THE ROLE OF CALCIUM UTILIZATION OF INTESTINAL FLORA ON URINARY CALCIUM EXCRETION

[1]F. Y. Lambrecht, [2]S. Kavukçu, [2]B. Kasap, [2]A. Soylu, [3]M. Yücesoy, [2]M. Türkmen, [3]N. Esen, [1]P. Ünak

[1]Ege University Institute of Nuclear Sciences, Department of Nuclear Applications, Izmir- TURKEY
[2]Dokuz Eylül University, School of Medicine, Department of Pediatrics, Division of Pediatric Nephrology and [3]Department of Microbiology, İzmir-TURKEY

ABSTRACT

We aimed to evaluate whether Ca^{+2} utilization of intestinal flora (IF) has an effect on urinary excretion of Ca^{+2} (UECa) levels. 0.1g/ml feces samples of children who underwent UECa examination in the last year were implanted in broths. 5 µL of labeled ^{45}Ca was added to the samples and incubated. 200 µL of the samples were filtrated by 0.45 micrometer membrane and rinsed by 200 µL pure water. ^{45}Ca activity in the membrane was counted and defined as percent activity per bacteria ($^{45}Ca_{act}$ %/CFU). Levels of aerobic and anaerobic $^{45}Ca_{act}$ %/CFU and their correlations with UECa were compared between hypercalciuric (Group I) and normocalciuric (Group II) patients. $^{45}Ca_{act}$ %/CFU levels were similar between the groups ($p>0.05$). UECa were negatively but not significantly correlated to aerobic and anaerobic $^{45}Ca_{act}$%/CFU (p:0,079, r:-0.503; p:0.260, r:-0.420, respectively) in hypercalciuric patients. In normocalciuric patients, levels were correlated positively to aerobic and negatively to anaerobic $^{45}Ca_{act}$%/CFU again in an insignificant manner (p:0.509, r: 0.223; p:0.623, r:-0.257, respectively). Similar $^{45}Ca_{act}$%/CFU levels in both groups imply that calcium utilization of IF does not have a distinct effect on UECa but, although not significant, there is a negative correlation between UECa and bacterial ^{45}Ca/CFU levels in hypercalciuric patients.

Keywords: Intestinal flora, calcium utilization, hypercalciuria, ^{45}Ca

1. INTRODUCTION

Hypercalciuria is a well-known risk factor for renal stone diseases (RSD) and their recurrences in both children and adults (1-4). In idiopathic hypercalciuria (IH), increased intestinal absorption (IA) of Ca^{+2} appears as either the first or the last step of the pathomechanism (Fig.1) (5). Increased IA of oxalate and resultant hyperoxaluria and oxalate stones represents a good example for the relation of increased IA and RDS (6). Absence of "*Oxalobacter formigenes*" which is an intestinal oxalate degrading bacteria has been accused for increased IA of oxalate. It is important in the prevention of RDS since lower levels in the intestinal flora (IF) increase the plasma and urine concentrations of oxalate (7). No specific type of bacterium in the IF has been determined to have such interference for Ca^{+2} metabolisms. The only relationship between IF and Ca^{+2} absorption in the literature is limited to the increment of Ca^{+2} absorption through acidification of the caecal contents by microbial fermentation of indigestible carbohydrates (8-10). In this study, we aimed to investigate whether Ca^{+2} utilization of IF in patients with IH differ from the other patients with normal levels of urinary excretion of Ca^{+2} (UECa) and performed a preliminary study using ^{45}Ca.

2. MATERIAL AND METHODS

The data of children who had undergone urinary calcium excretion examination in the last year were evaluated. For calcium excretion studies, children had ingested a diet including 300 mg calcium and 2000 mg sodium diet with adequate amount of protein according to Recommended Dietary Allowances for seven days and 24-hour urine collection had been then obtained as defined before (11). For spot urine calcium/creatinine ratios, the second void of the day had been used.

The patients were called to bring their stool samples. None of the patients had recent exposure to antibiotics that might affect bacterial colonization of gut or to vitamin D, calcium supplements, medications that might affect calcium and phosphorus absorption or excretion while urine collection or feces sampling.

