Nanobacteria: A Possible Etiology for Type III Prostatitis

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Abbreviations and Acronyms

IL-1β = interleukin-1β

PBS = phosphate buffered saline

TNF-α = tumor necrosis factor-α

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Purpose: Nanobacteria are thought to be a pathopoiesis bacterium in urological disease. We observed pathological changes in nanobacteria infected prostates in Sprague-Dawley® rats and investigated the possible etiological relationships of nanobacteria and type III prostatitis.

Materials and Methods: We randomized 40 adult male Sprague-Dawley rats each to the control and model groups. Rat prostate infection models were reproduced by infusing nanobacteria suspension transurethrally. Rats were sacrificed 1, 2, 4 and 8 weeks later, respectively. Prostatic pathology, and the cytokines interleukin-1β and tumor necrosis factor-α were assessed. Nanobacteria isolation, culture and characterization were also analyzed.

Results: In model rats we observed prostatic acute inflammatory changes 1 to 2 weeks after nanobacteria infusion and chronic inflammatory changes after 4 weeks. At 8 weeks we noted microcalculous formation in the prostatic glandular cavity in 7 of the 10 model rats, which was not seen in controls. Interleukin-1β and tumor necrosis factor-α in prostatic tissues were higher in model rats than in controls at different time points ($p < 0.01$). In model rats interleukin-1β and tumor necrosis factor-α were higher 2 weeks after infusion than at 1, 4 and 8 weeks ($p < 0.05$). Prostatic tissue was nanobacteria positive in 35 model rats and in 0 controls.

Conclusions: Nanobacteria may be an important etiological factor for type III prostatitis.

Key Words: prostate; prostatitis; nanoparticles; chronic prostatitis; calculi; rat, Sprague-Dawley

Of all types of chronic prostatitis in men type III has the highest incidence, accounting for 60% to 90% of those with prostatitis.[1] However, the etiology of type III prostatitis remains unclear and, thus, it is difficult to treat the disease. Bacterial pathogens have not been found in prostatic tissue, urine or prostatic fluid by conventional culture but prostatic inflammation or inflammatory markers are often identified.[2] This suggests the existence of yet unknown infectious pathogens and the importance of identifying these pathogens to diagnose and treat type III prostatitis.

Nanobacteria, a kind of recently discovered bacteria, are thought to be associated with type III prostatitis pathogenesis.[3–6] Nanobacteria are globoid or racket-shaped bacteria with a diameter of 100 to 500 nm. They grow slowly and double every 3 to 5 days. Also, they grow only in cell culture medium. Nanobacteria tend to mineralize and form apatite crystals under physiological conditions. It is difficult to detect nanobacteria by ordinary methods but they can be observed by immunological methods or electron microscopy.[3] When nanobacteria are co-cultured in vitro with fibroblasts,

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they enter fibroblasts through endocytosis and cause cell death.[7] Nanobacteria infection causes chronic renal parenchymal inflammation, degenerative changes, calcification and apoptosis of renal tubular epithelial cells in animals[8,9] as well as lithogenesis in the gallbladder and kidney of animals.[10,11]

The detection rate of serum and urine nanobacteria antigens is significantly higher in patients with type III prostatitis than in healthy men or those with prostatic hyperplasia.[4] Nanobacteria were isolated from the prostatic fluid of men with type III prostatitis and the detection rate of nanobacteria was relatively higher in these patients.[5] Anti-nanobacterial therapy ameliorated clinical symptoms in patients with type III prostatitis and significantly decreased the nanobacteria detection rate.[5,6] Also, prostatic calculi and calcifications were often found in some young men with type III prostatitis, associated with inflammation and chronic pelvic pain syndrome symptoms.[12,13] The core of prostatic calculi is typically calcium apatite, which is the hallmark of nanobacterial action.[14] These findings led researchers to postulate a role for nanobacteria in the development of type III prostatitis. They thought that nanobacteria may be a pathogen of this disease.[5,6] Since to our knowledge there are no reports of the in vivo effect of nanobacteria on the prostate, we performed an in vivo study.

