Urol Res (2008) 36:149–155
DOI 10.1007/s00240-008-0141-9

ORIGINAL PAPER

Citraturic, alkalinizing and antioxidative effects of limeade-based regimen in nephrolithiasis patients

Piyaratana Tosukhowong · Chatchai Yachantha · Thosaphol Sasivongsbhakdi · Supoj Ratchanon · Suchada Chaisawasdi · Chanchai Boonla · Kriang Tungsanga

Received: 2 April 2008 / Accepted: 21 May 2008 / Published online: 17 June 2008

Abstract Potassium citrate has long been used as a prophylactic remedy for nephrolithiasis recurrence. Lemonade consumption is also suggested as an option. We compared the efficacy of consumption of solution containing manufactured lime powder with that of potassium citrate, on the improvement of metabolic risk factors, oxidative stress and renal tubular damage in nephrolithiasis patients. Patients with kidney stone were enrolled and randomly assigned to three treatment programs for 3 month period consisting of consumption of solution containing lime powder (Group 1, $n = 13$), potassium citrate (Group 2, $n = 11$) and lactose as placebo regimen (Group 3, $n = 7$). Lime powder and potassium citrate contained equal amounts of potassium (21 mEq) and citrate (63 mEq). After treatment, there was an increase in urinary pH, potassium and citrate in Group 1 and 2. Increased plasma potassium and red blood cell glutathione (R-GSH) and decreased urinary malondialdehyde were found in Group 1, but not observed in Group 2. R-GSH was decreased in Group 2. Urinary *N*-acetyl-β-glucosaminidase activity and fractional excretion of magnesium, as renal tubular damage indicators, were decreased only in Group 1. In Group 3, all measured parameters were unaltered except for an increased urinary chloride. In conclusion, consumption of our in-house lime powder exerted citraturic and alkalinizing actions as efficient as consumption of potassium citrate. In addition, it provided an antioxidative effect and was able to attenuate renal tubular damage. These pharmacological properties may be clinically useful to diminish the stone-forming potential in kidney stone patients and hence for preventing recurrent calculi.

Keywords Lime · Antioxidants · Nephrolithiasis · Oxidative stress · Renal tubular damage

P. Tosukhowong (✉) · C. Yachantha · C. Boonla
Department of Biochemistry, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, Thailand
e-mail: fmedpts@md2.md.chula.ac.th

T. Sasivongsbhakdi · S. Ratchanon
Department of Surgery, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, Thailand

S. Chaisawasdi
Pilot Plant Development and Training Institute,
King Mongkut's University of Technology Thonburi,
Bangkok 10330, Thailand

K. Tungsanga
Department of Medicine, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, Thailand

Introduction

Nephrolithiasis is a complex condition with a high recurrence rate [1]. Metabolic abnormalities, particularly hypocitraturia increase the probability of stone relapse [2]. Additionally, oxidative stress and renal tubular damage in nephrolithiasis patients are suggested to associate with stone recurrence [3–5]. Thus, therapeutic regimen that is capable of correcting metabolic abnormality, reducing oxidative stress and attenuating renal tubular damage is a potentially good remedy for clinical use.

Potassium citrate is currently recommended for prophylaxsis of nephrolithiasis [6, 7]. It provides alkalinizing and citraturic effects to an extent that the probability of stone relapse is reduced [6–9]. In a recent review, it is stated that up to 48% of alkali citrate-treated patients were withdrawn prematurely from the studies because of intolerable side effects [7]. Alternative regimen that provides less or no adverse effects is desirable. Natural products rich in citric acid, e.g., citrus fruits are considered as an option for nephrolithiasis treatment.

Lime (*Citrus auranlifolia*), an originally wild species from the Rutaceae family is common in Southeast Asian countries. Lime juice is rich not only in citrate, but also in various antioxidants such as ascorbic acid, polyphenols and flavonoids [10]. Increased urinary excretion of citrate after lemonade consumption has been demonstrated in nephrolithiasis patients [11, 12]. However, a recent crossover trial reported that lemonade did not significantly improve urinary citrate and acidic pH in stone-forming patients [13]. To ensure that lemon or lime-based regimen is clinically useful, additional evidence is required.

In this study, we evaluated the beneficial effects of a manufactured lime powder regimen in comparison with potassium citrate in patients with nephrolithiasis.

