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High urinary excretion level of citrate and magnesium in children: potential etiology for the reduced incidence of pediatric urolithiasis

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Abstract It is well known that the incidence of calcium oxalate (CaOX) urolithiasis is much lower in children than in adults [2, 21]. One purpose of this study was to compare the inhibitory activity on CaOX crystal growth and nucleation of urine from children (ufC) with that of urine from adults (ufA). Another was to measure low molecular weight urinary substances related to CaOX lithiasis, including citrate and magnesium, which have been identified as stone inhibitors. The excretion volume per body weight of uric acid, phosphorus, magnesium and citrate was all significantly higher in 24-h ufC than in 24-h ufA, but that of calcium and oxalate was not. The growth inhibitory activities against CaOX crystals of ufC and ufA were measured in a whole urine system. The diameter of the crystals produced in this system was smaller for ufC (3.68 μm) than for ufA (4.66 μm). We also examined the metastable limit for CaOX with fresh spot urine, which was 3.15 mmol/l in ufC and 0.41 mmol/l in ufA. These results indicate that ufC has a more powerful inhibitory effect on CaOX crystal growth and nucleation than ufA. We also found that the excretion rate of citrate and magnesium in ufC was much higher than in ufA. We suggest that these two stone inhibitors are very likely to elevate the inhibitory activity of ufC against CaOX crystal growth and nucleation. The lower incidence of CaOX lithiasis in children might thus be partly attributed to citrate and magnesium.

Key words Citrate · Magnesium · Calcium oxalate crystallization · Children · Inhibitory activity · Metastable limit

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Introduction

The incidence of urolithiasis in children compared with that in adults is very low in developed Western countries [2, 21]; in Japan also the ratio of children less than 10 years old with urolithiasis to all lithic patients is estimated at under 1% [23]. The reason for this fact may reside in quantitative or qualitative differences in urinary risk factors or urinary inhibitors between children and adults and a higher inhibitory activity of the urine from children (ufC) than that of the urine from adults (ufA). However, only a few studies on this subject have been reported [4]. We measured low molecular weight urinary risk factors and inhibitors of calcium oxalate (CaOX) lithiasis in children and adults. The metastable limits for CaOX as well as the inhibitory effects on CaOX crystal growth of ufC and ufA were examined and compared.

Materials and methods

We collected urine from 27 male children aged from 3 to 6 years (average 5 years), and 22 male adults aged from 24 to 65 years (average 34 years). The averages of their body weight, height and body mass index were 17.7 ± 3.25 kg, 106.4 ± 8.975 cm, 15.57 ± 1.83 kg/m^2, respectively for children, and 67.2 ± 7.98 kg, 172.2 ± 6.425 cm, 22.66 ± 4.62 kg/m^2, respectively for adults. None of them had any congenital metabolic disorders or any episodes of urolithiasis. We collected their 24-h urine samples together with 0.1 ml of 30% sodium azide to examine the growth inhibitory activity against CaOX crystals. For measuring the metastable limit of CaOX, freshly voided morning urine (non-fasting) was used. The urine volume, pH, creatinine, uric acid, calcium, inorganic phosphorus, oxalate, magnesium and citrate of all samples were measured.

Inhibition assay in a whole urine system

The method reported by Tawashi et al. [19] was used for the inhibition assay in a whole urine system. Each 24-h urine sample was filtered through a Millipore filter (0.22 μm). A quarter-milliliter of calcium chloride (1 M) was added to 50 ml of filtered samples. The pH was adjusted to 5.7 and then 2.5 ml of sodium oxalate (0.005 M) were added to each sample. These samples were kept at

37°C without shaking. After 3 h the particle size of the calcium oxalate crystals was determined with a Coulter Multisizer.

