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Hypocitraturia: Mechanism and Therapeutic Strategies

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Abstract

Citric acid is a weak tricarboxylic acid naturally found in citrus fruits. Biochemically, it is a vital intermediate in the Krebs cycle in all living organisms. In urine, citrate plays an important role in preventing stone formation. Hypocitraturia, a low urinary citrate excretion is a well recognized kidney stone risk factor. Hypocitraturia is the most prevalent metabolic abnormality (77-100%) found in kidney stone patients in the northeastern (NE) Thailand, occurred coincidently with hypokaliuria (77-91%) and hypokalemia (10-37%). Studies suggested that the NE stone patients had a state of potassium deficiency due to low dietary intake of potassium and high sweat loss. Acid loading test suggested that the hypokaliuric and hypocitraturic phenotypes did not caused by distal tubular acidosis. We hypothesize that potassium depletion triggers hypocitraturia in NE stone patients. In addition, low consumption of citrus fruit and intake of high-carbohydrate/low-fat diets were found to be a dietary habit of these patients. Potassium depletion causes intracellular acidosis in renal tubular cells. Two pathways of citrate breakdown, 1) entering Krebs cycle to produce CO2 (bicarbonate precursor) and 2) lysing by ATP-citrate lyase in cytoplasm to produce acetyl CoA (fatty acid precursor) and oxaloacetate (glucose precursor), are induced by intracellular proton. Reduced intracellular citrate in turn accelerates the reabsorption of urinary citrate by sodium-dicarboxylate cotransporter-1 (NaDC-1), leading to hypocitrauria. AA genotype of I550V polymorphism in NaDC-1 gene was associated with hypocitraturic phenotype in our kidney stone cohort. These dietary and genetic factors may act in concert to cause a severely low urinary citrate excretion in the NE stone patients. Our recent studies showed that patient with nephrolithiasis had increased oxidative stress, enhanced renal tubular injury and declined renal function. In addition, intrarenal inflammation was commonly observed in kidney biopsies of the NE stone patients. Regimen that is capable of increasing urine pH and citrate, together with reducing oxidative stress and renal tubular damage, is in need to be developed. Our pilot data demonstrated that 3-month treatment with lime powder regimen (LPR, containing 21 mEq of potassium and 63 mEq of citrate) significantly increased urinary pH, potassium and citrate, and also reduced degrees of oxidative stress and renal tubular injury. We hypothesize that our limeade-based regimen could be a novel efficient medication for treating kidney stone disease in Thailand. Nationwide multi-center clinical trials (phases 1, 2 and 3) of LPR in Thai kidney stone patients are currently being planned to conduct by our group.

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Introduction

Kidney stone disease (nephrolithiasis) has a considerable morbidity moreover its prevalence and incidence progressively increase across the world, possibly due to changes in global climate and dietary pattern (particularly high animal protein consumption)[1,2]. Kidney stone belt in Thailand currently comprises the Northeast (NE) expands toward the North, with the reported prevalence of 16.9%[3]. Beside a large economic impact, kidney stone frequently recurs posing a difficulty for management[4]. Multiple stone relapsing leads to progressively kidney loss and eventually end stage renal disease[5-7], hence lowering quality of life of the patients[8]. Since surgical intervention is symptomatic therapy and does not correct the cause of stone formation, development of effective medical regimen with minimal adverse effect (e.g., natural product-based regimen) to prevent calculogenesis (causative treatment) and recuperate kidney function is in need.

Etiology of nephrolithiasis is multifactorial simply classified as intrinsic (e.g., genetic susceptibility, kidney anomaly, age, sex and ethnics) and extrinsic (e.g., dietary habit, fluid intake, climate, physical activity, occupation and medication) causes. These factors have varied impacts on urinary composition and concentration. According stone forming activity, urine constituents are categorized into stone promoters and inhibitors. Stone promoting factors include urinary calcium, oxalate, phosphate, uric acid, cystine, as well as low urine pH and volume. Stone inhibitors act to hamper the formation of lithogenic crystals, which include urinary citrate, potassium, magnesium and a number of urinary proteins (e.g., Tamm-Horsfall protein, osteopontin). Monogenic kidney stone disease such as cystine and xanthine stones is very rare, contributing less than 2% of all stones.

