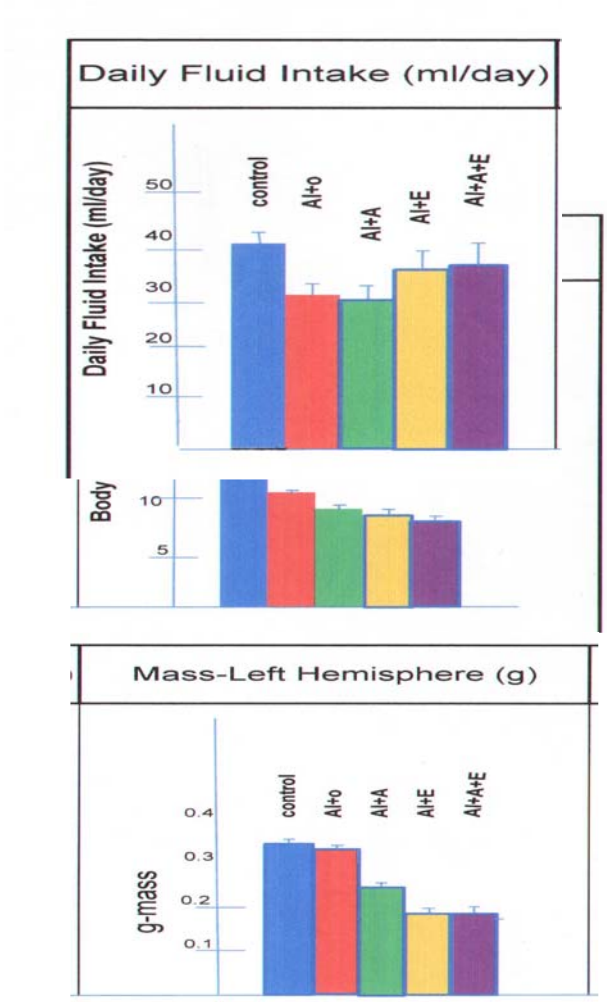

Fig. Shows the effect of oral intake of 5mM of aluminum sulphate in drinking water alone (control) with the addition of 1% acetic acid (Al+A), 10%ethanal (Al+E) or both (Al+A+E) on daily fluid intake (ml/day) mass of left hemisphere-g and body gain g/wk in rats (X±SD) for 12 weeks

Table (2): shows aluminum (Al) and Calcium (Ca) contents in plasma and brain in rats ingesting 5mM Al2 (so4)3 for 12 weeks

Mineral content	Plasma		Left hemisphere	
Groups n = 10	Al $mgdL^{-1}$	Ca $mgdL.^{-1}$	Al ppm	Ca ppm
Control no Al (C + O)	0.073±0.001↓ * +++	9.96±0.32 ↓ * -	2.27±0.14 ↓ * +++	415.01 ± 10.32 ↓ *
CotroI+AI (Al+O)	1.531±0.025 +	8.93±0.37 -	29,59±0,93 ++	592.35±10.71 ++
AI+Acetic Acid (Al+A)	1.613±0.02	8.92±0.46	44.58±0.95	1481.0±22,3
Control AI (Al+ O)	1.531±0.025 +	8.93±0.37 -	29.59±0.93 +	592,33±10.71 +++
Al± Ethanol (AI+E)	1,674±0.022	8.21±0.31	33.45±0.98	1537,12±2.1.28
Control+Al (Al+O)	1.531 ±0.025 ++	8.93±0.37 -	29.59±0.93 ++	592.35± 10.71 +++
Al+AceticA+Ethanol (Al+A+E)	1.758±0.026	8.31 ±0.29	35.87±0.84	1638.09±13.92

*** P- degree of significance**
- non significant
+ <0.05
++<0.01
+++<0.001

Table (3A, B): Shows the complete blood counts and plasma Al levels in rats ingested 5ml M of aluminum sulphate with 1% acetic acid and/ or 10% ethanol for 12 weeks (anb).

