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Changes in seminal fluid zinc during experimental prostatitis

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Summary. Patients with prostatitis have been found to have decreased prostatic fluid zinc (PF Zn). It is unclear whether changes of prostatitis are due to an inherent lack of Zn in some patients or whether the changes, including reduced PF Zn, are due to the infectious process itself. Six nonhuman primates were infected with 1×10^5 *E. coli* JR340, a clinical isolate known to cause prostatitis in the monkey. After infection, seminal fluid for culture and zinc assay was obtained by electroejaculation. Zinc was measured by colorimetric analysis. Blood counts, quantitative renal scans and urine cultures were also monitored. The animals were followed for 4 weeks and then sacrificed. The histopathology showed resolving prostatitis and fibrosis. Zinc levels dropped with acute infection, and variably recovered as the infection cleared. Controls showed slight variability with respect to PF Zn. It appears that PF Zn decreases as a result of the infectious process.

Key words: Nonhuman primate – Prostatitis – Seminal fluid – Zinc

Zinc has been known to be associated with the prostate gland for many years. It has been identified in a number of species of animals, notably the rat [9], monkey [15], and human [11]. In the normal human prostate gland it seems to be localized in a dorsolateral distribution, with higher concentrations laterally [7]. It is probably associated with a low-molecular-weight protein and has also been shown to exert antibacterial activity in prostatic secretions [3]. Fair et al. initially demonstrated this antibacterial activity and furthermore determined the association with Zn concentration [4]. In the past, a number of investigators have shown that patients with bacterial prostatitis have consistently lower Zn concentrations in their prostatic fluid (PF) than normals [4, 10]. It has yet to be shown, however, whether the lack of Zn is a primary defect in the gland and predisposes to prostatitis, or whether lower Zn concentrations are due to the disease process itself.

These concentrations of Zn in the normal PF are known to be bactericidal to the pathogens common in prostatitis. Zinc resistance occurs in this group of microorganisms, but it is not known whether this contributes to the virulence or pathogenesis.

Marmar et al. showed that patients with non-bacterial prostatitis had lower Zn concentrations but that this defect could be corrected by administering oral Zn [10]. It has also been shown that oral Zn does not correct this defect in the patient with bacterial prostatitis [8]. The question we are attempting to answer in this paper was raised by Dr. E. M. Meares: "... Whether men develop bacterial prostatitis because they have a secretory dysfunction of the gland or whether secretory dysfunction occurs as a result of infection remains unresolved" [12].

Materials and methods

We used four cynomolgus monkeys *(Macaca fascicularis)* and two baboons *(Papio anubis)* for this study. We have previously established a primate model of acute bacterial prostatitis and our method was used for this experiment [13]. The control animals for PF Zn were uninfected animals of the same species.

Histopathology control animals were monkeys that had undergone necropsy, for other reasons and had not been infected. The experimental animals were kept in cages approved by the American Association for Laboratory Animal Science at the Tulane Primate Research Center. Anesthesia was accomplished with ketamine hydrochloride, 3 mg/kg i.m. Animals had free access to Purina monkey chow and drinking water.

Bacteria

Our bacterium (*E. coli* JR340) is serotype 04, P fimbriated and type 1 fimbria positive after two passages in tryptose broth. The organism is non-hemolytic and motile. It is completely antibiotic sensitive and has remained stable under subculture. It was grown overnight in trypstose soy broth at 37°C in a shaker bath. The organisms were then removed and washed twice in phosphate-buffered saline. Using

