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Effects of Aluminum on Calcium Absorption, Growth and Bone Calcium Content

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ABSTRACT

The present study was designed to investigate the effect of aluminum on growth, calcium absorption and total bone calcium in male Sprague-Dawley rats. Rats were pair-fed on AIN-76A nutritionally adequate modified diet containing different levels of aluminum and calcium for three weeks. After three weeks of feeding, rats were fasted overnight. Half the rats in each group fed on different levels of Calcium and Aluminium were given ^{45}Ca orally by gavage and half by injection in the intraperitoneal cavity. Rats were anesthetized. Left femurs were collected and analyzed for ^{45}Ca activity while the right femurs were analyzed for total calcium content determination. Aluminum had an adverse effect on growth/food efficiency ratio and on bone calcium content while more ^{45}Ca was taken up by the femurs in the calcium deficient groups whether or not supplemented with aluminum as compared with calcium adequate group. The intake of ^{45}Ca by the femur of rats fed on deficient Calcium (0.02%) and 0.25% Aluminum Chloride was less compared with those fed on deficient Calcium and 0% Aluminum Chloride. This indicated the toxic effect of Aluminum on bone mineralization.

Key Words: Aluminum; Calcium absorption; Bone growth

INTRODUCTION

Calcium is an essential dietary mineral for the formation of bones. Ninety-nine percent of the body calcium exists in the bones and the remaining 1% is in the soft tissues as an intracellular regulator. Osteoporosis, a disorder of reduced bone mass which results in vulnerability to fractures, affects more than 25 million Americans (NIH, 1987). This disease could be completely prevented with adequate absorbable dietary calcium in addition to weight bearing exercise and adequate estrogen (Weaver, 1992). Similar research in India and other developing countries show the prevalence of osteoporosis in populations (Nordine, 1966). However, in Pakistan there is a need for research to find out the situation.

Toxicity of aluminum is associated with a number of disorders in man. Excess aluminum consumption has been postulated as a precipitating factor in Alzheimer's disease, microcytic anemia, senile dementia and bone disorders (Ganrot, 1986; Salusky *et al.*,1991; Yumoto *et al.*, 1992; Davenport *et al.*, 1993). In renal patients, aluminum has been used to control hyperphosphatemia. However, this treatment causes dialysis encephalopathy and dialysis osteodystrophy (Salusky *et al.*, 1991). Aluminum toxicity is not limited to renal dialysis patients. Whenever aluminum excretion is less than its intake, toxicity can occur. Most aluminum absorbed in the body is deposited in bones. Aluminum accumulation is associated with a variety of bone disorders including osteomalasia and possibly osteoporosis.

People normally consume 1 to >100 mg aluminum daily, but this level may reach as high as 5g in certain medical conditions such as in patients using phosphate binders and or antacids (Ecelbarger *et al.*, 1994). Aluminum is thought to interfere with the normal absorptive process of calcium; however, the effect has not been quantified and the level of aluminum that is toxic is not known. The mechanism of aluminum interference on calcium absorption is also not certain. One of the long term consequences of excess aluminum exposure is bone disorders. The objective of the present study was to find out the effect of aluminum on calcium absorption, growth and bone total calcium content.

MATERIALS AND METHODS

Animals, diet and experimental procedures. Forty-eight weanling (25–30 g) male Sprague- Dawley rats (Harlen, Indianapolis IN) were acclimated on nutritionally adequate powder chow for two days. When they grew 30 g or more, they were randomly assigned into three groups (n= 16/group) and given an AIN-76A nutritionally adequate modified diet containing different levels of aluminum and calcium for three weeks (Table I). Group 1 was given adequate calcium (0.5%) and no aluminum, group 2 was given 0.02% calcium and

Table I. Composition of the AIN–76 modified diets fed to rats for three weeks

Ingradients (g/Kg diet)	Different levels of Ca and Aluminum		
	0.5% Ca 0% Al	0.02% Ca 0% Al	0.02% Ca 0.25% Al
Casein	200	200	200
Sucrose	32	329.98	327.48
Cornstarch	325	325	325
DL–Methionine	3	3	3
Cellulose	50	50	50
AIN–Vitmix	10	10	10
Corn oil	50	50	50
Choline bitartrate	2	2	2
Mineral mix	30	30	30
$CaCO_3$	5	0.02	0.02
$AlCl_3$	0	0	2.5

0 aluminum, and group 3 was given 0.02% calcium and 0.25% aluminum as AlCl3. Rats were pair fed to control the effect of different amount of food consumed by the rats on different dietary treatment. Groups 1 and 2 were given everyday the average quantity of two days food eaten by the rats in group 3. All the rats had ad libitum access to deionized water. Each rat was weighed twice a week.

