Original Article

The preventive and therapeutic effect of potassium citrate combined estradiol on lithangiuria

Yun Liu, Yunfei Li, Shaofeng Zhang, Wei Gan

Department of Urinary Surgery, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, China

Received April 11, 2017; Accepted July 31, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: Urinary calculus is a common disease. Potassium citrate is usually used to prevent lithangiuria. Urinary calculus formation is affected by sex hormone. This study investigated the preventive and therapeutic effect of potassium citrate combined estradiol on lithangiuria. SD rats were randomly divided into three groups, control group, model group established by administration of 3% melamine (Mel), and potassium citrate combined estradiol group. The weight, 24 h urine volume, water intake, Mel level, Scr, BUN, UAIt, ratio of kidney and weight, and calculus formation rate were measured. TNF-α and interleukin (IL)-1β levels were detected by real-time PCR and ELISA. Rats from model group displayed reduced body weight, increased 24 h urine volume, water intake, Mel level, Scr, BUN, UAIt, ratio of kidney to body weight, but decreased urine potassium and urine citric acid compared with those parameters in control group (P < 0.05). TNF-α and IL-1β levels were significantly elevated in model group compared those in control group (P < 0.05). The rats in combination treatment group exhibited reduced calculus formation rate, 24 h urine volume and water intake, increased body weight, urine potassium, and urine citric acid, but declined levels of Mel, Scr, BUN, UAIt, ratio of kidney to weight as well as urinary calcium compared with those parameters in model group (P < 0.05). Potassium citrate combined estradiol treatment suppresses urinary calculus formation induced by Mel, inhibits TNF-α and IL-1β secretion, reduces urine calcium, as well as improves urine potassium and urine citric acid, leading to improved renal function.

Keywords: Urinary calculus, melamine, potassium citrate, estradiol, renal function

Introduction

Urinary calculus is a worldwide common disease in urinary surgery [1]. Its incidence keeps rising that occurs at each age stage and mainly in males [2, 3]. China is one of the three high-prevalence areas of calculus. Following increased incidence and recurrence rate, urinary calculus seriously threatens global health and social economy [4, 5]. Multiple factors affect urinary calculi, such as environment, gender, genetic factors, metabolic abnormalities, abnormal immune state, dietary, and lifestyle [6, 7]. Melamine (Mel) is a kind of triazine nitrogen heterocyclic compound that can polymerize with other substances through hydrogen bond under vacuum or acid solution [8]. Mel can be discharged in urine directly without metabolism in the body. In addition, Mel can damage kidney and form urinary calculus [9, 10]. Potassium citrate is a common drug to prevent urinary calculus. Except hypokalemia, it can be used to treat urinary calculus which is induced by low citric acid and elevated urinary calcium, and prevent new calculus formation [11, 12]. Urinary calculus exhibits significant difference in gender, suggesting that sex hormone may play a role in the formation of urinary calculus [13, 14]. Thus, estrogen drug can affect urinary calculus formation to a certain extent [15, 16]. However, the effect of application of potassium citrate and estradiol on the prevention and treatment of urinary calculus remains poorly understood. This study aimed to investigate the impact of potassium citrate combined estradiol on urinary calculus and related mechanism on rat urinary calculus model established by administration of Mel.

Materials and methods

Experimental animals

A total of 30 healthy SD rats aged 3 months and weighted 250 ± 30 g were purchased from experimental animal center, Hubei University of
Potassium citrate combined estradiol improves urinary calculus

**Methods**

**Experimental animal grouping and urinary calculus modeling:** The rats were randomly and equally divided into three groups with n = 10 in each group. The rats in model group were fed by fodder with 3% Mel and normal drinking for 8 weeks [17]. The rats in combined group were treated with 0.2 mg/kg potassium citrate through gavage once a day and 0.1 mM estradiol caudal via vein injection once a week during modeling. The experiment was in accordance with animal ethics.

**Sample collection:** After treatment, blood was extracted from the aorta to the vacuum biochemistry tube using the negative pressure acquisition method. After 30 min, blood was centrifuged at 3600 rpm at 4°C for 10 min. Next, the supernatant was stored at -20°C. The left renal tissue was obtained and stored at -80°C.

The urine was collected from the metabolism cage and stored at -20°C.

**Renal function detection:** BUN, Scr, urinary calcium, urinary potassium, and urinary citric acid were tested by automatic biochemistry analyzer. UAlb was determined by radioimmunoassay. Ratio of kidney to body weight was also calculated.

**Urine Mel concentration detection:** Urine Mel level was measured by high performance liquid chromatography. 5 ml trichloroacetic acid was added into 0.15 ml sample. Next, the sample was added with 5 ml ddH₂O and filtrated for detection. Phenomenex C8 was adopted. Heptane sulfonic acid sodium citrate buffer + acetonitrile (v/v, 90:10) was selected as mobile phase and the flow velocity was 112 ml/min. At last, a total of 10 μl sample was detected by a microplate reader (in triplicates) at a wavelength of 240 nm.