Inhibition assay in a diluted urine system

The growth inhibitory activity against CaOX crystals of each 24-h urine samples was also measured with a seed crystal method reported by Robertson et al. [14] with minor modifications. We produced 10 ml of a metastable solution on CaOX with ^{14}C-labeled oxalate mixed with the samples at concentration of 0.1%, 0.5%, 1%, 3%, and 5%. A quarter-milliliter of dispersed calcium oxalate monohydrate crystal solution (100 mg/dl) (Wako Chemical, Kyoto, Japan) was seeded to each sample, and the supernatant cpm was counted at 0 h (S_0). After incubation at 37°C with shaking for 4 h, the supernatant cpm was counted at 4 h (S_4). The count at 0 and 4 h in the control tube (with 0% urine) was adopted as C_0 and C_4. The percentage of inhibition (I) was then calculated as:

$$I = 100 \times \{(C_4 - C_0) - (S_4 - S_0)\}/(C_4 - C_0).$$

Metastable limit

The metastable limit of CaOX was measured with the microplate method reported by Kawamura et al. [5] and Suzuki et al. [18] with of non-fasting freshly voided morning urine samples to yield a final concentration of oxalate on the microplate of between 0 mmol/l and 10 mmol/l. The plate was kept at 37°C for 20 min and the maximal concentration of sodium oxalate which did not induce nucleation detectable with an inverted microscope was adopted as the measured CaOX metastable limit of each urine sample.

Results

Urinary low molecular weight substances

The excretion volume per body weight of uric acid and inorganic phosphorus was significantly higher in 24-h ufC than 24-h ufA, but that of calcium and oxalate was not. The volume of magnesium and citrate was twice as high in ufC as in ufA. The same results were recognized in regard to the concentration of these substances in 24-h ufC and 24-h ufA. Urine pH showed no difference between the two groups (Table 1).

Inhibitory activity

In the whole urine system, the average particle size of the CaOX crystals formed was significantly smaller in ufC (3.68 ± 1.04 μm) than in ufA (4.66 ± 1.36 ± μm), which means that the growth inhibitory activity against CaOX crystals of ufC was higher than that of ufA (Fig. 1). In the diluted urine system, 24-h ufC tended to inhibit CaOX crystal growth more effectively at all concentrations than did ufA, although no significant differences between ufC and ufA were recognized at any concentration (Fig. 2).

Metastable limit

The metastable limit of CaOX was significantly higher in spot-ufC (mean ± SD: 3.15 ± 3.31 mmol/l) than in spot-ufA (mean ± SD: 0.41 ± 0.38 mmol/l) (Fig. 3a).

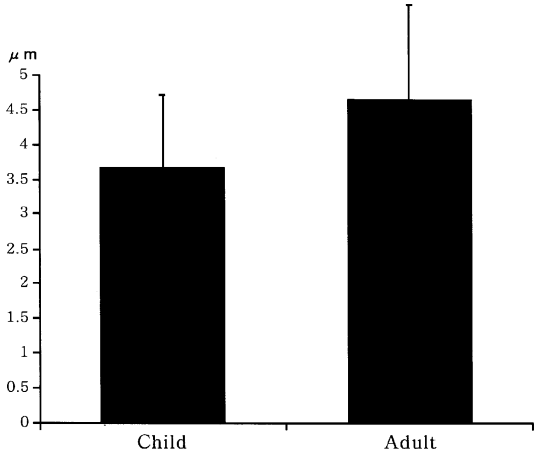

Fig. 1 The average calcium oxalate (CaOX) crystal size formed in the 24-h urine from children as measured with the whole urine system was larger than that in the 24-h urine from adults (unpaired *t*-test: $P = 0.0065$). The values are shown as means ± SD

Table 1 Urinary composition in 24-h urine from children and adults. All data are expressed as means ± SD. (*BW* body weight, *NS* not significant)