Materials and methods

Patients and study design

The study was a prospective, randomized, double-blinded, placebo-controlled trial conducted between July 2004 and October 2005. All patients reserved the right to withdraw from the study at any time and the withdrawers were followed up in accordance of the standard protocol. Informed consents were obtained from all participants and the research protocol was approved by the Institutional Ethics Committee, Faculty of Medicine, Chulalongkorn University.

At the beginning, a total of 39 post-operative patients with nephrolithiasis who came for follow up at the Department of Surgery, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok were recruited. Inclusion criteria were (1) the age was no greater than 60 years old, (2) the X-ray KUB film was negative for residual stones, (3) surgical removal of stone was longer than 3 month prior to the accrual (4) urine culture was negative. Patients with severe renal insufficiency (creatinine clearance <25 ml/min) or other clinically significant systemic illnesses, e.g., liver cirrhosis, jaundice, asthma, chronic lung disease, malabsorption syndrome, chronic diarrhea, malignancies, stroke, myocardial infarction and congestive heart failure were excluded.

Eligible patients were randomly assigned to three treatment arms, i.e., lime powder (Group 1), standard potassium citrate (Group 2) and placebo control (Group 3). At the beginning of a 3 month trial period, 13 patients were placed in each group. Patients in Group 1 were treated with in-house manufactured lime powder that contained 63 and 21 mEq of citrate and potassium, respectively. Group 2 patients received oral potassium citrate solution that contained potassium and citrate on an equimolar basis with that in Group 1. Group 3 patients received placebo (lactose), and served as controls. The three medications were in powder form packed in sachets. Patients were instructed to consume one sachet daily by dissolving the medication in 200 ml of water throughout the treatment period, and advised to increase water intake as well as avoid high salt- and high purine-diets. At the end of the study, the number of patients in Group 2 and 3 was reduced to 11 and 7 with dropout rates of 15 and 46%, respectively.

Manufacture of lime powder

The lime-based regimen used was made from natural lime, *Citrus auranliforia*, swingle. Lime fruits were thoroughly washed, cut in half and squeezed with a hand-squeezer to make fresh lime juice. Seeds were filtered out and the lime juice was processed to reduce bitterness. The lime juice was then freeze-dried using a freeze dryer (model FD8, Heto-Holten AS, Denmark). Physical characteristics of the juice made from the lime powder included color, odor and flavor resembled the original fresh lime juice. The chemical composition (pH, total soluble solids, ascorbic acid, citrate, calcium, sodium and potassium) in the re-dissolved lime juice was not different from that found in the fresh lime juice. The amount of citrate in lime powder reached the clinical dose of 63 mEq/5 g lime powder, but potassium content did not. Pharmaceutical grade potassium was added to achieve a clinical dose of 21 mEq/5 g lime powder. Lime powder of 5 g was packed into an aluminum sachet, completely sealed and kept at 4°C until used. It was stable for a year.

Amounts of ascorbic acid, total polyphenols and flavonoids in lime power were 27.30, 9.20 and 8.80 mg/sachet, respectively. These antioxidants were determined by HPLC, Folin–ciocalteau and modified a luminium-nitrate methods, respectively [14]. The radical-scavenging activity (RSA) of the solution of lime powder and potassium citrate was determined with diphenylpicrylhydrazyl (DPPH) method [15]. The RSA of lime powder was notably higher than that of potassium citrate ($21.5 \pm 0.85\%$ vs. $0.88 \pm 0.16\%$, at concentration 10 mg/ml). The RSA of lactose placebo was undetectable.

Blood and 24 h urine collection

Fasting heparinized blood and 24 h urine specimens were collected from each patient at the beginning and at the end of the 3 month follow up. Creatinine (Cr), sodium (Na), chloride (Cl), potassium (K), bicarbonate, calcium (Ca), phosphate, magnesium (Mg) and malondialdehyde (MDA) were measured in the plasma sample. Glutathione in red blood cells (R-GSH) was determined. Cr, Na, Cl, K, Ca, phosphate, Mg, uric acid, citrate, oxalate, proteins and *N*-acetyl-β-glucosaminidase (NAG) activity were also determined in the urine samples. Creatinine clearance (CCr) and

fractional excretion of magnesium (FE–Mg) as indicators of glomerular dysfunction and tubular reabsorption impairment, respectively, were determined.

Statistical analysis

Data were presented as mean ± standard deviation (SD) for variables with normal distribution and as median (interquartile range) for variables with skewed distribution. Difference between three independent groups was assessed by one-way ANOVA or Kruskal-Wallis test where appropriate. Two dependent groups were compared by Wilcoxon matched-pairs signed-rank test. Statistical analyses were performed using STATA version 8.0 (StataCorp, College Station, TX, USA). A two-sided $P < 0.05$ was considered significant.