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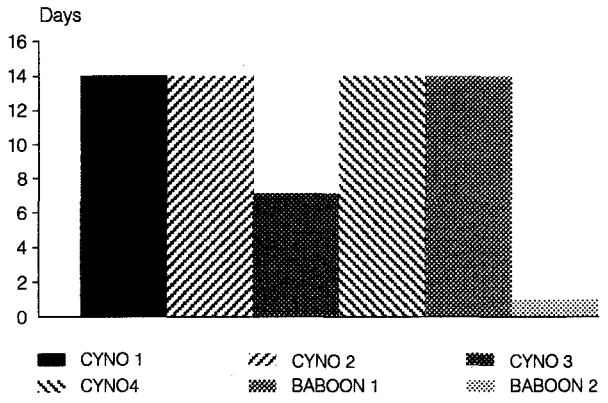

Fig. 1. Presence of bacteria in urine after inoculation with *Escherichia coli*

a Fisher Absorbance Spectrophotometer, the organisms were concentrated to 1×10^5/ml and resuspended in phosphate-buffered saline prior to inoculation.

Infection

Each animal was inoculated with 1×10^5 *E. coli* JR340. This organism is the same as was used in our previous animal model and was obtained from a patient with acute bacterial prostatitis [13]. The bacteria were deposited in the prostatic urethra, suspended in 1 ml normal saline. This technique involved insertion of a 5-F pediatric feeding tube into the bladder and withdrawal of the catheter until no urine was returned. By again withdrawing it approximately 1 cm past that point, the tip was positioned in the prostatic urethra, where the bacteria were deposited. The animals were followed up with cultures of the urine and prostatic fluid for a total of 4 weeks. The presence of the infecting organism (JR 340) in the urine or seminal fluid constituted bacteriuria and seminal fluid infection, respectively.

In addition, blood counts and quantitative renal camera studies were performed at weekly intervals using ^{131}I-hippuran [14]. Evidence of systemic infection and upper tract urinary infection was determined by these studies. Systemic infection was shown by elevations of the white blood count above the upper limit of normal and the left-shifted differential. Evidence of kidney infection was provided by the quantitative renal camera study as manifested by decrease in uptake and secretion of the radionuclide in the involved kidney. The reliability of this test has been described previously [14].

Ejaculation

The method of obtaining PF has been previously described [6]. Briefly, a probe was inserted into the rectum over the prostate of the monkey, and using a variable voltage of 3–10 V in a pulsatile fashion, ejaculation occurred. The prostatic fluid was collected in an aseptic container and was taken immediately for culture and Zn determination.

Determination of PF Zn

The PF obtained by electroejaculation was studied semi-quantitatively for Zn using a colorimetric analysis (EM Science, Cherry Hill, NJ) and by atomic absorption spectrophotometry. The latter method was used only on the initial specimens to insure accuracy of the dipstick test, and a close correlation was found. The PF was placed in a test tube and the dipstick was saturated with the fluid. The test stick was then placed in 32% sodium hydroxide solution and read colormetrically after 60 s. The upper limit of Zn measurable by this test is 250 mg/l (ppm). This was also compared against atomic absorption spectrophotometry on three samples of human prostatic fluid and found to be accurate compared with this method of determinng Zn concentration.

Electron microscopic studies

Utilizing a Philips transmission electron microscopy (TEM) with an EDAX Analyzer System, semi-quantitative amounts of Zn were determined on thin sections of these prostates (Tulate University, Department of Biology, New Orleans, LA). This attachment to the TEM enables the user to determine the elements in a sample and obtain the semi-quantitative value of that particular element.

Prostatic tissue samples were obtained from each animal at sacrifice and placed in Karnovsky's fixative for 1 h, then diced into 1-mm cubes and left overnight. They were subsequently washed six times in 0.2 M cacodylate buffer at 10-min intervals and postfixed in 1.0% osmium tetroxide in 0.1 M cacodylate buffer for 1 h. A 1:50 dilution of 1.0 M calcium chloride was added to the buffers to enhance the preservation of membranes. Dehydration was carried out in increasing concentrations of ethanol and embedded in Spurr's low-viscosity epoxy resin. A Sorvall MT-2 ultramicrotome was used to obtain silver to pale gold sections employing a Dupont diamond knife. Unstained sections were examined in the above Philips TEM. All procedures were conducted at room temperature except for the curing of Spurr's resin at 70°C [5].

