Original article

Applying quantitative micro-Raman spectroscopy to analyze stone compositions extracted from ureteroscopic lithotripsy urine

Chang-Chi Chang a,b, Yichun Chiu a,b, *

a Division of Urology, Department of Surgery, Zhong Xiao Branch, Taipei City Hospital, Taipei, Taiwan
b Department of Urology, School of Medicine, National Yang-Ming University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 26 April 2014
Received in revised form 5 April 2016
Accepted 6 April 2016
Available online xxx

Keywords:
Fourier transform infrared spectroscopy
micro-Raman spectroscopy
ureteroscopic lithotripsy
urinary stone
urolithiasis

ABSTRACT

Objective: In this study we applied quantitative micro-Raman spectroscopy (MRS) in binary stone compositions extracted from ureteroscopic lithotripsy (URSL) urine. Urolithiasis is a severe disease with a great impact. For ureteral stone patients, a basket retractor is used to catch the stone for analysis when patients undergo URSL. However, this can cause potential ureteral-related complications. The identification of the composition of a urinary stone is important for metabolic evaluation, decision making, and diet control. In this study, we applied a quantitative MRS method in a clinical setting to measure the composition of binary urinary stones in urine extracted via URSL.

Materials and Methods: This study was approved by the Institutional Review Board of Taipei City Hospital, Taipei, Taiwan from November 2008 to November 2009. Urine samples from fragmented stone sites in the patients’ ureters were collected via the URSL procedure by drawing at least 10 mL of urine with a syringe. The urine samples from 30 patients were analyzed using an MRS-based analysis method, both qualitatively and quantitatively.

Results: The post-URSL urine powder from the samples was successfully analyzed using a quantitative MRS-based method, which was based on a linear equation developed according to the ratios of the relative intensities of the Raman bands corresponding to binary mixtures of known composition. Fourteen urine samples from 30 patients disclosed binary composition, and the ratio percentages were obtained using the equation along with a quantitative MRS-based method.

Conclusion: We successfully applied the quantitative MRS-based method clinically to analyze the stone compositions extracted from URSL urine. This method decreases the need to use a basket to catch macrostones for Fourier transform infrared spectroscopy analysis, while also still providing quantitative and qualitative stone analysis information by using the microstones in urine.

Copyright © 2016, Taiwan Urological Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Urinary stones have been a major issue for centuries. Its prevalence rate is 8–15%,1–4 with rising incidence rates in the past 30 years.5 There is no doubt that urolithiasis is a significant problem worldwide, accounting for a considerable degree of morbidity and mortality. For frequent stone formers, treatment can be a painful experience. The exact mechanism of urinary stone formation is not fully understood; it may be caused by multiple factors.2 Apart from medical treatment, the prevention of recurrence is also very important. Statistical surveys show that 50–70% of urolithiasis patients experience a recurrence within 5 years, and 3% of patients experience renal failure due to urolithiasis.5 The medical therapy, metabolic evaluations, and diet control of urolithiasis patients are based on the results of exact stone analysis.2 Exact stone analysis represents an effective metaphylaxis of residual and recurrent stones, and is thus important for therapy. Currently, urinary stone analysis is mainly performed on stones that are at least visible to the naked eye in clinical settings.3 However, in recent years, the treatment of urinary stones has been completely revolutionized, and it is now unusual for a patient to undergo open surgery as the first line of treatment. With improvements to ureterorenoscopic lithotripsy (URS) and extracorporeal shock wave lithotripsy (ESWL), stone management has become more effective, less invasive, and more comfortable than before. In a URS procedure, a
Micro-Raman spectroscopy-based analysis of post-ureteroscopic lithotripsy (URSL) urine powder with the URSL procedure, from bench to bedside. The URSL laser provided the energy to fragment the ureteral stones; the micro-Raman spectroscopy system then analyzes the quantitative results of the urinary stones after the syringe/ filter procedure.

32 mL of 0.2M calcium chloride was added to 400 mL of 0.01M sodium citrate and magnetically stirred at 100 rpm at 23°C. Then, 16 mL of 0.05M sodium oxalate was rapidly added to this mixture, and stirring continued for 10 ± 15 minutes. The precipitate was then filtered off and washed repeatedly with distilled water. The residual solvent was removed by drying to constant weight at ambient temperature over calcium chloride granules and in a vacuum. 

