

Accumulation and Depletion of Aluminum from Various Tissues of Rats Following Aluminum Citrate Ingestion¹

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ABSTRACT. Accumulation of aluminum in various tissues has been associated with a variety of disorders such as Alzheimer dementia, Parkinsonism-dementia of Guam, dialysis dementia, amyotrophic lateral sclerosis, and vitamin D-resistant osteomalacia. Using 18 rats, an experiment was conducted to study the effect of aluminum citrate (AL-C) ingestion on tissue accumulation of aluminum and the effect of time on tissue depletion of this and other minerals. Mature, male Sprague Dawley rats were given free access to a commercial rat diet formulated to meet all their nutrient requirements. Rats were blocked by weight and randomly assigned to 3 groups. In group one, 6 untreated rats were euthanized, and brain, liver, kidney, and bone (tibia) tissues were collected for mineral analysis. The remaining 12 rats were housed in individual cages. Over a 30-d period, these rats were dosed by stomach tube with AL-C solution at a rate of 7.0 mg of Al/100g of body weight. At the end of the 30-d dosing period, group two rats (6) were euthanized and brain, kidney, liver, and bone (tibia) tissues were collected for mineral analysis. The remaining 6 rats were fed the same diet for an additional 30 d but the AL-C dosing was terminated. At the end of this 30-d period these rats were euthanized and tissues were collected for analysis as described above. Data were analyzed as a randomized complete block design with each euthanized group acting as a treatment. Aluminum citrate ingestion increased Al concentration in brain ($P < 0.08$) and kidney ($P < 0.02$). Aluminum citrate ingestion resulted in higher brain ($P < 0.07$), and kidney ($P < 0.07$) Ca and Cu ($P < 0.01$) as well as kidney ($P < 0.06$) Mg concentrations. Aluminum citrate withdrawal tended to decrease concentration of Al in brain ($P < 0.15$) and did decrease Al in kidney ($P < 0.03$). The accumulation of Al in brain, tibia, and kidney tissues is apparently a reversible process.

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INTRODUCTION

Humans and animals live in an environment that is abundant in aluminum (Al). Aside from oxygen (49.3%) and silicon (25.8%), aluminum (7.6%) is the most abundant element by weight in the earth's crust (Petrucci 1997). Many of the beverages and food items consumed by humans and animals are prepared, stored, and served in Al vessels. Over the last 3 decades a number of investigators have associated the accumulation of Al in various tissues with a variety of disorders such as Alzheimer dementia, Parkinsonism-dementia of Guam, dialysis dementia, amyotrophic lateral sclerosis, and vitamin D-resistant osteomalacia in humans (Alfrey and others 1972; Crapper and others 1973; Perl and others 1982; Burnatowska-Hledin and others 1983). Also, research on ingestion of Al in laboratory animals and livestock has shown an adverse effect on P, Ca, and Mg metabolism and Al accumulation in different tissues (Slanina and others 1984, 1986; Allen and others 1986, 1990, 1991). Normally, people with Alzheimer's have high levels of Al in their brain tissue (Crapper and others 1973). Additionally, Al regulation of serine protease activity has been implicated in Alzheimer disease (Clanberg and Joshi 1993). In light of this information, ingestion of Al in humans has become a major concern in recent decades. Although inheritance appears to play a role in some people with Alzheimer's (Miller 1993), it is not yet clear if Al accumulation is the cause/cofactor or the result

of this disease. No literature was found to show if once the dietary Al ingestion has been stopped there is a reduction of Al concentration in the brain and/or other tissues. Therefore, this research was conducted to: 1) determine the effect of Al-citrate ingestion on Al concentration in the brain, liver, kidney, and tibia of mature male rats and 2) whether accumulated Al in various tissues would be depleted once the Al ingestion had been stopped.