Results

Table 1 shows demographic data and baseline blood and urine biochemistry of the study population. There was no dropout in Group 1, whereas compliances in Group 2 and 3 were 11 (85%) and 7 (54%), respectively. Group 1, 2 and Group 3 consisted of 7 (54%), 6 (55%) and 4 (57%) males, respectively. Mean ages of the three groups were 47.1 ± 16.5, 47.8 ± 10.1 and 54.1 ± 8.6 years, respectively. Age and body mass indexes (BMI) were not significantly different.

The baseline blood and urine chemistry when compared among the three groups were not significantly different (Table 1). The oxidative stress status, as indicated by R-GSH, plasma (P)-MDA and urinary (U)-MDA, among the study groups were not significantly different. In addition,

Table 1 Baseline demographic and clinical characteristics of the study populations

Characteristics	Group 1	Group 2	Group 3	*P* value
n	13	11	7	
Drop-out patients (%)	0 (0.0)	2 (15.4)	6 (46.2)	
Male:Female (%)	7 (54):6 (46)	6 (55):5 (45)	4 (57):3 (43)	
Age (years) (mean ± SD)	47.1 ± 16.5	47.8 ± 10.1	54.1 ± 8.6	0.442
BMI (kg/m^2) (mean ± SD)	24.0 ± 3.5	24.8 ± 1.6	25.9 ± 5.0	0.500
P-Creatinine (mg/dl)	1.1 (0.2)	1.1 (0.4)	1.1 (0.2)	0.473
P-Sodium (mEq/l)	138.0 (2.0)	140.0 (2.0)	138.0 (3.0)	0.152
P-Potassium (mEq/l)	3.9 (0.5)	4.1 (0.3)	4.0 (0.8)	0.095
P-Chloride (mEq/l)	104.0 (2.0)	105.0 (4.0)	105.0 (6.0)	0.268
P-Bicarbonate (mEq/l)	20.7 (1.6)	20.2 (2.3)	22.0 (3.1)	0.302
P-Calcium (mg/dl)	9.4 (0.5)	9.5 (1.0)	9.1 (0.7)	0.391
P-Phosphate (mg/dl)	3.9 (0.7)	3.8 (1.0)	3.5 (0.2)	0.185
P-Magnesium (mg/dl)	2.1 (0.3)	2.1 (0.4)	2.3 (0.7)	0.946
Urine volume (ml)	2250 (1500)	2120 (1650)	2440 (1217)	0.808
U-pH	6.04 (0.53)	6.05 (0.59)	5.98 (0.31)	0.281
U-Creatinine (g/day)	1.2 (0.5)	1.2 (0.5)	1.2 (1.6)	1.000
U-Sodium (mEq/day)	141.1 (59.0)	160.1 (125.7)	127.7 (80.7)	0.538
U-Potassium (mEq/day)	25.2 (14.9)	34.9 (17.1)	29.3 (28.6)	0.342
U-Chloride (mEq/day)	113.4 (81.9)	123.4 (115.0)	101.8 (72.1)	0.582
U-Calcium (mg/day)	238.6 (124.6)	178.9 (180.6)	154.6 (112.8)	0.845
U-Phosphate (g/day)	0.5 (0.3)	0.6 (0.5)	0.7 (0.4)	0.780
U-Magnesium (mg/day)	79.5 (47.3)	71.8 (78.0)	75.0 (24.0)	0.841
U-Uric acid (mg/day)	612.0 (339.6)	468.5 (452.1)	867.2 (461.9)	0.124
U-Oxalate (mg/day)	23.8 (14.3)	37.7 (21.0)	40.0 (64.8)	0.099
U-Citrate (mg/day)	56.0 (70.0)	69.7 (125.0)	49.8 (57.1)	0.889
R-GSH (μmol/g Hb)	5.8 (1.2)	6.9 (1.6)	6.4 (2.3)	0.075
P-MDA (μmol/l)	2.7 (1.6)	3.0 (3.0)	4.3 (3.0)	0.474
U-MDA (μmol/g Cr)	10.7 (4.6)	5.7 (11.2)	10.3 (12.9)	0.117
CCr (ml/min)	71.5 (25.7)	94.7 (38.8)	83.1 (45.1)	0.463
FE–Mg (%)	2.8 (3.1)	3.4 (2.0)	3.2 (2.6)	0.559
U-NAG activity (U/g Cr)	4.7 (3.1)	3.9 (3.3)	4.3 (7.2)	0.490
U-Proteins (g/day)	0.16 (0.31)	0.15 (0.26)	0.22 (0.19)	0.611