For the detection of two mixed urinary stone compositions, we derived the calibration curves for each mixed group. We analyzed the seven most prevalent urinary stone mixtures based on their prevalence from clinical surveys. We then established standard calibration curves according to the known mixture concentration from the pure synthetic compound, thus producing an equation for quantitative measurement. A 20 mW helium–neon laser, 632.8 nm, was used for Raman excitation. The laser spectral line width was further spectrally purified with a laser line band-pass filter. A notch filter (HSPF-632.8-1.0; Kaiser, Ann Arbor, MI, USA) was used to block the excitation light while allowing the Raman signals to enter the spectrometer system. An 80-cm focal length spectrometer system (HR800, Jobin Yvon, Longjumeau Cedex, France) was used with an 1800 g/mm holographic grating to provide spectral resolution at 1 cm⁻¹. A liquid nitrogen-cooled charged-coupled device two-dimensional array detector was used to measure the Raman signal by integration at 1 second, with a total of 4 seconds for the complete scanning from 400 cm⁻¹ to 1800 cm⁻¹. The standard urinary stone compounds were measured on a microscope slide with a 50× microscope objective lens and a 1000-μm confocal hole. After evaluation of the standard mixtures, we applied quantitative analysis to the clinical urinary stone powders in the extracted urine after URSL. For post-URSL urine powder measurements, we drew 0.5-mL urine from the bottom of the 10-mL urine sample, and placed it on a microscope slide. Then, we used a hair dryer to dry the sample before the MRS measurement. This specimen was photobleached for 30 minutes before Raman analysis. Table 1 discloses the equation of the quantitative RS analysis to show how to perform the quantitative analysis.
3. Results

Table 1 summarizes the calibration equation for different binary mixtures of prevalent urinary stone compositions. The calibration equations for HAP/COD, uric acid/COM, and uric acid/HAP are much simpler because these compositions have no overlapped Raman band. However, COM/COD, COM/HAP, and DCPD/HAP have overlapped Raman bands. Therefore, we need to take the effect of the overlap into consideration. Based on these calculated models, we can measure the ratio between the intensity of the Raman band, which is contributed by different compositions to another with different concentrations and can calculate the proportion $K$. We found that we can plot $\frac{I}{x}$ or $\frac{x}{I}$ versus $I_x$ ($x$ is the concentration of one composition and 1-x is the other one, $I_x$ is the intensity ratio of the 2 compositions), as a straight line with the coefficient of correlation around 0.99. The ratios of the relative intensities of Raman bands corresponding to binary mixtures of known composition and content concentration yielded a linear regression and R-squared value in each group is 0.996 (COM/COD), 0.998 (HAP/COD), 0.991 (HAP/COD), 0.978 (uric acid/COM), 0.988 (COD/uric acid), 0.997 (uric acid/HAP), and 0.993 (HAP/DCPD). We used measured Raman intensity to estimate the percentage concentration of each compound.

The results of analysis of the post-URSL urine powder from 30 patients revealed 16 single compositions, two CODs, nine COMs, two HAPs, two uric acids, and one magnesium ammonium phosphates (struvite), and there were 14 patients with binary compositions. Table 2 shows the results of the 14 clinical samples with binary compositions and the ratio concentration calculated with a quantitative MRS-based analyzed method. There were six samples with COM/COD, five samples with HAP/COM, one sample with HAP/COD, and two samples with HAP/DCPD.

4. Discussion

Currently, FTIR is the most commonly applied and convenient urinary stone analysis method, but it is very sensitive to water content. The FTIR approach requires extensive sample preparation, including 10 cycles of sample grinding and 10 cycles of pressing the sample pellet, to ensure maximum absorbance with a potassium bromide transmission technique. It is not easy to prepare extensive samples of urinary stones after URSL and ESWL. A urinary stone is often neither retrieved by the patient nor the urologist for stone analysis. Patients who pass urinary stones spontaneously are unable to catch them in their voided urine. As a result, an MRS-based approach would be the most promising urinary stone analysis method since it only requires minimal sample preparation, and has no band overlap, while also boasting better signal-to-noise ratio.

With improvements to URSL and ESWL, microuninary stones and stone powders have become the end results of the lithotripsy. Although automated total internal reflection-FTIR is currently under study for the quantitative analysis of small urinary stones, the analysis process is complicated, time consuming, and still needs a pellet to work on. The MRS method, however, is superior for identifying the stone composition of microuninary stones or stone powders since it does not need pellets to work. The MRS-based method eliminates the inconvenience associated with stone collection by enabling direct stone analysis within the urine powders. It could directly measure multiple points on the micro urinary stones or urine powders. Therefore, the development of the MRS method provides a feasible approach for direct, quick, and convenient stone analysis of the post-URSL urine, and is very useful for clinical stone analysis. A skillful doctor could still choose to use a basket retractor to catch the stone sample for analysis. However, to avoid the possibility of ureteral injury, we could only collect the urine from the lithotripsy site, but these two decisions would not influence the outcome of the result of stone analysis.

In the post-URSL urine powder evaluation, we applied, at a minimum, 10-points sampling and averages in the MRS measurements. By conducting multiple-point sampling and averaging on the urine powder specimen, the MRS approach would produce analysis results that were relatively close to the urinary stone composition. This method can give a quantitative analysis of the mole ratio for each compound by combining the calibration curves of the known mixtures. There are a variety of urinary stone compositions, and we used the most prevalent ones from the clinical perspective. By doing so, we could quantitatively measure more than 95% mixed urinary stone composition.