MATERIALS AND METHODS

Using 18 male rats (avg wt 314 g), an experiment was conducted to study the effect of aluminum citrate (AL-C) ingestion on tissue concentration of Al and the effect of withdrawal time on tissue depletion of this and other minerals once AL-C ingestion was stopped. Mature male rats (Sprague Dawley) were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN, and housed in individual cages in an environmentally controlled room (22° C, 45% relative humidity, 14 h of light and 10 h of darkness). Prior to the start of the experiment, all rats (18) were fed (30 d) a commercial rat diet (Purina Rat Chow) formulated to meet all their nutrient requirements. After the 30-d preliminary period, rats were blocked by weight and randomly assigned to 3 groups. Block weights were 266, 270, 275.67, 278.33, 285, and 286.67 with 3 rats per block. Protocol for this research was approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee. Six rats (group one) were killed using carbon dioxide gas, and brain, liver, kidney, and bone (tibia) collected for mineral analysis. Over a 30-d period, the second and

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third groups of rats (12) were dosed via stomach tubes, with Al-C solution. Aluminum citrate was prepared by chelating reagent-grade anhydrous $AlCl_3$ with citric acid monohydrate in deionized water (Allen and others 1991). Rats were dosed at a rate of 7.0 mg of Al/100g of body weight (Allen and others 1991). At the end of the 30-d dosing period 6 rats (group two) were killed using carbon dioxide gas and brain, kidney, liver, and tibia were collected for mineral analysis. The remaining 6 rats were fed the same diet (Purina Rat Chow) for an additional 30 d, but the Al-citrate dosing was terminated. At the end of this 30 d, the remaining 6 rats (group three) were killed using carbon dioxide gas and the same tissues collected for mineral analysis. Although food consumption was not recorded, food intake was monitored to ensure that rats were consuming food and were healthy. Rats were weighed at the beginning and the end of each 30-d period. To reduce the possibility of mineral contamination, all tissue samples were collected using stainless steel (SS) scalpel blades, scissors, and forceps. Also, all samples were rinsed with a small amount of deionized water after collection to remove any blood or other possible contaminants. Facia was removed from the tibia using an SS scalpel and forceps and was rinsed with deionized water. All tissues were then lyophilized prior to wet ashing (perchloric acid) using the procedure described by Allen (1971). In addition, bone samples were extracted with ether to remove the lipid material prior to wet ashing. Mineral concentrations were determined using an Inductively Coupled Plasma Optical Emission Spectroscopy instrument. Data were analyzed as a randomized complete block design with each euthanized group acting as a treatment. Means were separated using the Fisher Least-Significant-Difference Test (Wilkinson and others 1993).

RESULTS AND DISCUSSION

Over the 30-d dosing period rats in group two gained 80.84 g while those in group three gained 60.66 g. Also, group three rats gained 61.34 g during the 30-d period that dosing was stopped. The effect of Al-C infusion and withdrawal on Al concentration in various tissues of mature rats is presented in Table 1. Concentration of Al in the brain of rats was higher ($P < 0.08$) after 30 d of Al-C ingestion compared to pre-ingestion of Al-C (6.45 versus 10.76 ppm). These values are within the range reported by Slanina and others (1984) for rats and Burnatowska-Hledin and others (1983) for humans. These results are also in agreement with those reported by Allen and others (1991) working with wethers lambs. Aluminum concentration in brain tissue 30 d after Al-C ingestion was stopped was similar ($P < 0.51$) to pre-ingestion concentrations (7.91 versus 6.45 ppm).

Aluminum concentration in the tibia tended to increase numerically (21.97 versus 32.84 ppm) with Al-citrate ingestion ($P < 0.33$) compared to pre-ingestion levels and to decrease ($P < 0.36$) numerically (32.84 versus 23.31) when Al-C infusion was stopped. This insignificant ($P > 0.05$) post infusion increase is in contrast to the results reported by Slanina and others (1984) and Allen and others (1991) and probably due to the fact that

TABLE 1

Effect of Aluminum Citrate Ingestion and Withdrawal on Al Concentration (ppm) in Various Rat Tissues^a