	Child	Adult	*P* value
pH	6.69 ± 0.384	6.37 ± 0.515	NS[b]
Uric acid (mg/dl)	58.50 ± 20.00	45.76 ± 19.13	0.0284[b]
Uric acid/BW (mg/kg)	15.20 ± 5.15	9.204 ± 2.922	< 0.0001[a]
Calcium (mg/dl)	12.20 ± 6.22	12.43 ± 6.32	NS[b]
Calcium/BW (mg/kg)	3.071 ± 1.417	2.482 ± 1.050	NS[b]
Phosphorus (mg/dl)	95.40 ± 31.90	66.22 ± 31.79	0.0025[b]
Phosphorus/BW (mg/kg)	24.69 ± 8.35	12.91 ± 4.27	< 0.0001[a]
Oxalate (mg/dl)	1.811 ± 1.344	2.130 ± 1.577	NS[b]
Oxalate/BW (mg/kg)	0.432 ± 0.280	0.439 ± 0.302	NS[b]
Magnesium (mg/dl)	10.18 ± 4.14	6.241 ± 3.160	0.0006[b]
Magnesium/BW (mg/kg)	2.669 ± 1.150	1.263 ± 0.526	< 0.0001[a]
Magnesium/calcium (mg/mg)	0.942 ± 0.168	0.531 ± 0.045	< 0.0001[a]
Citrate (mg/dl)	64.75 ± 28.64	40.28 ± 18.40	0.0002[a]
Citrate/BW (mg/kg)	16.82 ± 6.75	8.978 ± 6.794	0.0002[b]
Citrate/calcium (mg/mg)	6.474 ± 3.295	5.787 ± 9.366	0.0067[a]

[a] Mann-Whitney U-test,
[b] unpaired *t*-test

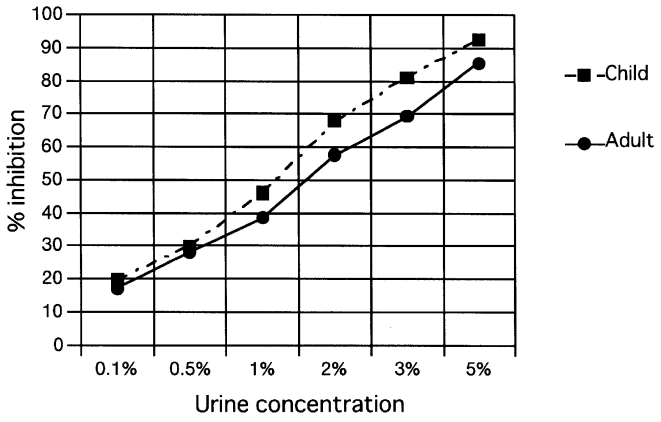

Fig. 2 The growth inhibitory activity measured in the diluted urine system did not show any significant differences at any concentration between the 24-h urine from children and that from adults(unpaired *t*-test)

Non-parametric data is shown in Fig. 3b (ufC: median value 1.75 mmol/l, range 0.30–10.0 mmol/l, ufA: median value 0.30 mmol/l, range 0–1.50 mmol). We also investigated the ratio to creatinine and concentrations of low molecular weight substances in this spot urine. There was no significant difference in the pH between spot-ufC and spot-ufA. The ratio of all substances except for calcium to creatinine in spot ufC was significantly higher than in spot-ufA. As for the concentration of these substances, a significant difference was recognized only in the calcium concentration between spot-ufC and spot-ufA. But magnesium/calcium and citrate/calcium ratios were significantly higher in ufC than in ufA (Table 2).

