Electron Microscopy and Spectroscopy of Citrate Induced Calcium Oxalate Crystal Structure and Hydration State Changes, and Implications for Kidney Stones

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In the USA alone, 20 million people are currently have kidney stones (KS) or are predicted to develop KS during their lifetimes. The most common KS symptom is excruciating pain, but symptoms often include nausea and emesis, blood in urine, difficulty urinating, and frequent urination. KS may require removal of the kidney and are associated with Chronic Kidney Disease as well as Cardiovascular Diseases [1]. Approximately 80% of KS contain Calcium Oxalate (CaOx) as the major phase with CaOx monohydrate (COM) as the most prominent hydration state [2]. Therefore, medical KS research primarily focuses on CaOx biomineralization and potential CaOx treatments.

Citrate, a common KS treatment and dietary inhibitor, was previously thought to function by lowering the effective CaOx supersaturation within the urine. However, in vitro studies of both human and artificial urine have shown that addition and supersaturation of CaOx does not cause CaOx biomineralization [3]. This finding suggests a far more complex mechanism by which citrate and other KS treatments prevent formation of CaOx KS. Insight into the mechanism or mechanisms by which citrate inhibits CaOx biomineralization would allow optimization of medical treatment protocols and discernment of the desired properties of improved next-generation KS treatments.

CaOx KS research has taken two broad approaches: 1) in vitro studies of CaOx crystallization within human or synthetic urine, and 2) organismal in vivo studies and clinical studies. These approaches are essential for determining the efficacy of a CaOx KS treatment. However, these approaches do not provide fundamental data on the manner in which CaOx treatments affect the formation of CaOx. This study implements nanoscale resolution electron microscopy techniques, including High Resolution Transmission Electron Microscopy (HR-TEM), Energy Dispersive X-Ray Spectroscopy (EDS) elemental analysis, Electron Energy Loss Spectroscopy (EELS) chemical analysis, and Select Area Electron Diffraction (SAED) analysis of crystal structures to provide a fundamental understanding of the formation of CaOx mineralization and the influence of citrate.

To determine the impact of citrate on CaOx solubility and crystal structure, control CaOx samples were synthesized by chemical reaction between 0.125M:0.125M sodium oxalate (NaOx) and calcium chloride (CaCl₂), while CaOx experimental samples were synthesized by chemical reaction between 0.125M:0.125M NaOx and CaCl₂ in the presence of 1M citrate. This citrate concentration is substantially higher than medically achievable concentrations. In each experiment, six control CaOx samples and six citrate treated CaOx samples were synthesized. A control sample and an experimental sample were analyzed after the solutions settled for 30 minutes, and other samples were subsequently analyzed at 3, 6, 12, 24, and 72 hours. At each time point, the supernatant was removed by pipette without disturbing the sample pellet. The remaining sample was then centrifuged and any remaining supernatant removed by pipette. The sample was then rinsed with ultrapure water and dried by evaporation. Alterations in the final weight of CaOx as a result of the rinsing procedure are minimal due to the 0.67mg/L solubility of CaOx in water, while the more soluble sodium chloride byproducts and
any dissolved calcium or oxalate are removed.

Compared to control CaOx synthesized without citrate, citrate treated CaOx showed no significant change in the total weight synthesized. EDS analysis of the sample (Figure 1) showed that samples were not contaminated by sodium or chloride. However, SAED (not shown) and HR-TEM (Figure 2) showed alterations in the crystal structure from monoclinic COM in the untreated CaOx samples to tetragonal CaOx dihydrate (COD) in the citrate treated CaOx samples.

The preliminary results shown here suggest that citrate may act by influencing the crystallization of CaOx to a less mechanically stable structure. Furthermore, a previous study by Gan et al. showed that COM better adheres to cell walls as compared to COD [4]. This suggests that citrate may inhibit the formation of KS by altering the crystal structure and preventing cell adherence [5].

References:

[5] The authors acknowledge funding from the National Science Foundation CAREER Award, grant No. DMR 1564950. This work made use of instruments in the Electron Microscopy Service (Research Resources Center, UIC)