Parameter / Groups	RBC ($10^6/mm^3$)	Hemoglobin Hb($g^{d1}\text{-}^{1}$)	PCV (%)	MCV (f)**
Control (noAl) (C-O)	5.44±0.19↓ **** -	11.09 ±0.26 ↓ -	31.25 + 0.61 ↓ -	54.58 + 0.92↓ -
Control +AI (Al+O)	5.97 + 0.35 ↑	11.37 + 0.64↑	31.48+ 1.64 ↑	53.51 + 1.72 ↑
Control +AI (Al+O)	5.97 + 0.35	11.37 + 0.64 -	31.48+1.64 +	53.51 + 1.72 +
Control+Acetic acid (Al+A)	6.78+ 0.21 +	12.14 + 0.22	34.61 ±0.95	51.35+1.15
Control+AI (C+Al)	5.97 + 0.35 ++	11.37 + 0.64 ++	31.48 + 1.64 ++	53.51 ± 1.72 ++
Al+Ethanol (Al+E)	7.56 + 0.11	13.22 + 0.23	37.58 + 0.48	49.75 + 0.83
Control+AI (A+O)	5.97 + 0.35 +++	11.37 + 0.64 +++	31.48+ 1.64 +++	53.51 + 1.72 ++
Al+Acetic A+ Ethanol (Al+A+E)	8.84 + 0.716	15.14+ 1.34	42.44 + 3.71	48.58+1.64
Trend of generalized effects	Increase	Increase	Increase	Decrease

Table (3): B

Parameter / Groups	MCH (P)**	Platelets (10^3 /mm^3)	MPV (f)	Plasma Al mg $^{dl\ -1}$
Control (noAl) (C-O)	18.82 ± 0.59 ↓	1175.21 ±42.35 ↓	7.62 ± 0.25 ↓	0.07 ±0.001 ↓
Control +AI (Al+O)	18.87 ±0.82 ↑	1131.12 ± 52.13 ↑	7.41 ±0.51 ↑	+++ 1.53±0.025↑
Control +AI (Al+O)	18.87± 0.82	1131.12 ±52.13	7.41 ±0.51	1.53 ± 0.025
Control+Acetic acid (Al+A)	- 18.02 ±0.48	+ 1063.92± 54.68	- 7.46± 0.43	- 1.61 ±0.024
Control+AI (C+Al)	18.87 ± 0.82	1131.12 ±52.13	7.41±0.51	1.53 ±0.025
Al+Ethanol (Al+E)	+ 17.44 ± 0.38	++ 832.61 ± 14.34	- 7.25±0.91	- 1.69 ±0.022
Control+AI (C+Al)	18.87 ±0.82	1131.12 ±52.13	7.41 ±0.51	1.53 ±0.025
Al+Ethanol (Al+E)	+ 17.28 ± 0.57	+++ 634.21 ± 84.28	+ 6.73 ± 0.51	++ 1.75 ± 0.025
Trend of generalized	Decrease	Decrease	Decrease	Increase

*** n=10/ group**
**** f- femtoliter (10^{-15}liter)**
*****p- picrogram(10^{-12}g)**
******p- degree of significancey**
- **P<0.05**
- **+ p<0.01**
- **+++p<0.001**

Discussion:

The reported results revealed that the acidified Al solution with acetic acid as a drinking fluid increased Al absorption which confirmed previous finding when lemon juice as citric was used instead (10). The addition of acetic acid or may be other weak organic acid as citric and lactic acids enhanced solubility of Alsalts while phosphorus and silicone reduce it (11). The elevated enhancement is likely to involve the formation of aluminum-organic complexes which permeate paracellarly by opening the tight junction that present in between gastric lining cells (12). Apparently, the addition of ethanol also enhanced Al absorption through gastric cell membrane as ethanol being a typical fat soluble compound. This new finding ought to be confirmed.