Treatment	Brain	Tibia	Liver	Kidney
Pre-infusion ^b	6.45	21.97	3.59	3.73
Al-infusion ^c	10.76	32.84	4.56	7.47
Post-infusion ^d	7.91	23.31	4.95	4.30
SE ^e	1.28	3.82	0.94	0.87
Fisher's Least Significant-Difference test				
Probability, pre versus Al-infusion	0.08	0.33	0.43	0.02
Probability, Al-infusion versus post	0.15	0.36	0.76	0.03
Probability, pre versus post	0.51	0.90	0.30	0.62

^aSix observations per treatment, ^bPrior to the start of Al infusion, ^c30d after Al infusion started, ^d30d after infusion was stopped, ^eRepresents pooled standard error of the means

the length of Al ingestion in this experiment was half as long as that reported by Allen and others (1991) and Slanina and others (1984). In the present experiment, concentration of Al in tibia was unchanged ($P < 0.90$) between pre-ingested and 30 d after Al-ingestion had been terminated (21.97 versus 23.31).

Liver concentration of Al was unchanged between pre-ingestion, and the two post-ingestion periods (3.59, 4.56, and 4.95 ppm, respectively). Allen and others (1991) and Alfrey and others (1972) reported increased liver concentrations of Al due to Al-C ingestion.

TABLE 2

Effect of Aluminum Citrate Ingestion and Withdrawal on Ca Concentration (ppm) in Various Rat Tissues^a

Treatment	Brain	Tibia	Liver	Kidney
Pre-infusion ^b	670	294,000	313	381
Al-infusion ^c	2,348	247,000	304	509
Post-infusion ^d	1,434	219,000	282	499
SE ^e	585	32,000	30	45
Fisher's Least Significant-Difference test				
Probability, pre versus Al-infusion	0.07	0.33	0.84	0.07
Probability, Al-infusion versus post	0.30	0.57	0.61	0.88
Probability, Pre versus Post	0.38	0.14	0.48	0.09

^aSix observations per treatment, ^bPrior to the start of Al infusion, ^c30d after Al infusion started, ^d30d after infusion was stopped, ^eRepresents pooled standard error of the means

TABLE 3

Effect of Aluminum Citrate Ingestion and Withdrawal on P Concentration (ppm) in Various Rat Tissues^a

Treatment	Concentration (ppm)			
	Brain	Tibia	Liver	Kidney
Pre-infusion ^b	16,000	112,000	9,335	10,359
Al-infusion ^c	19,000	103,000	8,779	10,793
Post-infusion ^d	16,000	99,000	8,786	8,817
SE ^e	1,800	11,500	241	442
Fisher's Least Significant-Difference test				
Probability, pre versus Al-infusion	0.24	0.64	0.13	0.50
Probability, Al-infusion versus post	0.30	0.81	0.98	0.10
Probability, pre versus post	0.86	0.49	0.14	0.03

^aSix observations per treatment, ^bPrior to the start of Al infusion, ^c30d after Al infusion started, ^d30d after infusion was stopped, ^eRepresents pooled standard error of the means

Kidney concentration of Al was most vividly affected by Al-C ingestion and withdrawal. Ingestion of Al-citrate increased ($P < 0.02$) Al concentration in kidney tissue (7.47 versus 3.73 ppm) and its withdrawal resulted in a reduction ($P < 0.03$) of Al in the kidney (7.47 versus 4.30 ppm). The noted increase in kidney Al may be due to the fact that urine is the main route of excretion of the excess absorbed Al. Consequently, there may have been some urine present in the kidney tissue. No differences ($P < 0.62$) were noted between pre- and 30-d post Al-C ingestion levels of kidney Al (3.73 versus 4.30 ppm).