**ELISA:** ELISA was used to test TNF-α and IL-1β contents in the serum. A total of 50 μl diluted standard substance were added into each well to establish standard curve. Next, the sample was added with 5 ml ddH₂O and filtrated for detection. Phenomenex C8 was adopted. Heptane sulfonic acid sodium citrate buffer + acetonitrile (v/v, 90:10) was selected as mobile phase and the flow velocity was 112 ml/min. At last, a total of 10 μl sample was detected by a microplate reader (in triplicates) at a wavelength of 240 nm.

**Real-time PCR:** Total RNA was extracted from renal tissue by Trizol and reversely transcribed into cDNA. The primers were designed using PrimerPremier 6.0 software and synthetized by Sangon (China).

**Main reagents and instruments**

Mel (chemical purity ≥ 98%) was purchased from Fujian chemical company. Potassium citrate and estradiol were obtained from Huari pharmaceutical company (Hunan, China). Serum creatine detection kit was from Roche. TNF-α and IL-1β ELISA kits were purchased from R&D (USA). Rat urine protein detection reagent was from Beijing Furui. RNA extraction kit and reverse transcription kit were purchased from Axygen (USA). Labsystem Version 1.3.1 microplate reader was obtained from Bio-rad (USA). CX5CE automatic biochemistry analyzer was purchased from Beckman (Germany). AB-17900 HT Real-time PCR was bought from ABI (USA). Surgical instrument was bought from Suzhou medical apparatus factory. EASY-nLC™ 1200 system high performance liquid chromatography was from Thermo Fisher (USA). Operation microscope was obtained from Zhenjiang optical instruments company. LZJ-4D operating microscope was got from Optical Instrument Company (Zhenjiang, China). Other reagents were purchased from Sangon (China).
Potassium citrate combined estradiol improves urinary calculus

**Table 2. General index analysis**

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>Model group</th>
<th>Potassium citrate group</th>
<th>Combined group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>497.6±45.3</td>
<td>311.9±32.6*</td>
<td>381.3±41.2*</td>
<td>381.3±41.2*</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>11.5±3.2</td>
<td>26.3±3.1*</td>
<td>20.1±4.1*</td>
<td>16.3±3.4*</td>
</tr>
<tr>
<td>Water consumption (ml)</td>
<td>22.5±0.9</td>
<td>45.8±1.8*</td>
<td>36.8±0.6*</td>
<td>26.5±1.6*</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control; *P < 0.05, compared with model group; *P < 0.05, compared with potassium citrate group.

**Table 3. Renal function detection**

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>Model group</th>
<th>Potassium citrate group</th>
<th>Combined group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney/body weight (mg/g)</td>
<td>2.4±0.3</td>
<td>5.6±0.8*</td>
<td>4.1±0.7*</td>
<td>3.5±1.2*</td>
</tr>
<tr>
<td>Scr (µmol/L)</td>
<td>84.5±14.7</td>
<td>1561.6±51.5*</td>
<td>1191±79.6*</td>
<td>791±69.2*</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>7.1±0.8</td>
<td>14.7±1.6*</td>
<td>12.5±1.4*</td>
<td>10.1±0.6*</td>
</tr>
<tr>
<td>UAlb (mg/24h)</td>
<td>0.4±0.3</td>
<td>1.7±0.6*</td>
<td>1.1±0.2*</td>
<td>0.8±0.2*</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control; *P < 0.05, compared with model group; *P < 0.05, compared with potassium citrate group.

Invitrogen (Table 1). Real-time PCR was performed including 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. GAPDH was selected as an internal reference. The relative expression of mRNA was calculated by 2^ΔΔCt method.

**Statistical analysis:** All data analyses were performed on SPSS19.0 software and presented as mean ± standard deviation (SD). Comparison of difference among different groups was performed by one-way ANOVA Newman-Keuls multiple comparison post-hoc analysis. P < 0.05 was depicted as statistical significance.

**Results**

**General conditions and urinary calculus formation**

Rat general conditions and urinary calculus formation rate were observed. The rats in control exhibited good mental state, glossy hair, and normal eating, drinking, activity, as well as urine output. 8 out of 10 rats formed urinary calculus after Mel induction. Rats in model group presented significant spirit drooping, hair removal, water quantity increase, urine output elevation, and weight loss compared with those in control group (P < 0.05). Potassium citrate single or combined estradiol obviously reduced calculus formation rate (40% and 20%, respectively), improved general conditions, perfected hair color, increased weight, as well as declined water consumption and urine volume compared with rats from model group (P < 0.05) with combined treatment showing better effects (Table 2).

