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Property changes of urinary nanocrystallites and urine of uric acid stone formers after taking potassium citrate

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ABSTRACT

The property changes of urinary nanocrystallites in 20 cases of uric acid (UA) stone formers after 1 week of potassium citrate (K_3cit) intake were comparatively studied by X-ray diffraction analysis, Fourier transform infrared spectroscopy, nanoparticle size analysis, and transmission electron microscopy. Before K_3cit intake, the urinary crystallites mainly contained UA and calcium oxalate. After K_3cit intake, the components changed to urate and UA; the qualities, species, and amounts of aggregated crystallites decreased; urine pH, citrate, and glycosaminoglycan excretions increased; and UA excretion, Zeta potential, and crystallite size decreased. The stability of crystallites followed the order: controls > patients after taking K_3cit > patients before taking K_3cit. Therefore, the components of urinary stones were closely related to the components of urinary crystallites.

1. Introduction

The efficiency of extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and ueteroscopic lithotripsy (URS) for stone removal has been established. However, these methods cannot be used for the prevention and recurrence of stone formation [1–3].

Based on the chemical composition of urinary stones, they can be divided into acidic (such as uric acid (UA) and cystine), alkaline (such as magnesium ammonium phosphate), and neutral (such as calcium oxalate (CaOxa) and calcium phosphate) stones. Urinary stones with different components vary not only in their chemical properties but also in their physical properties such as hardness. For instance, the relative hardness values are 4–5 for calcium oxalate monohydrate (COM), 3–4.5 for cystine and apatite, 2.5 for UA as well as 2.0 for magnesium ammonium phosphate and CaOxa. However, the same methods and the same drugs are currently used to treat stone patients without distinguishing the composition of the stones, thereby leading to different curative effects and lower cured rates. If the type of urinary stone (acidic, alkaline, or neutral) can be determined before stone removal and treatment, the appropriate shocking time and shocking frequency during ESWL can be selected based on the stone hardness. The personalized treatment can thus lead to increased curative success [4].

UA stones are the most common acidic stones, and the disease incidence of UA stones ranks second only to CaOxa stones in various countries and regions. For instance, the incidence rates of UA stones are 5%–10% in the USA, 17–25% in Germany, and 18%–40% in Israel [5–7]. In China, Sun et al. [8] determined the components of 5248 cases of urinary stones between 1999 and 2008 in Nanjing, Jiangsu province. They concluded that the incidence rate of UA stones was 6.12%. Another study found that in Guangdong province, south China, the proportion of UA stones rapidly increased from 13% in the 1980s to about 25% in the 2000s [9].

Potassium citrate (K_3cit) is one of the main drugs used to prevent and treat the formation of UA stones. However, the property changes of urinary crystallites in stone patients after taking K_3cit have not been reported. Hence, in this paper, the property changes of crystallites and the component changes of urine in 20 cases of UA stone formers after taking K_3cit were comparatively studied to determine the relationship between urinary crystallites and urolithiasis.

2. Materials and methods

2.1. Reagents and instruments

All the chemicals used in this work were of analytical purity. All glass vessels were cleaned with double-distilled water.

Nanoparticle size analyzer (Malvern company, Britain). TECNAI-10 transmission electron microscope (PHILIPS, Netherlands). Image Pro Plus 5.02 (Media Cybernetics, USA). D/max-γA X-ray diffractometer (Rigaku, Japan). Fourier transform infrared spectrometry (Nicolet Company, USA).

2.2. Clinical data

2.2.1. Collection and component characterization of urinary calculus

The participants in the study included 20 lithogenic patients (12 men and 8 women; mean age = 60.7 years; range = 24–71 years) and

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20 randomly selected healthy humans with no prior history of urinary stones (12 men and 8 women; mean age = 46.5 years). Urinary stones were collected after surgery, disinfected by 75% alcohol, cleared by distilled water, and placed in a dust-free incubator at 45 °C to dry. The urinary stones were then ground into powder by agate mortar for X-ray diffraction (XRD) and Fourier transform infrared (FT-IR) characterization. Results showed that urinary stones were mainly composed of uric acid (UA) with 90% to 100% mass fraction. Other urinary stone components included CaOxa and calcium phosphate.

2.2.2. Collection and treatment of urine

Fresh urine from UA stone patients were collected before and a week after K_3cit intake (2.538 g daily dosage) for pH measurement. A 2% (weight/volume) NaN_3 solution was subsequently added to the urine samples (10 mL/L urine sample) as an antiseptic.