The noticeable increase in volume of Al solution drank by animals taking the ethanol is attributed to the diuretic effect of ethanol which inhibits vasopressin hormone synthesis. The increased Al deposited in the brains of all animals ingesting the solution despite the presence of βββ is based on previous finding where the rise in plasma Al levels distrupts the barrier allowing more metal to permeate (13). The increase in Al deposited in brain as well in bone causing the latter to demineralize and release of Ca to be deposited in the brain with Al. This bone resorption creating a case of osteomalacia with increased risk of bone fragility fractures. (14)

Despite the liberated Ca from bones, plasma level was stable with optimum homeastasis. The progressive decrease in left hemisphere mass indicates undoubtedly that Al producing necrosis of brain neurons leading to brain atrophy. This finding has been observed earlier in Alzheimers diseased patients suffering from dementia with the presence of Al in the neuronal tangles mainly in the hippocampus that characterized the histopathologic changes observed in AD brains. (15, 16) .The published reports about the effect of elevated plasma Al on blood indices are inconsistant in experimental animals because of different protocols used with respect to Al solubility, dose, duration and route of administration(10,17,18). Patient under prolonged hemodialysis and children under infusion nutrition were with elevated plasma Al and low in Hb content with hypochromia (19). In the current study where 5mM of $Al_2 (SO_4)_3$. $18H_2O$ was given orally for 10 weaks to mature male rats of Sprague Dowley showed trend of rise in RBC count, Hb content and PCV percentage especially in animals taking the alcohol where plasma Al was relatively high coinsided with microcytosis and to a limited extent hypochromia. Further toxic effects of Al were microthrombocytosis and drastic thrombocytopenia and which is reported for the 1st time and so it needs to be confirmed. The possible mechanism could explain the reduced platelets count that Al in acidic

an amphoteric element that is behaves as an anion in alkaline medium and as cation in acidic medium binds to oxygen donor as acetate or lactate causing peroxidative damage of platelets membranes (20,21). The peroxydative damage of platelets membranes is likely the eause of thrombocytopenia. Further more, the elevated plasma Al may depress thrombocytopoietic hormone synthesis and/or blocks its action. This humoral regulator of platelets production is an acidic glycoprotein produced in the liver. Kidney and bone marrow. (22)

Conclusion:

The reported data that is oral intake of 5mM of $Al_2(SO_4)_3$ $18H_2O$ in drinking solution acidified with 1% acetic acid and/or the addition of 10% ethanol in drinking solution for 10 weeks build up a toxic plasma level induced hypochromia, microcytosis with severe thrombocytopenia and microthrombocytosis. This is in addition to elevated brain Al and Ca deposite with reduced mass or generalized atrophy and possible dementia. These findings should caution the general public to avoid using uncoated Al containing utensils and excessive use of acidified dressing and the over use of alcohol with the meals.

References:

1-Rogers, N. J.: Carson, K. C.; Dilworth M. J.; Hughes, M. N.;Poole, R. K. Alleviation of aluminum toxicity to Rhizobium Leguminosarum b v. viciae by hydroxyamate siderophore vicibactin. Biometals March 2001; 14(1): 29-66.

2-Yokel R. A. and Florence RL. Aluminum bioavailability from additive leavening agent acidic sodium aluminum phosphate, incorporated into a baked good, is lower than from water. Toxicology Oct. 2006; 277(1-2): 86-93.

3-Soni, M. G.; white. S. M.; Flamm. W.G. and Burdok, G.A. safety evaluation of dietary aluminum. Requlotory Toxicol pharmacol 2001; 33:66-79.

4-Divine. K. K.; Lewis. J. L.; Grant, P. G. and Bench. G. Quantitative particle-induced X-ray emission imaging of rat olfactory epithelium applied to permeability of rat epithelium to inhaled aluminum. Chemical research in toxical, 1999; 12: 575-81.