Kidney stone is not a homogeneous disease. Basically, stones are classified according to crystalline composition into four main types viz. calcium oxalate (CaOx), calcium phosphate (CaP), uric acid (UA) and magnesium ammonium phosphate (MAP). CaOx is the most prevalent stone reported in all countries, including Thailand. Our recent data showed that CaOx mixed with CaP was the most prevalent form of kidney stones in Thailand[9], and a rising prevalence of UA stone in northeastern (NE) Thailand was emerged[10].

Hypocitraturia in Thai kidney stone patients: what extent?

Metabolic stone risk factor or metabolic abnormality is defined as imbalance of urinary stone promoters and inhibitors that favors crystal formation. Hypercalciuria and hyperoxlauria are commonly detected in western kidney stone cases[11], but based on our study these two metabolic disorders are much less common in Thai nephrolithiasis patients[12]. The universal metabolic risk factor for kidney stone formation is hypocitraturia, generally defined as urinary citrate excretion < 320 mg (1.67 mmol)/day. The global prevalence of hypocitaturia in stone patients is of 20-60%[13]. However, in Thai nephrolithiasis patients a higher prevalence of hypocitraturia (77-100%) is reported[12,14]. Due to differences in dietary habit, genetic background and ethnics (as well as laboratory variation), excretion of urinary citrate in western populations is higher than that in the Thais. We reported an appropriate reference to define hypocitraturic phenotype at urinary citrate < 200 mg/day with sensitivity and specificity of 84% and 64%, respectively[14]. Food frequency questionnaire revealed that Thai nephrolithiasis patients (mostly from NE) consumed low amount of citrus fruits-this could be one of contributing factors for hypocitraturia [9,10]. Although it remains high, prevalence of hypocitaturia in Thai nephrolithiasis patients trends to be decreased over time (92% in 1992 vs. 84% in 2009).

This may be due to people get educated to consume more citric acid-containing food.

Is potassium depleted in Thai kidney stone patients?

Potassium depletion causes low urinary citrate excretion possibly via low intracellular pH, luminal acidification and upregulated Na-citrate cotransporter [15-17]. Rats fed with potassium-deficient diet shows significantly rapid decreases in urinary citrate and pH[18,19]. In NE nephrolithiasis patients, occurrences of hypokaliuria and hypokalemia were 83-91% and 10-37%, respectively[20,21]. Urinary citrate was positively correlated with urinary and serum potassium[21,22]. Acid loading test demonstrated that hypokaliuria and hypocitraturia in NE renal stone patients were infrequently due to distal renal tubular acidosis. These data suggests that huge fraction of NE nephrolithiasis patients is considered to be potassium deficient, which consequently causes hypocitraturia. Sriboonlue et al. reported that dietary potassium intake among these patients was inadequate moreover high amount of potassium loss through sweat was observed, suggesting the etiology of potassium depletion in these population[20,23,24]. Thus, potassium citrate is a drug of choice for treating the NE renal stone patients.

In addition to potassium, study by Reungjui and coworkers showed that magnesium was found to be depleted in NE renal stone patients[25]. Urinary potassium was linearly correlated with urinary magnesium. Magnesium deficiency was associated with low urinary citrate excretion. Magnesium supplementation in magnesium-depleted renal stone patients significantly increased urinary excretion of citrate. The authors suggested that a severe hypo-citraturia in NE stone patients was plausibly aggravated by dual deficiencies of potassium and magnesium. However, the mechanism of magnesium on citrate metabolism remains to be elucidated.

