IN VITRO RESISTANCE TO HUMAN PLATELET MICROBICIDAL PROTEIN AMONG URETHRAL STAPHYLOCOCCAL AND ENTEROCOCCAL ISOLATES WITH ITS CORRELATION WITH PROSTATITIS

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Abstract

The study was carried out to test the in vitro activity of human platelet microbicidal protein (hPMP) on most commonly isolated urethral pathogens and compare the same with clinical isolates from cases of chronic prostatitis (CP). Urethral isolates of Staphylococcus aureus (n=19), coagulase negative staphylococci (n=40) and Enterococcus faecalis (n=16) from patients with or without CP were tested. The hPMP susceptibility of bacterial strains was determined by exposing bacterial cells to serial dilutions of hPMP. A significantly higher proportion of CP-strains of coagulase negative staphylococci (91.3% vs 5.88%) was resistant to hPMP than was that of non-CP strains (P < 0.001). Among CP-strains of S. aureus studied, 77.8% were considered resistant to the bactericidal action of hPMP. All nine CP-strains of E. faecalis were highly resistant to hPMP. Most non-CP urethral isolates of S. aureus, coagulase negative staphylococci and E. faecalis were susceptible to the bactericidal action of hPMP while CP isolates of all species were significantly more resistant to hPMP. Data from the present study may have significant implications in understanding the pathogenesis of CP.

Key words: coagulase negative staphylococci, Enterococcus faecalis, platelet microbicidal protein, resistance, prostatitis, S. aureus

A number of microorganisms are able to infect the tissues of the reproductive tract in humans.1,2 Bacterial infection of prostate is the most common urologic condition that may occur as a result of ascending urethral infection.3 Other possible routes of prostatitis include invasion by bacteria by limphogenous or haematogenous spread. As it is difficult to exactly establish the significance of various microorganisms in the pathogenesis of chronic prostatitis (CP), it is imperative to delineate both microbial and host factors that contribute to its development.3

The key role of endogenous cationic antimicrobial peptides in host defense against bacteria, fungi, eukaryotic parasites and viruses has been emphasized recently.4 The antibacterial peptides have also been found in human platelets.5 These peptides are secreted at sites of infection and exert microbicidal activity against many pathogens, including Staphylococcus aureus.6 Wu et al showed that in vitro resistance of clinical staphylococcal and viridans group streptococcal strains to rabbit platelet microbicidal protein correlates with the diagnosis of infective endocarditis.7

However, the relationship between microbial susceptibility to human platelet microbicidal protein (hPMP) and clinical source of urogenital infections has not yet been addressed. The most common causative agents of CP are S. aureus, coagulase-negative staphylococci (CNS) or Enterococcus faecalis.3 The present study aimed at in vitro detection of hPMP resistant phenotypes of urethral isolates along with their comparison with isolates from patients with or without CP.

Materials and Methods

Preparation of hPMP

hPMP was prepared by techniques modified from those of Yeaman et al.8 In brief, healthy human platelet rich plasma was obtained by low speed centrifugation of 1 day outdated platelets from the blood donor department of the Orenburg Regional Medical Center. The platelet rich plasma was then dispensed into polypropylene tubes and centrifuged again at 250 x g for 30 min at 25°C. The sedimented platelets were washed three times with Tyrode’s buffer (138 mM NaCl, 3.6 mM KCl, 10 mM NaHCO3, 0.4 mM Na2HPO4, 10 mM MgCl2, and 6 mM glucose, adjusted to pH 7.3 with phosphoric acid). The washed platelets were suspended in 5 volumes of ice cold 30% acetic acid, and stirred in melting ice for 24 hours. The washed platelets were suspended in 5 volumes of ice cold 30% acetic acid, and stirred in melting ice for 24 hours. The resulting extract was centrifuged at 10000 x g for 15 minutes, and hPMP rich supernatant was recovered. hPMP bioactivity assays were performed with Bacillus subtilis ATCC 6633, an indicator organism highly sensitive to the bactericidal action of hPMP.9 Exposure of 10⁸ washed human platelets per mL to 5 mL of ice cold 30% acetic acid resulted in mean supernatant protein concentrations of ~90 mg/mL. The hPMP susceptibility of bacterial strains was determined by exposing bacterial cells to serial dilutions of hPMP. B. subtilis was grown in tryptic soy broth (TSB) at 37°C for 18 hours; organisms were harvested by centrifugation, washed twice in phosphate buffered saline.