200 µL feces samples were implanted in brain heart infusion broth for aerobic bacteria and thioglucolate broth for anaerobic bacteria. Five µL of ^{45}Ca (37 MBq/0.46 mL as $^{45}CaCl_2$ in aqueous solution, Amersham Biosciences UK), which is a pure beta emitter with a physical half life of 164 days was added to the samples. Before adding to the samples, initial ^{45}Ca activity of 5 µL $^{45}Ca_{act}$ was counted by a GM detector (Ludlum, Model 44-7 End Window) for 100 seconds. After it was added to 200 µL the samples, the samples were incubated for 24 hours at 37 °C.

Binding of calcium to bacteria was determined by filtration method as described previously (12,13). The samples were filtrated using 0.45 micrometer membrane and rinsed by 200 µL pure water to get rid of the ^{45}Ca which was not absorbed by the bacteria. $^{45}Ca_{act}$ of bacteria in the membrane was also counted by GM counter for 100 seconds. Activity% was calculated from ratio of the $^{45}Ca_{act}$ activity in the membrane to the initial $^{45}Ca_{act}$ activity. The amounts of bacteria in the samples were determined quantitatively as colony forming unit (CFU). Then, activity% per CFU ($^{45}Ca_{act}$%/CFU) was calculated for both aerobic and anaerobic bacteria in each sample.

3. RESULTS

A total of 29 patients brought their stool samples. The mean age of the patients was 7.5±4.3 (1.5-16) years. 18 of them were male (M/F: 18/11). Serum calcium and plasma potassium levels were in normal ranges in all patients. Number of patients was similar in Group I (n:14) and Group II (n:15). The $^{45}Ca_{act}$ %/CFU levels of aerobic or anaerobic bacteria were not different between the two groups (p>0.05) (Table 1).

Table 1. Comparison of $^{45}Ca_{act}$ %/CFU levels of aerobic or anaerobic bacteria between Groups 1 and 2.

	Group I	Group II	p
Aerobic $^{45}Ca_{act}$ %/CFU	n:14 3.80±4.63	n:15 3.03±2.78	0.983
Anaerobic $^{45}Ca_{act}$ %/CFU	n:10 4.86±8.74	n:7 0.99±0.53	0.601

Absorptive form:

↑ intestinal absorption → ↑ serum Ca^{+2} → ↓ iPTH → ↓ tubular Ca^{+2} resorption → hypercalciuria

Resorptive form:

↓ tubular Ca^{+2} resorption → ↓ serum Ca^{+2} → ↑ iPTH → ↑ calcitriol → ↑ intestinal absorption

Fig.1: Pathomechanisms of absorptive and resorptive forms of idiopathic hypercalciuria.

4. DISCUSSION

Hypercalciuria is a risk factor for RSD and its prevalence in children with RSD is about 40% (1). There are hypercalcemic, normocalcemic and hypocalcemic types of hypercalciuria. When normocalcemia is accompanied with normal or low plasma phosphate levels and abnormally high urinary calcium excretion, it is known as IH which is the most frequent type among patients with RSD (2, 14). In our group of patients, all had normal calcium and normal or low plasma phosphate levels. Thus, hypercalciuria in all patients were defined as idiopathic.

Two main pathophysiological hypothesis has been proposed for IH: Primary intestinal hyperabsorption of Ca^{+2} leading to a decrease in PTH secretion has been defined as "absorptive hypercalciuria" and primary renal tubular leak of Ca^{+2} which stimulates PTH secretion has been defined as "resorptive hypercalciuria" (15). It is suggested that both two forms are endpoints of a continuous spectrum with considerable overlaps and thus use of the tests (i.e. calcium loading test) to differentiate one form from another are not recommended in the routine clinical work-up (5,16,17). Consequently, we did not distinguish the type of IH in our patients.

Furthermore if the dietary Ca^{+2} intake is lower, the site of absorption moves to the upper parts of intestine where the number of floral bacteria is the lowest. However, we thought that any significant difference

between hypercalciuric and normocalciuric patients should support our theory. We used ^{45}Ca for evaluation studies of calcium binding to bacteria for that purpose (13, 18, 19).

In this study, similar $^{45}Ca_{act}$ %/CFU levels in both hypercalciuric and normocalciuric patients may imply that Ca^{+2} utilization of IF does not have a distinct effect on UECa. Nevertheless, although not significant, negative correlation between urinary Ca^{+2} and bacterial $^{45}Ca_{act}$ %/CFU levels, especially in hypercalciuric patients, exhibits a need for further investigation on this topic. That was a preliminary study and we are planning to repeat the study in a larger series and specify all the bacteria in the IF. In case the negative correlation between UECa and $^{45}Ca_{act}$ %/CFU is found significant, the bacteria responsible for the Ca^{+2} utilization may be identified by this way.