Data presented as median (interquartile range) except age and BMI variables

BMI body mass index, *P* plasma, *U* urine, *R* red blood cell, *GSH* glutathione, *Hb* hemoglobin, *MDA* malondialdehyde

CCr, creatinine clearance; FE–Mg, fractional excretion of magnesium; NAG, *N*-acetyl-*β*-glucosaminidase. *P* values obtained from one way ANOVA (for age and BMI) or Kruskal–Wallis test

CCr, FE-Mg, U-NAG activity and U-proteins did not significantly differ among the study populations. These data ensured the baseline equality among the three groups.

Table 2 shows changes in blood chemical profiles after the treatments. Treatment with the lime powder regimen caused significantly increased plasma potassium while the others did not.

Changes in urinary parameters in the three groups are shown in Table 3. After treatments, urine pH was significantly increased in Groups 1 and 2, but urine volume was unchanged in all groups. Like urine pH, urinary excretions of potassium and citrate were significantly increased in Groups 1 and Group 2. A significant rise of urinary chloride was found in Group 3, but it was unchanged in Groups 1 and 2.

Whether the regimens were able to reduce oxidative stress status in stone patients were explored. Levels of P-MDA were unchanged after treatment in all groups (Table 2). A significant reduction of U-MDA was observed in Group 1 ($P = 0.011$), but this change was not seen in Groups 2 and 3 (Fig. 1). Significant increase in R-GSH was observed only in Group 1 ($P = 0.007$). Unfortunately, R-GSH was significantly decreased in Group 2 ($P = 0.016$). In Group 3, R-GSH and U-MDA were unaltered.

Improvements of kidney function after the three treatments were investigated as shown in Table 4. The CCr and U-proteins compared between before and after treatments were unchanged in all groups. U-NAG activity ($P = 0.004$) and FE–Mg ($P = 0.003$) were significantly decreased in Group 1, but they remained unchanged in Groups 2 and 3.

Table 2 Changes in blood biochemistry in studied groups over the trial period of 3 months

Variables	Group 1			Group 2			Group 3		
	Base-line	3 months	*P*	Base-line	3 months	*P*	Base-line	3 months	*P*
P-Creatinine (mg/dl)	1.1 (0.2)	1.1 (0.3)	0.456	1.1 (0.4)	1.2 (0.4)	0.054	1.1 (0.2)	1.2(0.3)	0.702
P-Sodium (mEq/l)	138.0 (2.0)	140.0 (5.0)	0.149	140.0 (2.0)	141.0 (4.0)	0.822	138.0 (3.0)	140(2.0)	0.121
P-Potassium (mEq/l)	3.9 (0.5)	4.15 (0.4)	0.033*	4.1 (0.3)	4.2 (0.3)	0.531	4.0 (0.8)	4.0(0.4)	0.932
P-Chloride (mEq/l)	104.0 (2.0)	103 (4.0)	0.674	105.0 (4.0)	103 (3.0)	0.326	105.0 (6.0)	105(6.0)	0.203
P-Bicarbonate (mEq/l)	20.7 (1.6)	21.0 (4.7)	0.727	20.2 (2.3)	21.9 (2.8)	0.476	22.0 (3.1)	22.2(1.8)	0.612
P-Calcium (mg/dl)	9.4 (0.5)	9.0 (0.6)	0.438	9.5 (1.0)	9.5 (1.3)	0.532	9.1 (0.7)	9.2(0.4)	0.715
P-Phosphate (mg/dl)	3.9 (0.7)	4.0 (0.7)	0.220	3.8 (1.0)	4.0 (0.9)	0.306	3.5 (0.2)	4.0(0.7)	0.149
P-Magnesium (mg/dl)	2.1 (0.3)	2.1 (0.1)	0.442	2.1 (0.4)	2.1 (0.2)	0.964	2.3 (0.7)	2.1(0.4)	0.866
P-MDA (μmol/l)	2.7 (1.6)	2.5 (1.6)	0.249	3.0 (3.0)	2.7 (1.6)	0.790	4.3 (3.0)	3.1(1.9)	0.311

Data presented as median (interquartile range)