Renal handling of citrate and hypocitraturia

Citrate is freely filtrated through renal glomerular[13,15,17]. Reabsorption of citrate chiefly takes place in the proximal tubules. In human, it has been estimated that 60-90% of filtered citrated is reabsorbed [15,17]. Sodium-dicarboxylate cotransporter-1 (NaDC-1), first cloned by Pajor[26,27] is responsible for reabsorption of citrate. Its binding to 3 sodium ions allows the protein to transport 1 divalent citrate ($citrate^{-2}$). The uptake activity is highly pH dependent, faster in acidic condition [28]. Citric acid is a weak tricarboxylic acid whose carboxyl groups have pK values of 3.1, 4.8 and 6.4, thus at physiologic blood pH (7.4) or in slightly acidic urine (pH >6.4) it exists largely as $citrate^{3}$[29]. Acidic urine (pH less than 6.4) would generate more $citrate^{-2}$ that favors citrate reabsorption by NaDC-1 leading to hypocitra-turia.

Intracellular citrate is catabolized by two pathways, 1) Krebs cycle in mitochondria and 2) breakdown by ATP-citrate lyase (lipogenic enzyme) in cytoplasm[13]. The products of the former pathway are 3 NADH, 1 GTP, 1 FADH2 and 2 CO_2 (bicarbonate precursor). The latter generates Acetyl-CoA (fatty acid precursor) and oxaloacetate (glucose precursor via gluconeogenesis). Both uptake of citrate to mitochondria and cytoplasmic breakdown of citrate by ATP-citrate lyase are upregulated by proton (low intracellular pH). Therefore, in low cell pH or intracellular acidosis condition citrate breakdown is increased, leading to low intracellular citrate concentration. This creates citrate gradient between cell and urinary lumen that facilitates citrate reabsorption and eventually causes hypocitraturia.

Chronic metabolic acidosis and potassium depletion have been demonstrated to cause renal intracellular acidosis[30,31]. Transport of citrate across renal brush border membrane significantly increased

in rats loaded with acid water (NH4Cl) compared to controls and rats loaded with alkali water ($NaHCO_3$) [32]. Rats fed with low-potassium diet showed decreased fractional citrate excretion, but increased citrate reabsorption activity of NaDC-1[16]. Melnick et al. demonstrated that chronic metabolic acidosis and hypokalemia (both caused hypocitraturia in rats) increased renal cortical ATP-citrate lyase activity and protein expression[33]. Rats fed with high-carbohydrate/low-fat diet was also showed an increase in liver ATP-citrate lyase mRNA[34]. On the other side, activity and abundance of renal mitochondrial aconitase (enzyme that converses citrate to isocitrate in Krebs cycle) was increased in rats with chronic metabolic acidosis and potassium depletion, which contributed to hypocitrauria[35]. In sum, acid-base change primarily modulates urinary citrate excretion. Chronic potassium depletion and metabolic acidosis cause intracellular acidosis that increases breakdown of citrate in renal tubular cells, leading to increased reabsorption of urinary citrate and hypocitraturia. High-carbohydrate/low-fat diet might contribute hypocitraturia via upregulation of ATP-citrate lyase[23]. Tosukhowong et al. demonstrated that NE renal stone patients supplemented with potassium magnesium citrate (42 mEq K, 21 mEq Mg and 63 mEq citrate) for 1 month had significantly increases in urinary pH, potassium, magnesium and citrate, but leukocyte ATP-citrate lyase and mitochondrial acontase were significantly decreased[36].

Other factors known to influence on urinary citrate excretion include high animal protein intake (causes acidosis), high sodium intake (produces mild acidosis), starvation (increased urinary citrate reabsorption for ATP synthesis) renal tubular acidosis, diarrhea/malabsorption, and medications (e.g., ACE inhibitors, acetazolamide, amilorde, calcitonin, lithium and vitamin D).