A mixed pure synthetic composition could be measured by using the calibration curve. For a clinical urinary stone sample from post-URSL urine powder, we measured at least 10 points to include as many important composition elements as possible. This MRS-based approach represents a major shift from the current "no stone analysis results due to the risk of ureteral related injury" mentality, instead transferring to a "producing results of urine with microstone analysis" after URSL, while also providing quantitative measurement. It could detect urinary stone composition in urine after URSL only on the condition that urinary stones exist in the urine. It still works even if the collected urine amount is less than 10 mL, only if the microstones are in the urine. The majority of the clinical urolithiasis is one or two components, this study applied the method in the clinical aspect to check the efficacy. The theory to analyze triple components has already been established, but to date...
The table shows the 14 clinical sample analyses with binary compositions from the post-ureteroscopic lithotripsy urine powders. The Raman spectra are also shown in the right column, and ratio concentrations could be obtained from the quantitative micro-Raman spectroscopy-based equation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Composition</th>
<th>Intensity</th>
<th>Concentration (%)</th>
<th>Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COM / COD</td>
<td>5437 / 3048</td>
<td>59.4 / 40.6</td>
<td><img src="image" alt="Spectra1" /></td>
</tr>
<tr>
<td>2</td>
<td>COM / COD</td>
<td>245.1 / 206.4</td>
<td>45.5 / 54.5</td>
<td><img src="image" alt="Spectra2" /></td>
</tr>
<tr>
<td>3</td>
<td>HAP / DCPD</td>
<td>131.7 / 123.2</td>
<td>27.7 / 72.3</td>
<td><img src="image" alt="Spectra3" /></td>
</tr>
<tr>
<td>4</td>
<td>COM / COD</td>
<td>181.2 / 335.8</td>
<td>30.5 / 69.5</td>
<td><img src="image" alt="Spectra4" /></td>
</tr>
<tr>
<td>5</td>
<td>COM / COD</td>
<td>126.5 / 64.1</td>
<td>61.4 / 38.6</td>
<td><img src="image" alt="Spectra5" /></td>
</tr>
<tr>
<td>6</td>
<td>HAP / DCPD</td>
<td>92.6 / 119</td>
<td>34.5 / 65.5</td>
<td><img src="image" alt="Spectra6" /></td>
</tr>
<tr>
<td>7</td>
<td>HAP / COM</td>
<td>4329 / 3566</td>
<td>51.1 / 48.9</td>
<td><img src="image" alt="Spectra7" /></td>
</tr>
<tr>
<td>8</td>
<td>HAP / COM</td>
<td>3361 / 602.6</td>
<td>85.3 / 14.7</td>
<td><img src="image" alt="Spectra8" /></td>
</tr>
<tr>
<td>9</td>
<td>HAP / COM</td>
<td>2960 / 546.7</td>
<td>84.9 / 15.1</td>
<td><img src="image" alt="Spectra9" /></td>
</tr>
<tr>
<td>10</td>
<td>HAP / COM</td>
<td>45.6 / 114.5</td>
<td>13.2 / 86.8</td>
<td><img src="image" alt="Spectra10" /></td>
</tr>
<tr>
<td>11</td>
<td>HAP / COD</td>
<td>2546 / 7253</td>
<td>50.4 / 49.6</td>
<td><img src="image" alt="Spectra11" /></td>
</tr>
<tr>
<td>12</td>
<td>COM / COD</td>
<td>1361 / 1423</td>
<td>45.3 / 54.7</td>
<td><img src="image" alt="Spectra12" /></td>
</tr>
<tr>
<td>13</td>
<td>HAP / COM</td>
<td>3202 / 555.2</td>
<td>85.7 / 14.3</td>
<td><img src="image" alt="Spectra13" /></td>
</tr>
<tr>
<td>14</td>
<td>COM / COD</td>
<td>260.1 / 73.2</td>
<td>70.7 / 29.3</td>
<td><img src="image" alt="Spectra14" /></td>
</tr>
</tbody>
</table>

COD = calcium oxalate dehydrate; COM = calcium oxalate monohydrate; DCPD = dicalcium phosphate dehydrate; HAP = hydroxyapatite.

has not gained popularity in clinics. In this era of minimally invasive urological surgery, a MRS-based approach could be an alternative for micro urinary stone analysis to avoid ureter-related complications. It is promising to apply this method to measure the urine after ESWL both qualitatively and quantitatively.

A quantitative MRS-based method was successfully established and applied for the measurement of the most prevalent compositions of microurineary stones from patients’ urine after URS. This method only requires minimal sample preparation to gain enough information, and also avoids basket-related ureteral complications.

Conflicts of interest

The authors have no conflict of interest.

References