P plasma, *MDA* malondialdehyde

P values obtained from the Wilcoxon matched-pairs signed-rank test. * Statistically significant

Table 3 Changes in urinary parameters in studied groups over the trial period of 3 months

Variables	Group 1			Group 2			Group 3		
	Base-line	3 months	*P*	Base-line	3 months	*P*	Base-line	3 months	*P*
Urine volume (ml)	2,250 (1,500)	2,680 (1,220)	0.196	2,120 (1,650)	2,460 (1,800)	0.929	2,440 (1,217)	2,850 (1,300)	0.150
U-pH	6.04 (0.53)	6.68 (0.57)	0.028*	6.05 (0.59)	6.54 (1.04)	0.003*	5.98 (0.31)	6.00 (1.15)	0.865
U-Creatinine (g/day)	1.2 (0.5)	1.3 (0.2)	0.311	1.2 (0.5)	1.07 (1.13)	0.306	1.2 (1.6)	1.3 (1.1)	0.203
U-Sodium (mEq/day)	141.1 (59.0)	190.2 (106.1)	0.600	160.1 (125.7)	148.1 (108.9)	0.657	127.7 (80.7)	166.8 (113.0)	0.499
U-Potassium (mEq/day)	25.2 (14.9)	49.0 (17.7)	0.005*	34.9 (17.1)	55.6 (25.2)	0.003*	29.3 (28.6)	41.1 (14.0)	0.398
U-Chloride (mEq/day)	113.4 (81.9)	144.7 (91.8)	0.075	123.4 (115.0)	113.2 (159.3)	0.790	101.8 (72.1)	150.1 (77.8)	0.028*
U-Calcium (mg/day)	238.6 (124.6)	119.0 (152.0)	0.927	178.9 (180.6)	139.7 (95.4)	0.424	154.6 (112.8)	176.7 (180.4)	0.735
U-Phosphate (g/day)	0.5 (0.3)	0.7 (0.4)	0.600	0.6 (0.5)	0.7 (0.6)	0.657	0.7 (0.4)	0.5 (0.7)	0.866
U-Magnesium (mg/day)	79.5 (47.3)	82.5 (42.9)	0.917	71.8 (78.0)	61.4 (58.5)	0.929	75.0 (24.0)	77.9 (40.7)	0.735
U-Uric acid (mg/day)	612.0 (339.6)	707.9 (167.0)	0.507	468.5 (452.1)	702.0 (381.6)	0.182	867.2 (461.9)	803.7 (855.2)	0.866
U-Oxalate (mg/day)	23.8 (14.3)	18.8 (6.7)	0.279	37.7 (21.0)	23.0 (17.0)	0.131	40.0 (64.8)	33.2 (30.6)	1.000
U-Citrate (mg/day)	56.0 (70.0)	270.0 (319.5)	0.002*	69.7 (125.0)	387.4 (239.2)	0.010*	49.8 (57.1)	70.7 (85.5)	0.091

Data presented as median (interquartile range)

U urine. *P* values obtained from the Wilcoxon matched-pairs signed-rank test. * Statistically significant