Genetic influence on urinary citrate excretion has been demonstrated. Shah et al. categorized citraturic pattern into low, intermediate and high excretors[37]. Pedigree analysis (six families) suggested three excretor phenotypes, with a codominant mode of inheritance. Mossetti et al. reported association of hypocitraturic phenotype with allelic variants of vitamin D receptor (BsmI and TaqI)[38]. Recent study by Okamoto et al. showed that B allele of I550V polymorphism of NaDC-1 gene was associated with low urinary citrate excretion in recurrent kidney stone formers[39]. We have investigated the exonic polymorphisms of NaDC-1 gene in Thai nephrolithiasis patients to find the association with hypocitraturic phenotype (ongoing research). GG genotype of rs11567842 SNP in NaDC-1 gene (it is bb genotype reported by Okamoto et al.) was found to associate with high excretion of urinary citrate.

Anti-lithogenic role of citrate

Although the precise mechanism of kidney stone formation is not fully understood, urinary crystals, tightly stacked together by organic matrix, are building blocks of stone. Driving force of the crystallization is supersaturation of lithogenic ions, particularly calcium, oxalate and phosphate. Citrate, potassium and magnesium effectively reduce the urinary saturation of these lithogenic salts mainly by chelating mechanism, thereby defined as stone inhibitors[40]. Citrate is currently known as the most potent stone inhibitor. It inhibits calcium oxalate and formation by forming a soluble calcium citrate. In vitro evidences demonstrated that citrate can inhibit crystal growth and agglomeration[41] and also binding of crystals to renal epithelial cells[42]. In vivo, inhibition of calcium oxalate crystal deposit in rat kidneys by citrate has been demonstrated[43]. These suggest that citrate could be a therapeutic agent for preventing urinary stone formation.

In 1984, Pak and colleagues investigated the

citraturic response of oral potassium citrate in 22 normal volunteers and 21 uric acid and calcium oxalate nephrolithiasis patients (a pioneer clinical study), and found that urinary citrate is successfully raised by potassium citrate supplement. Potassium citrate of 60 mEq/day restored normal urinary citrate (>320 mg/day) in hypocitraturic nephrolithiasis patients[44].

Mechanism of hypocitraturia in NE kidney stone patients: derived from our research experiences

Based on our research experiences, we believe that dietary (intake of low-potassium diets, high-carbohydrate/low-fat diets, and low intake of citrus fruits), environmental (hot weather, outdoor working, sweat loss) and genetic (polymorphism of NaDC-1 gene) factors act in concert to cause hypocitraturia in NE renal stone patients. Chronic potassium depletion due to low-potassium diet intake and sweat loss causes intracellular acidosis in renal tubular cells. Low cell pH induced breakdown of cytoplasmic citrate through 1) Krebs cycle in mitochondria and 2) lysis by ATP-citrate lyase in cytoplasm. A low concentration of citrate in cytoplasm leads to increased urinary reabsorption citrate and consequently hypocitraturia. Acidic pH is also increased activity of NaDC-1 and protein abundance. In addition, citrate-2 is predominant in acidic urine (pH <6.4), which favors to be transported by NaDC-1. Putative mechanism of hypocitraturia in NE renal stone patients is depicted in Figure 1.

Do we have a suitable regimen for treating NE renal stone patients?

Increased fluid intake and dietary modification are general advice for nephrolithiasis patients. The well accepted regimen for medical therapy of kidney