Zn sensitivity of bacteria

The sensitivity of infecting organisms to Zn was tested on 27 separate organisms. Each was a clinical isolate taken from a patient with acute or chronic prostatitis, and each was *Escherichia coli.* Control organisms were 7 *E. coli* specimens taken from female patients with urinary tract infections. The organisms were grown in tryptose soy broth for 24 h. They were washed two times in sterile tris-hydroxymethylaminoethane (TRIS) buffer. Zinc chloride was added to sterile 0.01 *N* TRIS solution in serial dilutions from 250 parts per million to 15 parts per million (ppm). To each of these solutions, 1×10^6 organisms were added, and overnight incubation followed. A control tube contained only 0.01 *N* TRIS buffer. If growth was present in the dilution of 125 ppm or higher, the organism was considered Zn resistant.

Results

Infection occurred in all animals after a single inoculation. Bacteriuria was present from day 1 for up to 14 (mean 10.7) days (Fig. 1). Seminal fluid cultures remained positive for a mean of 11.2 days during the same time (Fig. 2). These values are consistent with our prior experience with this model and have been correlated with the histopathological changes of acute prostatitis [12]. One animal died of gram-negative septicemia on day 10 of the study. White blood counts paralleled the course of bacteriuria, indicating that a systemic infection was present and resolved. Peak counts were observed from day 7 to day 14 and returned to normal in all cases by day 21. Renal scans demonstrated upper tract involvement in three of the six animals.

Absolute concentrations of Zn prior to infection varied widely in their baseline values, ranging from 20 to

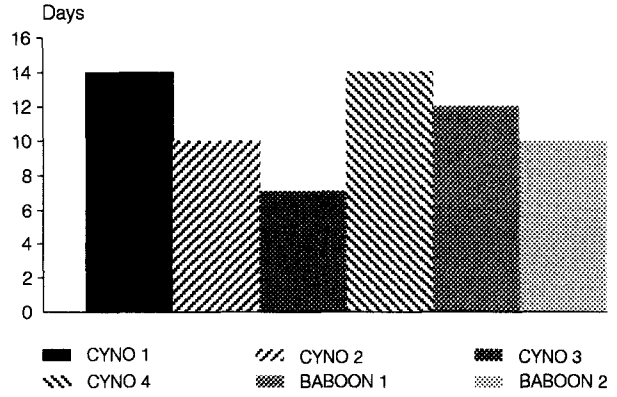

Fig. 2. Presence of *E. coli* in seminal fluid after inoculation

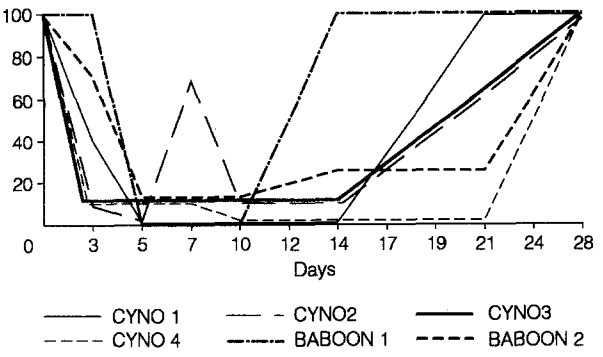

Fig. 3. Changes in zinc level in prostatic fluid over time following inoculation with *E. coli*

250 ppm. The PF Zn levels reached their nadir by day 7 and returned to approximately 100% of their baseline value by day 28 (Fig. 3). No rebound of PF Zn amounts to levels greater than pre-infection values was observed, however.