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(PBS pH 7.2), and resuspended in PBS prior to use. Bacteria were diluted to $10^4$ CFU/mL in PBS. Two fold serial dilutions of hPMP were prepared in PSB and 900-µL hPMP aliquots were transferred to a low protein binding protein microtiter tubes (Costar Glass Works, Corning, USA). To each of the tubes, 100 µL of the bacterial suspension was added. The tubes were incubated on a rotary shaker (300 rpm) at 37°C. After 1 hour, aliquots of 200-mL were plated on blood agar plates. Microbicidal activity of hPMP was assessed the next day after counting colonies on the agar plates and defined as the concentration of protein which retained ≥ 50% lethality for *B. subtilis*. The bioactivity of such hPMP preparations against *B. subtilis* ranged from 0.9 to 1.2 µg/mL. Control samples were found to possess no anti *B. subtilis* bioactivity.

**Determination of hPMP susceptibility of urethral strains**

Well characterized urethral isolates of *S. aureus* (n=19), CNS (n=40) and *E. faecalis* (n=16) from patients with or without CP were kindly provided by Serge Cherkasov (Orenburg State Medical Academy) and Michail Kuzmin (Orenburg Regional Medical Center). The diagnosis of patients and cases from which the isolates were initially obtained as CP and non-CP were made by the contributing investigators, using standard clinical parameters, prior to knowledge of an isolate’s hPMP susceptibility. The hPMP susceptibility of bacterial strains was determined by exposing $2 \times 10^3$ bacterial cells to serial dilutions of hPMP of 5 µg/mL to 15 µg/mL as described by Yeaman *et al.* To define the proportion of strains considered hPMP susceptible and hPMP resistant, a survival of ≤ 50% of the initial inoculum at hPMP concentration of 5 µg/mL was considered a relative hPMP susceptibility breakpoint, on the data of Wu *et al.*

**Statistical analysis**

The proportion of CP and non-CP isolates that were hPMP susceptible or hPMP resistant were compared and differences between groups were assessed by using Student’s t test. A P value of ≤ 0.05 was considered significant.

**Results**

Of the 40 urethral CNS isolates tested, 23 and 17 were from CP and non-CP cases respectively. A significantly higher proportion of CP strains was resistant to hPMP than that of non-CP strains (P < 0.001) (Table 1). In addition, 16 of 17 (94.1%) non-CP strains exhibited either low level resistance or susceptibility to 10 µg/mL hPMP (Table 2). However, 16 of 23 CP-strains (69.6%) were resistant to higher bactericidal concentrations of hPMP (> 15 µg/mL).

Among the 19 *S. aureus* isolates studied, 10 urethral isolates were from patients with CP, while 9 isolates were from patients without CP. Of the CP strains tested, 7 of 9 (77.8%) were considered resistant to the bactericidal action of hPMP compared with only 2 of 10 of the non-CP isolates (20%, P < 0.001). Furthermore, only 33% CP strains were resistant to 15 µg/mL of hPMP.

Of the 16 *E. faecalis* strains tested, 7 were from patients without clinical symptoms of CP, while 9 were from patients with CP. All 9 CP strains were highly resistant to hPMP.

**Discussion**

At local sites of microbial infections, platelets, neutrophils, or macrophages release large amounts of different bactericidal peptides. Chronic bacterial prostatitis is characterized by recurrent urinary tract infections and persistence of bacteria in prostatic secretory system despite the presence of multiple antibacterial peptides in prostatic fluid. There is urgent need to understand the virulence properties of bacteria that are associated with chronic infection of the prostate. Identifying such a factor(s) would be helpful in devising effective treatment strategies.

In the present work, we studied the hPMP bactericidal activity against the most frequent bacterial CP pathogens. Most non-CP urethral isolates of *S. aureus*, CNS and *E. faecalis* were susceptible to the bactericidal action of hPMP, while CP isolates of all species were significantly more resistant to hPMP. Similar findings were obtained by Yeaman *et al* for bacteraemic isolates from patients with or without infective endocarditis.

These findings suggest that the phenotypic trait of hPMP resistance may be important for bacterial pathogens to induce and perpetuate chronic infections of different localization by surviving or avoiding microbialproteins mediated clearance.

Data from the present study may have significant implications in understanding the pathogenesis of CP, as well as the role of antibacterial peptides in prostatic fluid.
as for future improvement in the prevention and therapy of CP.

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References


