An Additional Potential Factor for Kidney Stone Formation during Space Flights: Calcifying Nanoparticles (Nanobacteria): A Case Report

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Abstract

Spaceflight-induced microgravity appears to be a risk factor for the development of urinary calculi due to skeletal calcium liberation and other undefined factors, resulting in stone disease in crewmembers during and after spaceflight. Calcifying nanoparticles, or nanobacteria, reproduce at a more rapid rate in simulated microgravity conditions and create external shells of calcium phosphate in the form of apatite. The questions arises whether calcifying nanoparticles are niduses for calculi and contribute to the development of clinical stone disease in humans, who possess environmental factors predisposing to the development of urinary calculi and potentially impaired immunological defenses during spaceflight. A case of a urinary calculus passed from an astronaut post-flight with morphological characteristics of calcifying nanoparticles and staining positive for a calcifying nanoparticle unique antigen, is presented.

Introduction

Human exposure to microgravity results in a number of physiological changes including musculoskeletal deconditioning with potential loss of muscle and bone mass[1-3], and potentially associated regional bone mineral losses reflected in increased urinary calcium excretion during and after spaceflight[4-6]. Also, cellular/molecular alternations in immune system function have been observed during spaceflight, which may predispose to impaired clearance of bacterial species[7-13]. There have been 11 post-flight and 1 in-flight urinary calculi observed in astronauts and cosmonauts in a population screened against stone formation pre-flight [14, 15]. Despite the obvious increased urinary calcium

excretion, the mechanism driving urinary calculus formation in astronauts during and/or after space missions in microgravity is not well understood[16, 17]. In prior studies a unique calcifying agent, named calcifying nanoparticles (CN) or nanobacteria (NB), has been hypothesized to have an active role in calcium phosphate-carbonate deposition in kidney. [18-23]. Previous studies have also shown an increased multiplication rate of NB while in microgravity-simulated conditions.[24] In this case report the results of the analysis of urinary calculus from a crew member occurring several years following the last of his previous 4 space missions.

Case History

The cumulative duration of the crewmember's previous space missions was 43 days, the details of the missions admitted to protect the crewmember's privacy. The last mission was less than 5 years prior to the onset of symptoms. This patient had no history of prior urinary tract infections or urinary calculi.

HPI: The 50+ year old, male patient presented with sudden onset of abdominal pain, 2 days prior to stone passage. The pain was predominantly in the right lower quadrant, with history of prior appendectomy. In addition to pain, he reported vomiting several times, with no diarrhea. He described the pain as intermittent, but severe, 8/10, so colicky in nature.

Physical Examination: Vital signs: Afebrile, Pulse 75, Respirations 20 and B/P 165/96 upon presentation to the flight medicine clinic. His physical examination revealed an age-

appropriate male alert and oriented, but in acute distress with right costovertebral angle and right upper quadrant tenderness to percussion and deep palpation. His bowel sounds were present. His remaining physical examination was unremarkable.

Laboratory: His CBC was within normal limits, with the Hgb 15.4 g/DL, Hct 45.8%, and WBC of 8100. His serum electrolytes were normal, including BUN 19 and creatinine 1.1, except for carbon dioxide of 21.7 mmol/L. His glucose and LDH were slightly elevated at 121 mg/DL and 176 U/L respectively. Urinalysis showed pH 6.5, S.G.-1.025, Hgb 3+, nitrite and leukocyte esterase neg., and microscopic showed 3-5 WBC/HPF, RBC-TNTC.

Imaging: CT Scan with and without contrast as per renal stone protocol. The left kidney and renal collecting system were unremarkable. The right renal parenchyma appeared normal however the right collecting system (pelvis and entire ureter) showed mild hydronephrotic changes consistent with a subacute distal obstruction at the level of the ureterovesical junction, where a 4 mm calculus was visualized on the non-contrasted images.

Course: The patient was diagnosed with a small, partially obstructing right ureteral calculus, and after he was hydrated with 2 liters of normal saline and given appropriate analgesia, his symptoms much improved and was discharged to home with a urinary strainer. He experienced episodic ongoing right flank and lower abdominal pain throughout the next 20 hours, however, the following day the stone was recovered while

voiding. One half of the stone was submitted for standard clinical laboratory analysis and found to be composed of 40% calcium phosphate (carbonate apatite) and 60% magnesium ammonium phosphate (struvite). The remaining stone material analyzed at the Johnson Space Center, as described below.

Methods

A urinary calculus captured by urinary straining methods passed per urethra from the patient was submitted for more detailed morphological and compositional analysis. In addition to standard compositional analysis, the calculus was examined for the chemical composition by FTIR and EDS. The morphology of crystals was observed by scanning electron microscopy (SEM). The kidney stone crystals were powdered and was demineralized in 1N HCl for 10 min, neutralized, sterile-filtered through 0.22 μm filter and cultured as described earlier.[18] The demineralized sample was also stained with immunofluorescence (IFS) technique using the IgG-1 class anti-nanobacterial monoclonal antibody, 8D10 (Nanobac Oy, Finland), as previously described.[18] Culture result was evaluated for the growth of NB with IFS after three weeks incubation. The culture was centrifuged at 14,000g for 30 min, and the pellet was treated with negative staining, and imaged with transmission electron microscopy TEM.[21]

Results

FTIR/EDS analysis showed that the urinary calculus was composed of a combination of apatite, (calcium phosphate), (see Figure 1-E) and struvite (magnesium ammonium

phosphate). These findings were in agreement with the clinical laboratory findings by X-ray diffraction methodology.