2.3. Properties and components characterization of urinary crystallites

(1) For the size and Zeta potential (ζ) detection of urinary crystallites: 30 mL of urine was filtered through a 1.2-μm microporous membrane to detect the ζ and size of urinary crystallites by nanoparticle size analyzer.
(2) For the XRD and FT-IR detection of urinary crystallites, 20 mL of anhydrous alcohol (V_{urine}:$V_{anhydrous\ alcohol}$ = 3:2) was added to 30 mL of urine samples. The solution was stirred and left undisturbed for half an hour to denaturalize and deposit the proteins. The urine was then filtered through a 1.2-μm microporous membrane, and about 100 μl of spare urine sample was placed on a clean hydrophilic quartz glass with a size of 12 mm × 12 mm using a microsyringe. Hydrophilic quartz glass was prepared by ultrasonic treatment with saturated KOH/methanol solution for 5 min, followed by washing with double-distilled water. The quartz slides were dried in an oven at 50 ± 5 °C for 2 h to volatilize the urine. This process was repeated thrice, and then the nanocrystallites were deposited onto glass slides. Subsequently, the glass slides were slowly immersed at a 45° angle in distilled water and gently shaken for 1 min to remove the soluble fractions of NaCl and urea. The glass slides were carefully taken out, and the water from the edges of the slides was dried using an absorbent paper. The slides were dried at 50 ± 5 °C in a vacuum desiccator for 1 d for XRD and FT-IR characterization.
(3) For the TEM detection of urinary crystallites, after ultrasound for 3 min, about 30 μL of the above spare urine sample was placed in a copper mesh. The copper mesh was stored in a desiccator for 24 h and then examined by TEM.

2.4. The determination of citric acid, GAGs, and uric acid in the urine

A Lixin blue colorimetric method [10] and ammonium metavanadate-activated catalytic–kinetic spectrophotometry [11] were used to detect the content of glycosaminoglycans (GAGs) and citrate in urine. By exploiting the ability of UA to reduce Fe (III) into Fe (II) and the ability of Fe (II) to coordinate with phenanthroline yielding orange-red complexes, the UA content was detected by spectrophotometry [12].

2.5. Statistical analysis

The experimental data were analyzed using SPSS version 16.0 software. The experimental data were expressed as the mean ± standard deviation ($\bar{x}$ ± SD). Data differences between two groups were analyzed by a *t*-test, and the *p* value was used to assess the statistical significance. $P < 0.05$ was deemed to indicate a significant difference, $P < 0.01$ indicated an extremely significant difference, and $P > 0.05$ indicated no significant difference.

3. Results and discussion

3.1. Morphology changes of urinary crystallites

Transmission electron microscopy (TEM) was used to observe the morphology of urinary crystallites in UA stone formers before and after taking K_3cit. Representative photographs are shown in Fig. 1. Before K_3cit intake, the edges and corners of urine crystallites were sharp and some crystallites were aggregated. After K_3cit intake, the crystallites became blunt, and the amount of aggregated crystallites declined. Before K_3cit intake, the average size of urinary crystallites of UA stone patients was about 325 ± 150 nm (Fig. 1a–c). After K_3cit intake, the crystallite size decreased to about 170 ± 100 (Fig. 1d–f).

3.2. Component changes of urinary crystallites

The XRD patterns of urinary crystallites in all 20 cases of UA stone formers before and after taking K_3cit were analyzed. Three representative results are shown in Fig. 2.

(1) Before K_3cit intake (Fig. 2a, c, and e), the main components of urinary crystallites were UA and COM. The diffraction peaks at 3.93, 3.59, 3.24, 3.10, 2.73, 2.25, and 2.18 Å were assigned to the ($\bar{2}$ 11), ($\bar{3}$ 01), (021), ($\bar{1}$ 21), (202), ($\bar{2}$ 22), and (600) faces of UA crystals, respectively. These results well agreed with previously reported ones [13–15]. The peaks at 2.49, 2.36, and 1.98 Å were assigned to the (112), (130), and ($\bar{3}$ 03) face of COM crystals.
(2) After the patients took K_3cit for 1 week, the urine crystallites (Fig. 2b, d, and f) were analyzed again by XRD and the following changes were observed.

First, the number of diffraction peaks of urinary crystallites decreased, indicating that the number of urinary crystallite species decreased [14].

Second, the intensity of the diffraction peaks of urinary crystallites obviously weakened, meaning that the mass of crystallites significantly declined [14]. For example, compared with the XRD patterns before K_3cit intake, the diffraction peaks assigned to the ($\bar{3}$ 01) and (021) faces of UA disappeared, and the peaks assigned to ($\bar{2}$ 11), ($\bar{1}$ 21), and (202) faces of UA decreased or disappeared. Thus, the quality of UA crystallites in urine remarkably decreased. Likewise, the diffraction peaks assigned to the (130) and (112) faces of COM weakened, indicating that the quality of COM crystallites in urine also decreased.

Third and last, a new characteristic diffraction peak of urate at 2.80 Å (Fig. 2d and f) and 3.40 Å (Fig. 2b) appeared.

3.3. FT-IR spectra of urinary crystallites

The component changes of urinary crystallites were further investigated by FT-IR spectroscopy. Three representative spectra are shown in Fig. 3, and the following changes were observed.

(1)Before K_3cit intake (Fig. 3a, c, and e), the following absorption peaks were observed: 3488–3048 cm^{-1} assigned to coordinated water; 1668, 1426, 1119, 989, 705, 618, 571, and 472 cm^{-1} assigned to UA; and 1332, 956, and 781 cm^{-1} assigned to COM. These results well agreed with previously reported ones [16–19]. Notably, the vibration peak of COM at 1619 cm^{-1} was covered by the strong absorption peak of UA.
(2) After K_3cit intake, the following changes in the absorption peaks were observed (Fig. 3b, d, and f).

First, some absorption peaks disappeared, indicating that the urine microcrystallite species decreased. For example, the absorption peak