After three weeks of feeding, they were fasted overnight (16 hours). Half the rats in each group were given 45Ca orally by gavage and half by injection in the intraperitoneal (IP) cavity. The oral dose was prepared as 25 mg calcium by dissolving calcium ascorbate in double deionized water, labelled with 6uCi of ^{45}Ca. The IP dose was prepared as 6 (Ci ^{45}Ca in normal saline (0.9%). Food was returned to the rats an hour later after the isotope administeration. Rats were anesthetized the following day by injection of xylene ketamine (0.2 ml/100g body weight). Left femurs were collected from each rat, cleaned from the adhering tissues and stored in the freezer for analysis later. Right femurs were collected cleaned and stored similarly as for the left femurs in the freezer at –800°C for total calcium content analysis later.

Analysis

^{45}Ca uptake in femur. Femurs were digested overnight in 2-3 ml concentrated nitric acid. After diluting in 25ml double deionized water, 1ml of the solution was combined with 10 ml scintillation cocktail for counting in (-Scintillaion counter (Beckman, LS6500 multipurpose Scintillation Counter, Fullerton, CA). The ^{45}Ca uptake in the femurs was calculated as a per cent of the ^{45}Ca dose administered orally or injected in the IP cavity.

Food efficiency ratio (FER). FER was calculated by dividing the weight gain of each rat over the total amount of food consumed per rat in three weeks period.

Total Calcium content of the femurs. Femur bones were defrosted, weighed and dried in vacuum oven at 700°C overnight. They were weighed again after drying and then ashed in the muffle furnace (Thermolyne Sybron Type 30400, Dubuque, IA) at 6000C. Total calcium content of the femurs was determined by the standard method using atomic absorption spectrophotometry (Perkin-Elmer 5100 PC, Norwalk, CT).

Statistical analysis. Comparisons of FER, ^{45}Ca uptake and calcium content of the femur bones of the rats from different treatment groups were evaluated by one way analysis of variance (ANOVA), followed by student-Newman-Keuls multiple range test to compare the means. Statistics were performed on the Statistical Analysis System (SAS). A p value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Per cent ^{45}Ca uptake in femurs. Per cent uptake of calcium from the oral and IP dose was calculated on per gram basis of the bone because of the fragility of the femurs. Removal of the femurs was extremely difficult and they were usually collected as broken bones. There was a significant difference between ^{45}Ca uptake in the femurs of rats on calcium-adequate no-aluminum diet vs. calcium deficient diet with or without aluminum from oral dose. However, from the IP dose, all the three groups were significantly different from each other in per cent dose of ^{45}Ca uptake (Table II).

Table II. Femur uptake of ^{45}Ca and total calcium content of femur in rats fed various levels of calcium and aluminum for three weeks.

Groups	Mean ± S.D. (n=8)		
	Uptake of ^{45}Ca/g femur		mg total Ca/g femur
	% Oral dose	% IP dose	
0.5% Ca, 0% Al	16.8±1.9 ^{b*}	26.1±3.8^{c}	93.3±2.2^{a}
0.02% Ca, 0% Al	35.3±6.4^{a}	41.7±4.0^{a}	53.2±19.7^{b}
0.02% Ca, 0.25% Al	36.6±4.2^{a}	36.4±2.9^{b}	30.3±5.3^{c}

*Mean values with different superscripts indicate difference ($P<0.05$).

Calcium content of Femurs. Group 1 had higher total calcium content in femurs of rats on calcium-adequate no-aluminum diet. Group 2 had significantly lower

calcium in their femurs compared to group1 but higher calcium than group 3 which was on the same calcium level with supplemental aluminum in the diet (Table II).

Food efficiency ratio (FER). A significant decrease was observed in the weight gain and FER of the rats fed low calcium, 0.25% aluminum or no aluminum as compared to the rats fed adequate-calcium no aluminum (Table III). However, group 2 ate less food than the other two groups despite of pair feeding.