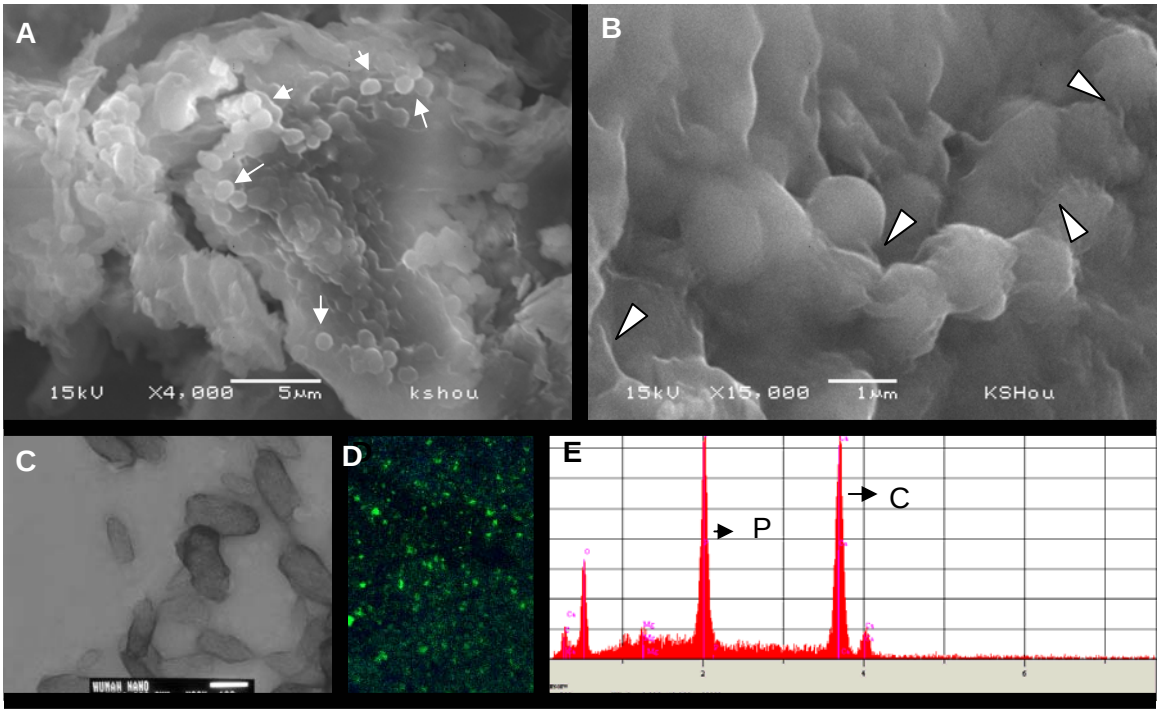
The SEM revealed NB-like small spherical apatite units as can be observed in Figure 1-A and B. The TEM, as shown in Figure 1-C, using a negative staining technique, revealed particles growing in culture from the stone material consistent with NB in morphology. Following demineralization the calculus material showed strong fluorescein signals with anti-NB antibodies by the IFS technique shown in Figure 1-D.

Conclusion. In the case report, a spontaneously passed urinary calculus from a spaceflight crewmember is associated with the presence of NB. Although the presence of this agent does not imply it played a causal role in the development of the calculus, the stone composition included 40% apatite by weight, and NB are known to accumulate apatite as the principle mineral component of its formed clusters, termed “igloos”[22, 25, 26].

With the observed increased rate of reproduction during simulated microgravity conditions[24], it is not inconceivable that NB infection may have a potential role in kidney stone formation in crew members during space flights. Therefore there are several points of evidence supporting this hypothesis: a) space crew may have some alterations in immune defense systems to infectious agents[8-13], b) NB have been detected in urinary calculi in earlier studies[19, 20, 23], c) NB multiply faster in microgravity conditions[24], d) several of Koch postulates for NB-induced urinary calculus formation were satisfied by previous research studies[19, 20, 26, 27], e) in this research NB antigens and

morphology were detected in the passed calculus of a crewmember after space flight. However, much additional work is required to determine the validity of the hypothesis that NB can induce urinary calculi. Such studies could include screening of the CN/NB antigen and antibody level in flight crew before and after flight, culturing of actively reproducing CN/NB from urinary calculi derived from stone patients, and clinical finding of the development of urinary stones, *de novo*, in a non-stone former recently infected with NB. The contribution of CN / NB to human disease, as a pathogen, has yet to be elucidated, however findings such a reported in this case, justify additional studies to determine if the role is causal.

Figure 1



A. SEM image of kidney stone from patient described. See the spherical apatite (calcium phosphate) depositions shown with white arrows. B. Higher magnification of apatite spherical formations in kidney stone under SEM. The arrow heads show the biofilm-like structures among the spheres which is a sign of bacterial formations. C. TEM, negative staining image of nanobacteria cultured from the kidney stone. D. Immunofluorescein staining of cultured nanobacteria from kidney stones by using anti-NB antibody, 8D-10. E. EDS analysis of the apatite spheres under SEM showing calcium and phosphate peaks as identical to cultured nanobacteria EDS results.

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