Acknowledgements The authors gratefully acknowledge the financial support received from the Dokuz Eylul University Department of Scientific Projects.
*This study have been just published in a journal.

5. REFERENCES

1. Stapleton FB, McKay CP, Noe HN. Urolithiasis in children: the role of hypercalciuria. *Pediatr Ann* 1987; 16: 980-981,984-992
2. Levy FL, Adams-Huet B, Pak CY. Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. *Am J Med* 1995; 98: 50-59
3. Pietrow PK, Pope JC 4th, Adams MC, Shyr Y, Brock JW 3rd. Clinical outcome of pediatric stone disease. *J Urol* 2002; 167: 670-673
4. Ozokutan BH, Kucukaydin M, Gunduz Z, Kabaklioglu M, Okur H, Turan C. Urolithiasis in childhood. *Pediatr Surg Int* 200; 16: 60-63
5. György R. *Idiopathic hypercalciuria in children.* In: Andreoli TE, Ritz E, Rosival L (eds) Nephrology, Hypertension, Dialysis, Transplantation, 1st edition, Hungarian Kidney Foundation, Budapest, 2005; 397-406
6. Kumar R, Mukherjee M, Bhandari M, Kumar A, Sidhu H, Mittal RD. Role of Oxalobacter formigenes in calcium oxalate stone disease: a study from North *India. Eur Urol* 2002; 41:318-322
7. Allison MJ, Dawson KA, Mayberry WR, Foss JG. Oxalobacter formigenes gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch Microbiol* 1985;141:1-7
8. Zafar TA, Weaver CM, Zhao Y, Martin BR, Wastney ME. Nondigestible oligosaccharides increase calcium absorption and suppress bone resorption in ovariectomized rats. *J Nutr* 2004; 134: 399-402
9. Suzuki T, Hara H. Various non-digestible saccharides increase intracellular calcium ion concentration in rat small-intestinal enterocytes. *Br J Nutr* 2004; 92: 751-755
10. Chonan O, Matsumoto K, Watanuki M. Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. *Biosci Biotechnol Biochem* 1995; 59: 236-239
11. Stapleton FB. Idiopathic hypercalciuria: association with isolated hematuria and risk for urolithiasis in children. The Southwest Pediatric Nephrology Study Group. *Kidney Int* 1990; 37: 807-811
12. Rose T, Sebo P, Bellalou J, Ladant D. Interaction of calcium with Bordetella pertussis adenylate cyclase toxin. *J Biol Chem* 1995; 270:26370-26376
13. Tsopelas C, Penglis S, Ruszkiewicz, Bartholomeusz FD. ^{99m}Tc-Alafosfalin: an antibiotic peptide infection imaging agent. *Nucl Med Biol* 2003; 30: 169-175
14. Albright F, Henneman P, Benedict PH, Forbes AP. Idiopathic hypercalciuria: a preliminary report. *Proc R Soc Med* 1953; 46: 1077-1081
15. Pak CY. Physiological basis for absorptive and renal hypercalciurias. *Am J Physiol* 1979; 237: F415-23
16. Aladjem M, Barr J, Lahat E, Bistritzer T. Renal and absorptive hypercalciuria: a metabolic disturbance with varying and interchanging modes of expression. *Pediatrics* 1996; 97: 216-219
17. Heilberg IP, Martini LA, Draibe SA, Ajzen H, Ramos OL, Schor N. Sensitivity to calcium intake in calcium stone forming patients. *Nephron* 1996; 73: 145-153

18. Duan Y, Fisher E, Malamud D, Golub E, Demuth DR. Calcium-binding properties of SSP-5, the streptococcus gordonii M5 receptor for salivary agglutinin. *Infect Immun* 1994; 62: 5220-5226

19. Steinmetz MO, Plüss C, Christen U, Wolpensinger B, Lustig A, Werner ER, Wachter H, Engel A, Aebi U, Pfeilschifter J, Kammerer RA. Rat GTP cyclohydrolase I is a homodecameric protein complex containing high-affinity calcium-binding sites *J Mol Biol* 1998; 279: 189-199.