Table III. The effect of Aluminum on appetite, weight gain and FER of rats fed various levels of Calcium and Aluminum for three weeks

Groups	Mean ±S.D. (n=16)		
	Food eaten (g)	Weight gain (g)	FER
0.5% Ca, 0% Al	196.24	78.0±3.8^{a*}	39.7±2.0^{a}
0.02% Ca, 0% Al	187.55	63.3±5.8^{b}	36.4±3.0^{ab}
0.02% Ca, 0.25% Al	196.24	66.3±5.4^{c}	32.0±3.1^{b}

*Mean values with different superscripts indicate difference ($P<0.05$).

The effect of aluminum and calcium on the bone health is well explained by the uptake of ^{45}Ca and the total calcium content in the femur. From both oral and IP dose, higher percent uptake of ^{45}Ca was observed in calcium deficient rats with or without supplemented aluminum (group 2 and 3) than in calcium adequate no aluminum group (group 1) (Table II). This is clearly due to the calcium deficient condition of the bones of group 2 and group 3 rats.

Comparing IP vs Oral ^{45}Ca uptake within the same group, femur uptake is higher from IP dose since intraperitoneal injection is considered as 100% absorption in the body (Koo *et al.*, 1993). However, uptake of ^{45}Ca from IP dose was significantly different in all the groups. Group 2 with low calcium no aluminum has significantly higher ^{45}Ca in their bones than group 3 with the same calcium intake supplemented with aluminum in their diet, indicating the toxic effect of aluminum on calcium uptake by the bones.

The total calcium content of the femurs is significantly higher in calcium adequate group as compared to low calcium groups (group 2 and 3), while the content further reduced with aluminum supplementation in group 3 (Table II). Bone calcium content is positively associated with bone strength (resistance to breaking). Fractures of bones occur when skeleton becomes weak. Bones from the rats on calcium-adequate no aluminum diet were strong and well shaped, while bones from the rats on calcium deficient with or without aluminum groups were spongy, soft and hollow. This weakness comes from the imbalance between formation and resorption of bones, from the impairment of bone mineralization or from lack of the availability of calcium to bones (calcium deficiency). Furthermore, inhibitory effect of aluminum on calcium absorption has been shown by a number of studies. A competitive absorption of calcium and aluminum has been proposed in the literature (Alder *et al.*, 1986). Aluminum has been found to affect bone development and cause resorption in dialysis patients (Alfrey *et al.*, 1979; Parisien *et al.*, 1988).

Aluminum also affects weight gain and FER negatively. Chronic feeding of aluminum at 0.1% level in the calcium deficient condition seriously affected the weight gain in rats fed for eight weeks (Konishi *et al.*, 1996). In a chick study, higher aluminum feeding to chicks at 0.3% level for two weeks in calcium adequate state had resulted in reduced food consumption and thus reduced weight gain (Dunn *et al.*, 1993).

Severe calcium deficiency also had adverse effect on food consumption of rats. In our study, despite pair feeding, the calcium deficient no aluminum group ate less food than calcium deficient with 0.25% aluminum or calcium adequate no aluminum group. This clearly suggests that weight reduction was due to lower food consumption (Table III). On the other hand, the significantly lower FER in group 3 with calcium deficiency was further aggravated with 0.25% aluminum supplementation, although their food intake was similar to the group 1 fed adequate calcium no aluminum.

It is imperative to understand the interaction of calcium and aluminum, because despite the growing knowledge about aluminum toxicity, awareness of the general public is very limited. Aluminum is frequently used in clinical conditions, a good substitute for aluminum as a phosphate binder has not yet been discovered. $CaCO_3$ used as a phosphate binder for the dialysis patients sometimes results in hypercalcemia which is controlled by administration of aluminum hydroxide (Grosso *et al.*, 1996).

Antacids and aspirin are the non-prescribed drugs which contribute an unaccountable high level of aluminum to the habitual users. Along with oral aluminum ingestion the calcium intake of general public is suboptimal. Increasing evidence suggests that adequate calcium intake in growing children is required to achieve the peak bone mass which helps prevent the risk of bone fractures later in life (Sandler *et al.*, 1985;

Halioua & Anderson, 1989). Besides the bone disorders, accumulation of aluminum in the aging brain cells creates dementia type of disorders, which could be reduced by decreasing the exposure to aluminum. Alzheimer's disease is a common old-age disorder, the cause and etiology of which is not understood.

CONCLUSION

It is, therefore, in the best interest to recommend the reduction of aluminum from food, water, drugs and cosmetics; and to find a better substitute for the phosphate binder for the dialysis patients.

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