In the control animals, high concentrations of Zn were observed ultrastructurally in the caudal prostate, with only negligible amounts seen in the cranial prostate. The seminal vesicles had similar levels to the cranial prostate, in that minimal amounts of Zn were identified there. In infected animals, Zn was found to be slightly lower in the caudal prostate than in the control group, with similar small amounts seen in the cranial prostate. Light microscopy confirmed the presence of prostatitis in these animals. Both intraductal and stromal infiltration of inflammatory cells was present. Numerous lymphocytes and some polymorphonuclear leukocytes were seen, with a predominance of lymphocytes. Occasional microabscess formation was also noted.

Eight of the prostatitis organisms (29.6%) showed Zn resistance, while three of the pyelonephritis organisms were found to be Zn resistant (42.9%). In each case there was excellent growth in the control flask, indicating that viable organisms were inoculated into the Zn dilutions. Approximately half of all the organisms showed some growth in the lowest dilution of Zn concentration.

Discussion

In the course of acute prostatitis, Zn levels in the prostatic fluid rapidly dropped to negligible levels and gradually returned to approximately their baseline values. Furthermore, ultrastructurally there was little change in the total Zn content of the gland itself, despite the presence of an inflammatory process. Cowan et al. have shown that infection does not decrease the total amount of Zn in the prostatic tissue [2]. These findings would be expected, considering that the Zn defect in bacterial prostatitis cannot be corrected by administering oral Zn. In addition, resolution of the infection allows return of Zn to the semen, possibly by restoration of the Zn-concentrating or Zn-secretory capacity. Our electron microscopic analysis seems to confirm this.

Since the studies of Fair et al. demonstrated antibacterial activity in the PF which was ultimately shown to be Zn [4], it was assumed that this was a mechanism for prevention of prostatitis. It was then unclear whether the findings of diminished PF Zn was an effect of the infection itself or whether this was a natural defect in certain individuals, which predisposed them to infection. While diminished PF Zn may be a natural variant in certain individuals, this study demonstrates that experimental acute infection of the prostate gland does indeed produce diminished Zn in the prostatic secretions. Furthermore, the fact that infection occurs after urethral inoculation shows that the antibacterial effect of the initial high PF Zn found in monkeys can be overcome by an adequate inoculum of bacteria. In our previous prostatitis study, 1×10^9 bacteria were used and produced the changes of acute and chronic prostatitis histologically [13]. We used a bacterial concentration of only 1×10^5 and were still able to produce the desired effects of prostatitis. The findings of decreased Zn concentrations in the prostatic secretions in the presence of positive PF cultures are different from the work of Branam et al. [1]. This study was conducted in dogs and showed that there was no demonstrable difference in PF Zn in infected versus uninfected animals. The difference we observed may be explained by the fact that our animals had an experimentally induced infection with a known pathogen. Their dog model used animals that were clinically well, but had bacteria isolated from the PF. In our experience, commensal organisms can frequently be found in the PF in the absence of infection or inflammtion and are not necessarily the hallmark of prostatitis. Anecdotally, however, it seems that the ejaculate has a reduced volume in infected animals, and that a plug of mucoid material is all that can be produced by electroejaculation. The volume of PF varies from animal to animal each time an ejaculate is produced; when there is only a mucous plug, however, very little Zn is present in this material.

The determination of Zn sensitivity of the bacteria causing prostatitis was performed to determine whether Zn resistance was a prerequisite for organisms to cause

prostatitis. It appears that these organisms are no more Zn resistant than other organisms causing urinary tract infections. It is difficult to determine whether Zn resistance has any role in the pathogenesis of bacterial prostatitis, however.

These studies also have a possible implication for the management of patients with prostatitis in that seminal fluid Zn may be semi-quantitatively measured in the office with this dipstick test. Since diminished Zn is a consistent finding in infected patients [4], this could be used clinically. This may facilitate the diagnosis of prostatitis in patients in whom the culture results are equivocal or possibly in situations where using the four-culture technique is too cumbersome or economically impossible. If the PF Zn did not respond to oral Zn administration, this would be evidence for an infectious process.

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