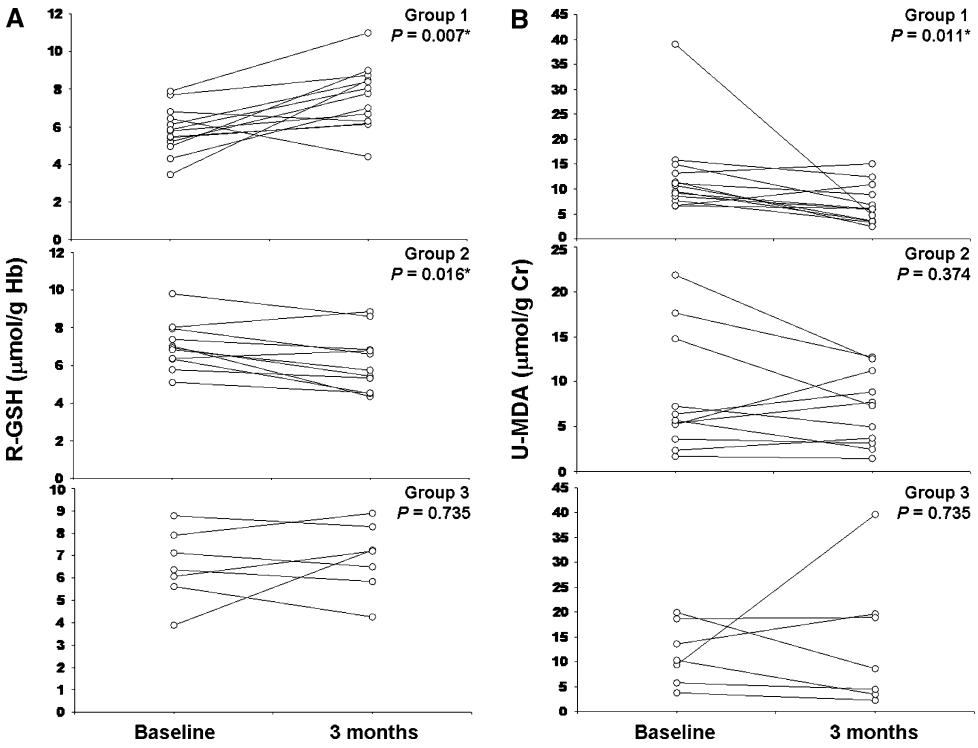

Fig. 1 Changes in oxidative stress biomarkers, red blood cell glutathione (R-GSH, **a**) and urinary malondialdehyde (U-MDA, **b**) in the three treatment groups over the trial period of 3 months. U-MDA was significantly decreased in Group 1, but remained unchanged in Groups 2 and 3. Level of R-GSH was significantly increased in Group 1, but it was significantly declined in Group 2. In Group 3, both R-GSH and U-MDA levels were unaltered after the intervention

Table 4 Kidney function biomarkers changed in studied groups after the treatment period of 3 months

Variables	Group 1			Group 2			Group 3		
	Base-line	3 months	*P*	Base-line	3 months	*P*	Base-line	3 months	*P*
CCr (ml/min)	71.5 (25.7)	76.1 (39.0)	0.650	94.7 (38.8)	81.4 (61.0)	0.657	83.1 (45.1)	80.6 (73.9)	0.612
FE–Mg (%)	2.8 (3.1)	2.1 (1.4)	0.033*	3.4 (2.0)	3.7 (1.8)	0.248	3.2 (2.6)	3.0 (2.8)	0.865
U-NAG activity (U/g Cr)	4.7 (3.1)	2.0 (3.0)	0.004*	3.9 (3.3)	3.4 (2.4)	0.859	4.3 (7.2)	3.6 (5.4)	0.499
U-Proteins (g/day)	0.16 (0.31)	0.15 (0.15)	0.235	0.15 (0.26)	0.13 (0.28)	0.823	0.22 (0.19)	0.17 (0.15)	0.933

Data presented as median (interquartile range)

U, urine; CCr, creatinine clearance; FE–Mg, fractional excretion of magnesium; NAG, *N*-acetyl-*β*-glucosaminidase. *P* values obtained from the Wilcoxon matched-pairs signed-rank test. * Statistically significant

Discussion

Potassium citrate is widely used for medical management of nephrolithiasis. Alkalinizing and citraturic responses are the main action of potassium citrate or its related formulations [4, 6, 7, 9, 16–21]. The drug efficiently elevates urinary citrate and increases urine pH that in turn reduce the rate of recurrence [7, 22, 23]. We previously demonstrated that treatment with potassium citrate in nephrolithiasis patients at 63 mEq/d effectively increased urinary potassium and citrate [4].

Alternatively, citrus fruit consumption has been suggested as remedy for nephrolithiasis. A small cross-over trial in eight healthy and three hypocitraturic nephrolithiasis men showed that orange juice consumption acted like potassium citrate to increase urinary pH and citrate [24]. However, it was inefficient to decrease urinary saturation of calcium oxalate. Lemon juice, which contains nearly five times the concentration of citrate compared to orange juice, was later shown to have a citraturic effect in 12 patients with calcium nephrolithiasis, and noted that lemonade ingestion was well tolerated [11]. Another retrospective case-control study that evaluated the long-term effect of lemonade in 11 hypocitraturic nephrolithiasis subjects compared with potassium citrate, concluded that lemonade-based therapy appeared to be an alternative for hypocitraturic stone patients who are unable to tolerate the potassium citrate drug [12]. However, a recent prospective cross-over trial in 21 kidney stone patients reported that potassium citrate, but not lemonade, increased urinary pH and citrate [13]. Due to low potassium content in the natural lemonade may be a cause as the authors suggested.