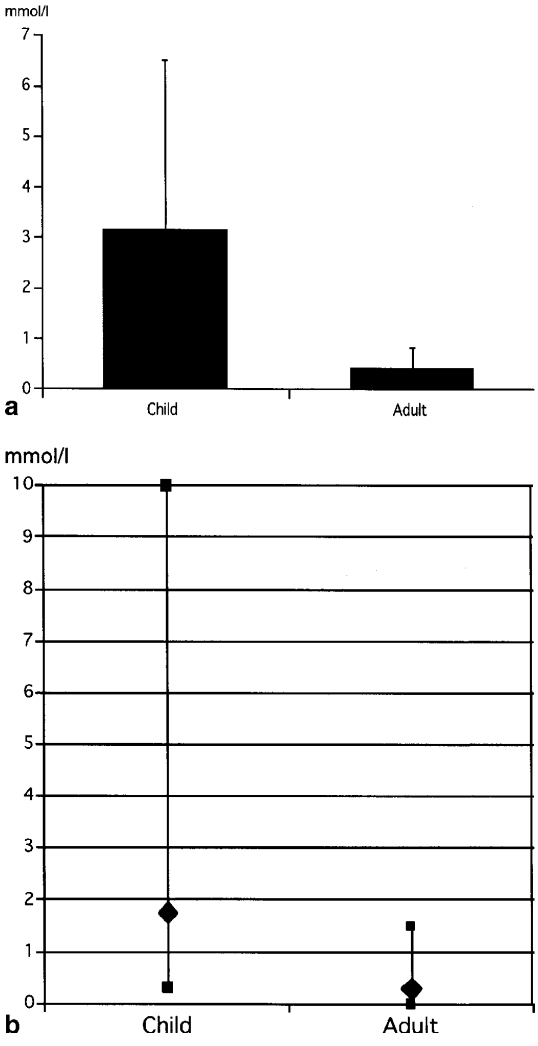

Fig. 3a,b The metastable limit of CaOX of urine from children was remarkably higher than that of urine from adults (Mann-Whitney U-test: $P < 0.0001$). Black columns indicate means ± SD (**a**); straight bars indicate the range of the samples (*diamond* median value) (**b**)

Discussion

The incidence of calcium oxalate lithiasis in childhood is remarkably low [2, 21]. Robertson et al. [13] calculated the risk index of CaOX lithiasis from urinary stone risk factors, and reported a low rate of stone formation risk in children. However, only a few papers about the difference in inhibitory activity between ufC and ufA have been published [4, 13]. We measured the low molecular weight urinary substances since we suspected that there might be some differences in the amounts between ufC and ufA.

We found in our experiment that the urinary excretion volume of uric acid was significantly higher in 24-h ufC than in 24-h ufA. Stapleton et al. [16] reported similar results to ours and they suggested the reason for the higher uric acid level in ufC than in ufA might be that the reabsorption of uric acid is less in children due to the immaturity of their nephrons. There is no clear consensus about the association between urinary urate and CaOX stone formation, but the salting-out effect, the epitaxy of CaOX induced by the precipitation of sodium urate, and the effect of sodium urate and uric acid crystals on inhibitors of CaOX crystallization reported by several authors seem to explain the possible role of uric acid as a CaOX stone risk factor [20].

The urinary excretion volume per body weight of calcium and oxalate in our study was not significantly higher in 24-h ufC than in 24-h ufA although Stapleton et al. [17] reported that the ratio of calcium to creatinine was higher in ufC than in ufA. As for substances in 24-h urine, we express the excretion level as a value per kilogram of body weight because the low urinary excretion volume of creatinine in children influences the ratio of the substances to creatinine. But in spot urine, with which we measured the metastable limit, we used the ratio of creatinine to compare the urinary excretion rate of the substances between children and adults because spot urine presents a variable density in every urination.

The excretion level of CaOX crystallization risk factors in 24-h ufC was not lower than that in 24-h ufA;

Table 2 Urinary composition of freshly voided morning urine (spot urine) from children and adults. All data are expressed as means ± SD. (*NS* not significant)

	Child	Adult	*P* value
pH	6.32 ± 0.659	6.28 ± 0.480	NS[b]
Uric acid (mg/dl)	46.93 ± 30.06	60.48 ± 20.84	NS[b]
Uric acid/creatinine (mg/mg)	0.893 ± 0.338	0.464 ± 0.181	0.0001[a]
Calcium (mg/dl)	6.120 ± 6.412	15.16 ± 11.01	0.001[a]
Calcium/creatinine (mg/mg)	0.122 ± 0.117	0.111 ± 0.059	NS[a]
Phosphorus (mg/dl)	65.80 ± 42.34	58.21 ± 60.03	NS[b]
Phosphorus/creatinine (mg/mg)	1.264 ± 0.604	0.321 ± 0.190	< 0.0001[a]
Oxalate (mg/dl)	2.700 ± 2.077	1.714 ± 1.116	NS[a]
Oxalate/creatinine (mg/mg)	0.0501 ± 0.0320	0.0138 ± 0.0090	< 0.0001[a]
Magnesium (mg/dl)	5.81 ± 4.64	7.59 ± 4.50	NS[b]
Magnesium/creatinine (mg/mg)	0.108 ± 0.063	0.049 ± 0.014	< 0.0001[a]
Magnesium/calcium (mg/mg)	1.527 ± 1.234	0.603 ± 0.379	0.0008[a]
Citrate (mg/dl)	35.73 ± 19.10	41.94 ± 21.39	NS[b]
Citrate/creatinine (mg/mg)	0.748 ± 0.346	0.303 ± 0.138	< 0.0001[a]
Citrate/calcium (mg/mg)	13.20 ± 11.85	3.585 ± 2.383	0.0023[a]