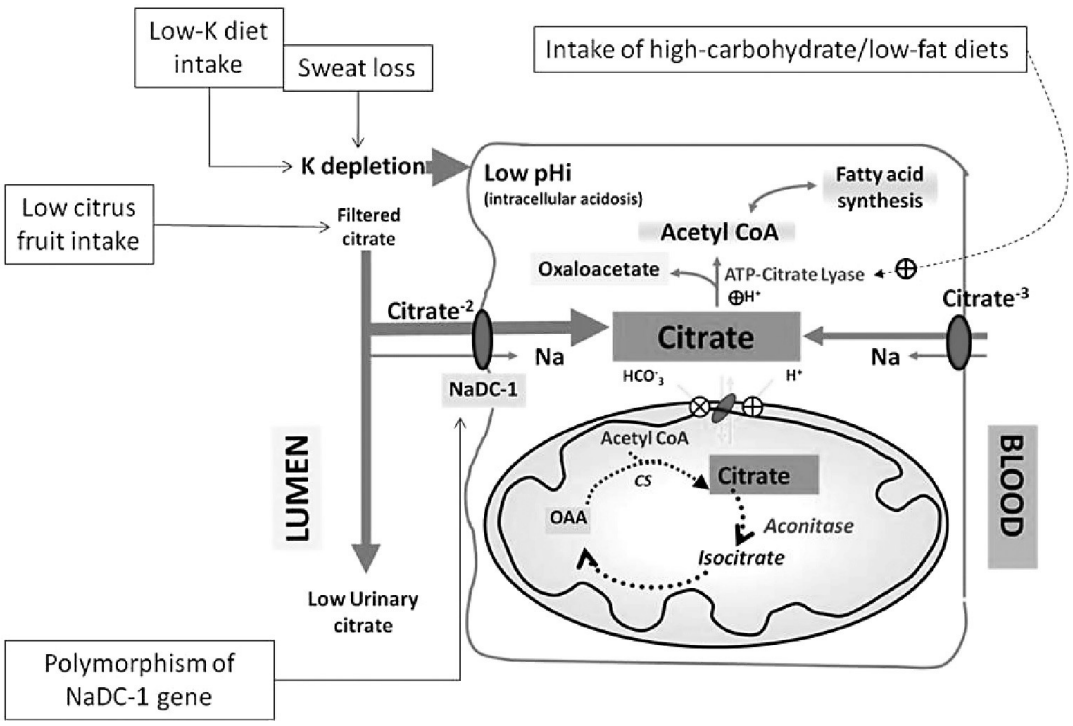

Fig 1 *Putative mechanism of hypocitraturia in NE renal stone patients. Dash line represents hypothesized pathway.*

stone is potassium citrate and its related preparation. To date, there are four prospective, randomized, controlled trials of alkali citrate for the treatment of stone recurrence. Fifty-four percent of alkali citrate-treated patients remained stone free through at least 1 year of follow up, while only 35% found in the control patients[13,45]. Alkalinizing urine and citraturic response are main action of potassium citrate. Side effect of alkali citrate therapy is not severe, usually involved gastrointestinal upset. Patients' complaints include abdominal bloating, diarrhea, nausea, and abdominal pain[13,45]. Oxidative stress and inflammation were documented in kidneys of nephrolithic rats[46,48]. We have demonstrated that patients with nephrolithiasis have increased oxidative stress, enhanced renal tubular injury and some degree of intrarenal inflammation[49-51]. Treatment with potassium citrate has minimal impact to improve these pathological changes[22]. Oxidative stress and renal tubular injury have been believed to increase a risk for stone recurrence. Therefore, natural-based regimen that is capable of alkalinizing urine, increasing urinary citrate and reducing oxidative stress and renal tubular injury (with a lesser side effect than potassium citrate) is required for kidney stone treatment. We have developed lime powder regimen (LPR) containing 21 mEq of potassium and 63 mEq of citrate for treating kidney stone disease in Thailand. Acute toxicity of LPR in mouse was performed, and its LD50 was >10 g/kg, suggesting that LPR has a very low toxicity. In addition, total antioxidant capacity of LPR is very high (by DPPH method). Our preliminary study in kidney stone patients found that consumption of LPR (dissolved in at least 250 ml water and drink daily) for three months efficiently increased urinary citrate and urine pH[52]. LPR was also reduced oxidative stress and renal tubular injury in kidney stone patients, while potassium citrate did not. There were no side effects and complaints from LPR-treated patients. Thus, treatment with LPR effectively reduced risks for kidney stone formation. We hypothesize that LPR could be a novel homemade-regimen for treatment of Thai kidney stone patients. Long-term outcome of LPR and its effect on stone recurrence rate are waiting to be investigated.

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