Efficacy of Mixtures of Magnesium, Citrate and Phytate as Calcium Oxalate Crystallization Inhibitors in Urine

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Abbreviations and Acronyms

COD = calcium oxalate dihydrate

COM = calcium oxalate monohydrate

COT = calcium oxalate trihydrate

SS = supersaturation

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Purpose: The main aim of the current study was to evaluate the effectiveness of mixtures of magnesium, citrate and phytate as calcium oxalate crystallization inhibitors.

Materials and Methods: A turbidimetric assay in synthetic urine was performed to obtain induction times for calcium oxalate crystallization in the absence and presence of different mixtures of inhibitors. The morphology of calcium oxalate crystals in the absence or presence of inhibitors and mixtures of the inhibitors was evaluated in 2 crystallization experiments at low and high calcium oxalate supersaturation. The crystals formed were examined using scanning electron microscopy.

Results: Examination of crystallization induction times revealed clear inhibitory effects of magnesium, citrate and phytate on calcium oxalate crystallization, supporting usefulness in the treatment and prevention of calcium oxalate nephrolithiasis. Significant synergistic effects between magnesium and phytate were observed. Scanning electron microscopy images revealed that phytate is a powerful crystal growth inhibitor of calcium oxalate, totally preventing the formation of trihydrate and monohydrate. In addition to crystallization inhibition capacity, citrate and magnesium avoided calcium oxalate crystallization by decreasing its supersaturation.

Conclusions: The synergistic effect between magnesium and phytate on calcium oxalate crystallization suggests that a combination of these 2 compounds may be highly useful as antilithiasis therapy.

Key Words: nephrolithiasis, calcium oxalate, phytic acid, magnesium, citric acid

Renal lithiasis is a highly prevalent pathological condition affecting about 10% of the global population.[1] The disease is multifactorial with factors classified into 2 groups, including renal morpho-anatomy and urine composition factors.[2,3] In the second group the main thermodynamic factor is SS. Several compounds, including calcium oxalate, uric acid and calcium phosphate, may be supersaturated in urine. Consequently urine is in a metastable state and tends to revert to a more stable condition through crystallization of these compounds. The ease of crystallization depends on several factors other than the degree of SS. The main kinetic factors involved are crystallization promoters (preformed particles that act as heterogeneous nucleants) and crystallization inhibitors.[4]

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Crystallization inhibitors are specific for each type of crystal. Three well-known calcium oxalate crystallization inhibitors are magnesium, citrate and phytate.[5–10] Magnesium also acts as a competitor of calcium in oxalate binding. However, magnesium oxalate is 2 orders of magnitude more soluble than calcium oxalate and, thus, it does not crystallize in the kidney to form stones.[6] Based on this competition between magnesium and calcium for oxalate binding magnesium salts have been studied for the treatment of calcium oxalate nephrolithiasis in rats and humans.[11,12] Similarly citrate complexes with calcium, leading to a reduction of free calcium in urine and consequently to decreased calcium oxalate supersaturation. Furthermore, citrate acts as an inhibitor of nucleation and crystal growth of calcium oxalate.[13,14] Phytate is a powerful crystallization inhibitor of calcium salts, inhibiting nucleation and crystal growth of calcium oxalate.[10]

Citrate and magnesium were selected in view of earlier studies showing effective application in the treatment and prevention of renal lithiasis. Recent studies also demonstrated that phytate is naturally present in human urine.[15–17] Studies in humans suggest that phytate absorption in the gut is low and does not exceed 2%, as is observed for bisphosphonates.[16] Ingestion of phytate significantly reduced the risk of calcium stones in humans.[18] A prospective study examined the association between dietary factors and the risk of incident symptomatic kidney stones in 96,245 females.[19] The results of that study showed that phytate intake was associated with a decreased risk of stone formation. However, under unbalanced dietary conditions phytate may affect the bioavailability and in consequence the levels of iron, zinc and calcium.[16]

In contrast to phytate, magnesium absorption is much higher. The body typically absorbs 11% to 65% of magnesium intake.[20] However, magnesium bioavailability depends on the salts used with magnesium chloride the salt with highest bioavailability.[21] Phytate is typically present in urine at between 0.2 to 2 μM.[16] Magnesium is usually present at around 90 mg/l in adults but it is higher (120 to 150 mg/l) in children.[22] On the other hand, citrate should be higher than 300 mg/l, which is usually considered the limit value for hypocitruria, and it can reach levels greater than 1,000 mg/l.[23]

A number of in vitro studies in the literature have described the effects of crystallization inhibitors.[24–26] However, these compounds have been usually studied separately, which does not reflect their combined physiological presence in urine. The main aim of this investigation was to evaluate the capacity of magnesium, citrate and phytate, and their binary mixtures to inhibit calcium oxalate crystallization in synthetic urine.

MATERIALS AND METHODS

Reagents, Solutions and Software

Synthetic urine components and magnesium sulfate heptahydrate were obtained from Panreac Química, Barcelona, Spain. Sodium citrate tribasic dihydrate and phytic acid dipotassium salt were obtained from Sigma-Aldrich®. Chemicals of analytical reagent grade purity were dissolved in ultrapure deionized water from a Milli-Q® system and filtered through 0.45 μm pore filters before use. Sodium oxalate stock solution (40 mM) was prepared by dissolving 1.34 gm sodium oxalate in 0.25 L water. Synthetic urine was prepared by mixing equal volumes of solutions 1 and 2 (table 1).[27] The pH of each solution was adjusted to 6.0. The final concentration of all ions in synthetic urine was 154 mM Na^+, 81 mM K^+, 43 mM NH_4^+, 5 mM Ca^{2+}, 246 mM Cl^-, 10 mM SO_4^{2-} and 16 mM PO_4^{3-}.

EQUIL® 2.0 was used to calculate the relative supersaturation of calcium oxalate in synthetic urine.

Turbidimetric Assay

Calcium oxalate crystal formation in synthetic urine and the effects of crystallization inhibitors were assessed with a kinetic turbidimetric system consisting of a Model 662 photometer (Metrohm, Riverview, Florida) equipped with a fiberoptic light guide measuring cell with an attached 2 × 10 mm light path reflector and monochromatic light at 550 nm. Crystallization was assessed at a constant temperature of 37C with magnetic stirring.

Synthetic urine (200 ml) without additives was added to a crystallization flask. The desired concentrations of magnesium or citrate (according to normal values in urine) were achieved by dissolving the corresponding amounts of magnesium sulfate heptahydrate or sodium citrate tribasic dihydrate, respectively, in 200 ml synthetic urine. To achieve the desired concentration of phytate the corresponding volume of 1 M phytate was added to the flask containing 200 ml synthetic urine. When the resulting solution reached a temperature of 37C in the thermostatic bath 2.84 ml sodium oxalate stock solution were added to achieve the desired SS of calcium oxalate and the timer was switched on. Final concentrations of calcium and oxalate were selected as 200 and 50 mg/l, respectively, to facilitate a short induction time. The pH of the final solution was measured at the beginning of each experiment. Absorbance of the solution at 550 nm was measured regularly until the end of the kinetic assay. Assays were performed in duplicate and

Table 1. *Synthetic urine composition*

	Concentration (mM)
Solution 1:	
$Na_2SO_4 \cdot 10\ H_2O$	19.34
NH_4Cl	86.73
KCl	162.60
$CaCl_2$	10.00
Solution 2:	
$NaH_2PO_4 \cdot 2\ H_2O$	15.45
$Na_2HPO_4 \cdot 12\ H_2O$	15.64
NaCl	223.08