We investigated the pharmacological effect of the manufactured lime-based regimen which has an equimolar content of potassium and citrate as a potassium citrate drug in the improvement of metabolic abnormality, oxidative stress and renal injury in nephrolithiasis patients. Both lime powder and potassium citrate therapies significantly elevated urinary pH, potassium and citrate while urinary excretions of calcium and oxalate were unaltered. These physicochemical changes may lead to decreased urinary supersaturation and crystal-forming potential, thus reducing the propensity to develop the recurrent calculi. Our data clearly demonstrate that the in-house lime powder regimen delivers an antilithogenic action for treating nephrolithiasis that is equivalent to standard potassium citrate.

Our results complemented Seltzer's study which found that consumption of lemonade significantly increased urinary citrate concentration and tended to decrease calcium excretion [11]. Lemonade used in Seltzer's study contained a large amount of citrate (84 mEq) but only small amounts of potassium (approximately 0.23 mEq), and the study was conducted over 6 days. Koff et al. [13] suggested that the low potassium content in natural lemonade may cause a poor alkalinizing effect. In their study, which was conducted for 5 days using lemonade with citrate concentration of 63 mEq, poor citraturic and alkalinizing responses of lemonade treatment were reported [13]. Despite an equal concentration of citrate was used in our and Koff's studies, an efficient citraturic response of our lime powder regimen may be explained by a much longer intervention period. The composition difference (lemon vs. lime) might be the other reason.

In the potassium citrate therapy, R-GSH was reduced and P-MDA and U-MDA were unaltered. In contrast, increase in R-GSH and decreases in U-MDA, FE–Mg and U-NAG activity were revealed in treatment with the lime powder regimen. Our findings indicate that lime powder regimen effectively improves oxidative stress status and attenuates the damage to renal tubular cells offering a better prophylactic mean for nephrolithiasis. Furthermore, any gastrointestinal adverse effects, common with potassium citrate treatment [6, 7], were not observed in patients who were treated with lime powder.

The lime powder regimen contained a number of antioxidants, e.g., vitamin C, polyphenols and flavonoids (described in Methods section). The radical-scavenging activity of the lime powder regimen is evidently higher than that found in potassium citrate. It is reasonable to accept that patients who were treated with lime powder regimen obtained more of these antioxidants than those treated with potassium citrate or placebo. Our data showed that lime powder therapy provided antioxidative response (indicated by increase in R-GSH and decreases in U-MDA, U-NAG activity and FE–Mg) whereas the other two therapies did not. Vitamin C supplement has been shown to increase glutathione in human lymphocytes [25], which might explain an increased R-GSH in lime powder-treated patients. We thought that patients treated with lime powder regimen develop antioxidative force to overcome oxidative tubular damage. Treatment with this lime-based regimen may diminish the predisposition to stone relapse. To elucidate whether our lime powder regimen can prevent stone recurrence, a long-term cohort trial using stone re-growth as an outcome awaits to be performed.

In this study, we found decreased R-GSH and unchanged P-MDA and U-NAG activity after the potassium citrate treatment. Our previous work, unchanged R-GSH, decreased P-MDA and increased U-NAG activity after potassium citrate treatment were found [4]. The major differences between these two studies are the trial period (1 vs. 3 months) and number of subjects ($n = 30$ vs. $n = 11$). The subjects recruited in the previous study were pre-operative kidney stone patients while those in this study were post-operative patients. This could be the reason for the discrepancies. However, the conclusion is fixed. Treatment with potassium citrate has a small effect on improvement of oxidative stress and is not efficient enough to reduce the damage to renal tubular cells in nephrolithiasis patients. Therefore, lime powder regimen may be a better option.

In conclusion, we demonstrated the ability of lime powder to decrease stone-forming potential by increasing urinary pH and citrate excretion, improving antioxidative status and reducing renal tubular damage in nephrolithiasis patients. Our data support the suggestion that consumption of lime juice is beneficial in reducing the probability of stone recurrence in renal stone patients. In addition, it may be a health promotion mean to prevent the development of kidney calculi in healthy people.

Acknowledgments The authors are grateful to Professor Yoshihide Ogawa and Dr. Rayhan Zubair Hossain at Department of Urology, University of Ryukyus, Japan for generous advices, and thankful to Dr. Phisit Prapunwatana for comments.

Conflict of interest statement Supported by Grants from Division of Nephrology, Faculty of Medicine, and from the Graduate School, Chulalongkorn University.