5-War, MK. Aluminum Toxicity and people with impaired renal function. In Alzheimer Disease and the Enviroment (Lordwalton editor). Rol socmed services, Round Table series. 1991; no. 26, pp. ????

6-Yokel, RA.; IncNameua, PJ. Aluminum toxicokinetic: An up dated minireview. Pharmacol toxicol, 2001, 88:159-67.

7-Mahieu, S.; del-Carmen-contini, M.; Gonzalez. M.; Millen, N., Zlies, N. M. Aluminum toxicity. Hematologic effects. Toxicol letters, Jan, 2000; 111(3): 235-42.

8-Flarend, R.; Bin, T; Zlmore. D. and Hem S. L. Aprelimory study of the dermal absorption of aluminum from antiperspirants aluminum 26: Food and chemical toxicol, 2001; 39: 163-68.

9- Gonzalez, R. J., Casares, M.; del-paula, M., pascual, T.; Giner, V. And miravalles, E. Biochemical and hematolosical changes in low-levels aluminum intoxicatio. Clin chom Lab med, March, 2000; 38(3):221-25.

10- Vittore. D., Nesse. A., Perez. G. And Garbossa, G. Morphologic and functional atteratium of erythroid cells in duced by long term ingestion of aluminum. J. Inorg Biochem, Aug. 1999; 76(2): 113-20.

11- Taylor, GA.; moore P.B.; Ferrier, IN.; Tyrer, SP. and Edwardson, JA. Gastrointestinal absorption of aluminum and citrate in man. J Inorg Biochem, 1998; 69: 165-69.

12- Yokel, R, A. Blood-brain barrier flux of aluminum, magnesium, iron and other metals suspected to contribute to metal induced neuron degeneration. J Al_3 heimers, Nov 2006, dis 10(2-3).

13- Mjoberg, B., Hellguist, E.; Mallmin, H. and Lindh, U. Aluminum, Alzheimers disease and bone fragility. Acta orthopaedice Scand, 1997; 68:511-14.

14- Altmonn, P.; Cunningham, J., Dhanesha, u.; Ballard, M.; Thompson, J. and March. F. Distrurbance of cerebral function in people exposed to drinking water contaminated with aluminum sulphote: retrospective study of camelfold water incidence. Brit med, J. 1999; 319:807-11.

15- Perl. D. P. and Moalem, S. Alzheimers disease, a personal prospective after 15 years. J Alzhemer's Dis. 2006, 9(3 suppl):291-300.

16- Deng, Z.; Coudray, C.; Gouzoux, L.; Muzur, A.; Rayssiguier, Y. and Pepia, D. Effect of oral aluminum and aluminum citrate on blood level and short-term tissue distribution of aluminum in the rat. Biol Trace Elements Res, Aug. 1998; 63(2):139-47.

17- Galud, MS.; German, S. L., Han. B. and keen, C. L. Lifelong feeding of a high aluminum diet to mice. Toxicol, Sept. 2000; 150(1-3):107-17.

18- Ganchev, T.; Dyankov, E., Zacharieva, R., Pachalieva, I. Velikova, M. And Kavaldjieva, B. Influence of aluminum on erythropoiesis, irox metabolism and functional characteristic of enythrocytes in rats Acta physid pharmacol Bulg, 1998; 23(1): 27-31.

19- Campbell, A. and Bondy, S. C. Aluminum induced oxidative event and its relation to inflammation: a role for the metal in Alzheimer's disease. Cellular and molecular Biol, 2000; 46: 721-30.

20- Rath, I. and Barz, W. the role of lipid proxidation in aluminum toxicity in soybear cells suspension cultures. Z. Naturforsch [C], Nov. 2000; 55(11-12): 957-64.

21- Kaushansky, K. the molecular mechanisms that control thrombopoiesis. J. Clin Invest, Dec. 2005; 15(12); 3339-3347.