**Renal function analysis**

Ratio of kidney to body weight, Scr, BUN, and UAlb levels were markedly increased in model group compared with rats in control (P < 0.05). Potassium citrate single or combined estradiol significantly reduced Scr, BUN, and UAlb levels compared with model group (P < 0.05) with better effects observed in combined treatment group (Table 3).
Potassium citrate combined estradiol improves urinary calculus

**Urinary calcium, urinary potassium, and urinary citric acid changes**

Urinary calcium was increased, while urinary potassium and urinary citric acid was reduced in model group compared with control (P < 0.05). Potassium citrate single or combined estradiol markedly declined urinary calcium as well as elevated urinary potassium and urinary citric acid compared with model group (P < 0.05). However, combined treatment demonstrated better effects (Figure 1).

**Urine mel content changes**

Mel content was obviously elevated in the urine of rats from model group compared with rats in control group (P < 0.05). Potassium citrate single or combined estradiol treatment significantly declined Mel level compared with model group (P < 0.05) with combined treatment demonstrating better effects (Figure 2).

**The impact of potassium citrate combined estradiol on the secretion of TNF-α and IL-1β expressions in renal tissue**

As TNF-α and IL-1 levels were demonstrated to be significantly increased in patients with stones in the urinary tract [18], we also test TNF-α and IL-1β mRNA expressions in renal tissue by Real-time PCR. TNF-α and IL-1β mRNA expressions were markedly upregulated in renal tissue of rats from model group compared with control (P < 0.05). Potassium citrate single or combined estradiol treatment significantly inhibited TNF-α and IL-1β mRNA expressions compared with model group (P < 0.05). However, combined treatment demonstrated better effects (Figure 3).

**The impact of tamsulosin on TNF-α and IL-1β expressions in the serum**

ELISA was used to detect TNF-α and IL-1β contents in the serum. TNF-α and IL-1β contents were obviously enhanced in the serum of rats from model group compared with control (P < 0.05). Potassium citrate single or combined estradiol treatment apparently reduced TNF-α and IL-1β contents compared with model group (P < 0.05) with combined treatment demonstrating better effects (Figure 4).
Potassium citrate combined estradiol improves urinary calculus

Discussion

As a common disease in urinary surgery, the incidence of urinary calculus is gradually increased, while its specific pathogenic factors and mechanisms have not been elucidated. Because of high incidence and recurrence rate, it is required to identify novel approaches on the treatment and prevention of urinary calculus [19]. Normal renal environment is acidity, thus in favor of Mel dissolution and self-assembly. Small molecular substance with weak acidity delays in the urine, thus forming urinary calculus and renal injury [19, 20]. Through administration of 3% Mel into SD rat, this study established urinary calculus rat model (90% success rate) with increased water intake and urine volume, Scr, BUN, UAlb, and ratio of kidney to body weight. Crystal inhibiting factor can block crystal growth, nucleation, or aggregation by adsorbing on the surface of crystal. Some crystallization inhibitors can also reduce the saturation level of stone materials in the urine [20]. Citric acid and potassium are important crystallization inhibitors existing in urine that can affect urine supersaturation. Therefore, potassium citrate may influence calcium oxalate, calcium phosphate, and urine pH to regulate urinary stone formation [21]. Since the urinary tract is in inflammatory state during calculus formation, it produces a large amount of inflammatory cytokines, thus accelerating calculus formation, leading to damage of renal function [22, 23]. On the other hand, estrogen is confirmed to inhibit urinary calculus [24]. However, the effect of combined application of potassium citrate and estradiol on the prevention and treatment of urinary calculus still remains poorly understood. It was showed that potassium citric is conducive to the excretion of urinary calculus [11, 12]. In this study, potassium citric suppressed inflammatory cytokines synthesis and secretion, improved general conditions, increased weight, reduced 24 h urine volume and water intake, declined Mel, Scr, BUN, UAlb, accelerated urine calcium discharge, elevated urine potassium and urine citrate acid, decreased ratio of kidney to weight, as well as declined calculus formation rate. However, combined treatment exhibited better improvement on the renal function by inhibiting inflammatory cytokines secretion and reducing calculus formation. This study for the first time reported that potassium citric combined estradiol restrained calculus formation rate, suppressed inflammatory cytokines secretion, and improved renal function. However, the exact mechanism by how potassium citric combined estradiol reduced calculus formation remains unclear and requires further investigations.

Conclusion

Potassium citrate combined estradiol treatment suppresses urinary calculus formation which is induced by Mel, inhibits inflammatory cytokines secretion, reduces urine calcium, as well as improves urine potassium and urine citric acid to improve renal function. It could be used to treat urinary calculus especially in female in menopause that require estrogen supplement.

Acknowledgements

This work was supported by Hubei provincial health and Family Planning Commission (No. WJ2015MB220).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yun Liu, Department of Urinary Surgery, Renmin Hospital, Hubei University of Medicine, No. 39, Chaoyang Road, Shiyan, Hubei, China. Tel: +86-719-8891088; Fax: +86-719-8891088; E-mail: yunliurn@163.com

References

Potassium citrate combined estradiol improves urinary calculus