References

1. Moe OW (2006) Kidney stones: pathophysiology and medical management. Lancet 367:333–344
2. Khan SR (2006) Renal tubular damage/dysfunction: key to the formation of kidney stones. Urol Res 34:86–91
3. Huang HS, Ma MC, Chen CF, Chen J (2003) Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology 62:1123–1128
4. Tungsanga K, Sriboonlue P, Futrakul P, Yachantha C, Tosukhowong P (2005) Renal tubular cell damage and oxidative stress in renal

stone patients and the effect of potassium citrate treatment. Urol Res 33:65–69
5. Boonla C, Wunsuwan R, Tungsanga K, Tosukhowong P (2007) Urinary 8-hydroxydeoxyguanosine is elevated in patients with nephrolithiasis. Urol Res 35:185–191
6. Pak CY, Fuller C, Sakhaee K, Preminger GM, Britton F (1985) Long-term treatment of calcium nephrolithiasis with potassium citrate. J Urol 134:11–19
7. Mattle D, Hess B (2005) Preventive treatment of nephrolithiasis with alkali citrate—a critical review. Urol Res 33:73–79
8. Preminger GM, Harvey JA, Pak CY (1985) Comparative efficacy of "specific" potassium citrate therapy versus conservative management in nephrolithiasis of mild to moderate severity. J Urol 134:658–661
9. Barcelo P, Wuhl O, Servitge E, Rousaud A, Pak CY (1993) Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. J Urol 150:1761–1764
10. Gaharagozloo M, Doroudchi M, Ghaderi A (2002) Effects of Citrus aurantifolia concentrated exact on the spontaneous proliferation of MDA-MB–453 and RPMI–8866 tumor cell lines. Phytomedicine 9:475–477
11. Seltzer MA, Low RK, McDonald M, Shami GS, Stoller ML (1996) Dietary manipulation with lemonade to treat hypocitraturic calcium nephrolithiasis. J Urol 156:907–909
12. Kang DE, Sur RL, Haleblian GE, Fitzsimons NJ, Borawski KM, Preminger GM (2007) Long-term lemonade based dietary manipulation in patients with hypocitraturic nephrolithiasis. J Urol 177:1358–1362, discussion 1362; quiz 1591
13. Koff SG, Paquette EL, Cullen J, Gancarczyk KK, Tucciarone PR, Schenkman NS (2007) Comparison between lemonade and potassium citrate and impact on urine pH and 24-h urine parameters in patients with kidney stone formation. Urology 69:1013–1016
14. Leong LP, Shui G (2002) An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem 76:69–75
15. Jayaprakasha GK, Patil BS (2007) In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem 101:410–418
16. Preminger GM, Sakhaee K, Pak CY (1988) Alkali action on the urinary crystallization of calcium salts: contrasting responses to sodium citrate and potassium citrate. J Urol 139:240–242
17. Koenig K, Padalino P, Alexandrides G, Pak CY (1991) Bioavailability of potassium and magnesium, and citraturic response from potassium–magnesium citrate. J Urol 145:330–334
18. Pak CY (1994) Citrate and renal calculi: an update. Miner Electrolyte Metab 20:371–377
19. Ettinger B, Pak CY, Citron JT, Thomas C, Adams-Huet B, Vangessel A (1997) Potassium–magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. J Urol 158:2069–2073
20. Tosukhowong P, Borvonpadungkitti S, Prasongwatana V, Tungsanga K, Jutuporn S, Dissayabutr T, Reungjui S, Sriboonlue P (2002) Urinary citrate excretion in patients with renal stone: roles of leucocyte ATP citrate lyase activity and potassium salts therapy. Clin Chim Acta 325:71–78
21. Zerwekh JE, Odvina CV, Wuermser LA, Pak CY (2007) Reduction of renal stone risk by potassium–magnesium citrate during 5 weeks of bed rest. J Urol 177:2179–2184
22. Hamm LL, Hering-Smith KS (2002) Pathophysiology of hypocitraturic nephrolithiasis. Endocrinol Metab Clin North Am 31:885–893, viii
23. Hofbauer J, Hobarth K, Szabo N, Marberger M (1994) Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis—a prospective randomized study. Br J Urol 73:362–365
24. Wabner CL, Pak CY (1993) Effect of orange juice consumption on urinary stone risk factors. J Urol 149:1405–1408
25. Lenton KJ, Sane AT, Therriault H, Cantin AM, Payette H, Wagner JR (2003) Vitamin C augments lymphocyte glutathione in subjects with ascorbate deficiency. Am J Clin Nutr 77:189–195