Tables 2, 3, and 4 present the concentration of Ca, P,

TABLE 4

Effect of Aluminum Citrate Ingestion and Withdrawal on Mg Concentration (ppm) in Various Rat Tissues^a

Treatment	Concentration (ppm)			
	Brain	Tibia	Liver	Kidney
Pre-infusion ^b	805	4,792	646	745
Al-infusion ^c	896	4,754	653	856
Post-infusion ^d	834	4,185	645	722
SE ^e	65	231	18	37
Fisher's Least Significant-Difference test				
Probability, pre versus Al-infusion	0.35	0.92	0.79	0.06
Probability, Al-infusion versus post	0.52	0.12	0.75	0.03
Probability, pre versus post	0.76	0.12	0.95	0.66

^aSix observations per treatment, ^bPrior to the start of Al infusion, ^c30d after Al infusion started, ^d30d after infusion was stopped, ^eRepresents pooled standard error of the means

and Mg, respectively, in various tissues of the rats. Brain and kidney concentrations of Ca were increased ($P < 0.07$) by Al-C infusion. Robertson and others (1983), working with rats, suggested that Al may cause osteomalacia by reducing the relative amounts of parathyroid hormone. This hypothesis may be somewhat substantiated by the lower ($P < 0.33$) numerical values of Ca in the bone due to Al-citrate ingestion (294,000 versus 247,000 ppm) in rats noted here and its increase in the kidney (381 versus 509 ppm) and brain (670 versus 2348 ppm).

Phosphorous concentrations of brain, bone, and liver

TABLE 5

Effect of Aluminum Citrate Ingestion and Withdrawal on Cu and Fe Concentration (ppm) in Various Rat Tissues^a

Treatment	Brain		Tibia		Liver		Kidney	
	Cu	Fe	Cu	Fe	Cu	Fe	Cu	Fe
Pre-infusion ^b	10.09	139.58	0.55	71.80	10.00	698.67	19.14	379.90
Al-infusion ^c	11.14	148.04	1.05	66.49	10.03	861.33	22.92	467.49
Post-infusion ^d	11.23	128.79	0.35	69.28	10.67	889.25	18.45	463.44
Se ^e	0.55	7.66	0.26	10.00	0.36	19.49	0.92	25.63
Fisher's Least Significant-Difference test								
Probability, pre versus Al-infusion	0.21	0.45	0.21	0.72	0.95	0.001	0.15	0.36
Probability, Al-infusion, versus post	0.72	0.11	0.08	0.85	0.24	0.34	0.01	0.91
Probability, pre versus post	0.12	0.34	0.59	0.87	0.22	0.001	0.61	0.40

^aSix observations per treatment, ^bPrior to the start of Al infusion, ^c30d after Al infusion started, ^d30d after infusion was stopped, ^eRepresents pooled standard error of the means

were not affected by Al-citrate infusion in rats (Table 3). These results are similar to those reported by Allen and others (1991). The reason for lower ($P < 0.03$) P concentration in the rats' kidneys after Al-citrate withdrawal is not clear.

Magnesium (Table 4) followed a pattern somewhat similar to that of Ca. This may be expected since both these minerals have similar ionic charges and belong to the same family in the periodic table of elements. Ingestion of Al-C caused an increase ($P < 0.06$) in the accumulation of Mg (856 versus 745 ppm) in the kidneys of rats. Kidney concentration of Mg was reduced ($P < 0.03$) when Al-ingestion was terminated (856 versus 722 ppm).

Concentration of Cu and Fe in various tissues studied are presented in Table 5. Similar to Ca, Cu concentration in the kidney appeared to be increased ($P < 0.15$) by Al-C infusion and was later decreased ($P < 0.01$) when Al-C infusion was terminated. Valdivia and others (1982) working with sheep, also reported an increase in kidney Cu due to Al-C infusion. A similar trend to kidney was noted for the concentration of Cu in the tibia of these rats. Liver Fe concentration increased ($P < 0.001$) due to Al-C ingestion and remained high after Al-C ingestion was stopped for 30 d. Similar increases in sheep liver Fe was reported by Valdivia and others (1982).

In summary, this study showed that Al ingestion may result in Al accumulation in the brain, bone, and kidney tissues of rats. However, the subsequent withdrawal of the dietary Al may also result in a reduction of the accumulated Al from these tissues. Further research with longer aluminum ingestion and withdrawal time would be needed to clarify whether the Al found in the brains of Alzheimer patients is the result of the disease or the cause/cofactor of it.

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