[a] Mann-Whitney U-test,
[b] unpaired *t*-test

nevertheless, the inhibitory activity of ufC on CaOX crystal growth was significantly higher than that of ufA, so that it was reasonable to assume that the ufC would include powerful stone inhibitors. We also found that the urinary excretion levels of magnesium and citrate, which are well-defined stone inhibitors, in 24-h ufC was much higher than those in 24-h ufA.

Magnesium inhibits oxalate absorption by forming a magnesium–oxalate complex, so that magnesium oxide administration has been found to lower urinary oxalate excretion [3]. Magnesium excreted in urine can form complexes with oxalate and thus reduce supersaturation [6, 8]. In our experiments, three times as much magnesium was found in ufC as in ufA. Fujisawa et al. [4] reported similar results to ours concerning urinary magnesium excretion in children [4]. Urinary magnesium thus might also play an important part in inhibiting CaOX crystal nucleation in ufC.

Citrate is one of the urinary constituents which is freely filtered in renal glomeruli and reabsorbed in the proximal tubules. Many reports have shown that the urinary excretion of citrate in patients with renal tubular acidosis or idiopathic hypercalciuria is reduced in comparison with that in normal controls and that the administration of potassium citrate is very effective for preventing stone recurrence in those patients [7, 10, 11, 15, 22]. Citrate is a very important stone inhibitor of CaOX and calcium phosphate (CaP). It lowers the supersaturation level of CaOX by forming complexes with calcium, and thus inhibits the nucleation and growth of CaOX and CaP crystals. Citrate also inhibits the aggregation and growth of CaOX crystals in two hypothesized manners. It has been suggested that citrate might influence the structure of Tamm-Horsfall protein (THP) and reduce its viscous binding power and self-aggregation by chelating protein-bound calcium. The other suggested role of citrate as a stone inhibitor is to affect the crystal surface directly as a crystal poison by binding to crystal surfaces [1, 8]. Serious consideration must thus be given to the fact that in our study higher urinary excretion levels of citrate as well as of magnesium were found in children than in adults. Why are there such differences in citrate and magnesium excretion between children and adults? Melnik et al. [9] proved differences in the activity of citrate synthesizing enzyme in liver between infant and mature rats, and suggested that such etiology could cause an increased excretion of urinary citrate and a reduced prevalence of urolithiasis in children [9].

Nucleation of the CaOX crystal proved to be much more strongly inhibited in spot-ufC than in spot-ufA in our experiment, judging from the higher metastable limit of CaOX in spot-ufC. There was no significant difference in the concentration of magnesium and citrate between the two groups; however, the magnesium/calcium (Mg/Ca) and citrate/calcium (Cit/Ca) ratios in ufC were significantly higher than those in ufA. Low Mg/Ca and Cit/Ca ratios have been considered to be an important cause for the formation of urinary calculi [11, 12]. We suppose that such a higher metastable limit in ufC may be partly due to higher Cit/Ca and Mg/Ca ratios in ufC.

Be that as it may, our study made it clear that ufC had a stronger inhibitory activity than ufA, although the excretion rate of urinary stone risk factors such as calcium, oxalate, and uric acid in ufC was not lower than that in ufA. This result led us to suppose that ufC would include powerful stone inhibitors, and that citrate and magnesium will be part of these inhibitors. In conclusion, as far as low molecular weight urinary substances are concerned, and considering the factors related to CaOX crystallization, citrate and magnesium might be of crucial importance as CaOX stone inhibitors, thus accounting for the reduced incidence of CaOX lithiasis in childhood.

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