MATERIALS AND METHODS

Nanobacteria Isolation, Culture and Preparation

As described previously,[5] urine and prostatic fluid samples were collected from outpatients with refractory type III prostatitis by aseptic technique using the Meares-Stamey method. Men with refractory type III prostatitis fulfilled certain criteria, namely 1) symptom history greater than 9 months, 2) appropriate antibiotic therapy failure and 3) anti-inflammatory therapy failure (quercetin or nonsteroidal anti-inflammatory drugs). All patients must have abstained from sexual activity for at least 4 days before sample collection. The urethral orifice was disinfected with benzalkonium chloride. Samples were cultured as described by Ciftçioglu et al.[7,15] Briefly, samples were diluted, filtered with a 0.45 μm, 0.22 μm Millex® pinhole filter and centrifuged, followed by primary culture in serum-free RPMI-1640 (Gibco®) medium at 37C in air containing 5% CO_2. Subcultures were done after 4 weeks of initial inoculation and kept under the conditions described. Cultures were harvested by centrifuged at 20,000 $\times$ gravity for 30 minutes at 4C, washed with PBS (pH 7.2) and prepared as a bacteria suspension in normal saline (1 McFarland U) for experiments.

Animals

We randomized 80 adult male Sprague-Dawley rats 3 to 4 months old and weighing 250 to 300 gm to the control (40) and model (40) groups. Rats were raised in a total of 8 cages at 20C to 23C with a 12:12-hour light/dark cycle. Animal use was approved by the committee for animal protection and use. Rats had free access to food and drink, and were allowed to adapt to the new environment for a week.

Animal Model Reproduction

Chronic bacterial prostatitis models were reproduced as described by Nickel et al.[16] Briefly, rats were anesthetized with ether. The genital area was cleaned with 70% ethanol and catheterized with a lubricated sterile polyethylene tube 0.9 mm in diameter and 2.5 cm long. Bacterial suspension (0.2 ml) was instilled into the prostatic urethra using an insulin syringe. In controls normal saline was infused instead.

Sample Collection

After nanobacteria infusion into the urethra the rats were raised under the same conditions. Ten rats each were sacrificed in 4 batches 1, 2, 4 and 8 weeks, respectively, after nanobacteria infusion. By aseptic technique the ventral and dorsal prostatic lobes were harvested and the prostatic capsule was removed completely, leaving only prostatic tissue.

Pathological Study

The remaining prostatic pieces were fixed in 10% formalin, dehydrated, embedded in paraffin and sectioned conventionally. Sections were deparaffinized, and conventional hematoxylin and eosin staining was done, followed by observation and photography under microscopy.

Prostate Ultrastructural Study

Prostatic tissue was sectioned randomly and sections were fixed in 2.5% glutaraldehyde. Sections were mounted on a membrane coated copper screen and stained with 3% phosphotungstic acid for 1 to 2 minutes, followed by observation and photography at the central laboratory at our institution using a Tecnai 10 transmission electron microscope (Philips, Eindhoven, The Netherlands) with an 80 kV working voltage. Some sections were fixed for 1 hour with 2.5% glutaraldehyde and dehydrated in an ethanol gradient, followed by cryodesiccation and metal plating. Sections were observed under a KYKY-EM3200 scanning electron microscope (KYKY Technology Development, Beijing, People's Republic of China) at 30 kV working voltage.

Cytokine Determination

By aseptic technique the ventral prostatic lobe was weighed and homogenized in ice-cold saline to prepare 10% homogenate. The homogenate was centrifuged at 4C and 3,000 rpm for 15 minutes. Supernatant (1 ml) was collected and stored at $-$20C before cytokine determination. TNF-α and IL-1β were determined by enzyme-linked immunosorbent assay (R & D Systems®). Standard preparation (0.1 ml) diluted 1:1 was added to each well of the antibody coated microplates, followed by the addition of 100 μl of sample into each well and incubation for 90 minutes at 37C. Microplates were washed with PBS/Tween and 0.1 ml biotin labeled antibody was added to each well, followed by 60-minute incubation at 37C. Microplates were washed with PBS/Tween and 0.1 ml peroxidase labeled avidin complex was added to each well, followed by 60-minute incubation at 37C. After a final wash 0.1 ml tetramethyl benzidine solution was added to