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·*Original Article*·

Distribution of secretory inhibitor of platelet microbicidal protein among urethral isolates with its correlation with prostatitis

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Abstract

Aim: To report the detection *in vitro* of secretory inhibitor of platelet microbicidal protein (SIPMP) phenotypes of urethral isolates along with a comparison with isolates from patients with or without chronic bacterial prostatitis (CBP). **Methods:** Urethral isolates of *Staphylococcus spp.* (n = 64), diphtheroids (n = 28), micrococci (n = 15), streptococci (n = 21), *Enterobacteriaceae* (n = 9) and *Enterococcus faecalis* (n = 19) from patients with or without CBP were tested. SIPMP production was tested by inhibition of platelet microbicidal protein (PMP) bioactivity against *Bacillus subtilis* and was expressed as percentage of inhibition of PMP bactericidal activity. **Results:** A significantly higher proportion of CBP-strains (57.78% *vs.* 16.67%) reduced PMP-induced killing of *Bacillus subtilis* than non-CBP strains did ($P < 0.01$). SIPMP levels of staphylococci and *Enterococcus faecalis* from the CBP group were significantly higher than those of the control group. **Conclusion:** These results suggest that SIPMP production is associated with the CBP source. Data from the present study might have significant implications for the understanding of the pathogenesis of CBP. *(Asian J Androl 2008 Mar; 10: 189–192)*

Keywords: bacteriology; platelet microbicidal protein; prostatitis

1 Introduction

A number of microorganisms are able to infect the reproductive tract tissues in humans with serious consequences for reproductive function [1, 2]. A most common result of microbial infection of the reproductive tract is chronic bacterial prostatitis (CBP), a condition that can lead to transient or permanent infertility [1–3]. CBP is a subtle illness, which is characterized by persistence of bacteria in the prostatic secretory system [2, 3]. As it is difficult to establish precisely the significance of various microorganisms in the pathogenesis of CBP, it is imperative to delineate both microbial and host factors that contribute to its development [1, 3, 4].

The major role of endogenous cationic antimicrobial peptides in preventing the onset of infection has been emphasized recently [5, 6]. Such peptides have also been found by several authors in human platelets and are designated beta-lysins [7], thrombocidins [8] and platelet microbicidal protein (PMP) [9, 10]. These pep-

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tides are secreted at sites of infection and exert microbicidal activity against many pathogens [7–10]. We have shown that resistance of urethral staphylococcal and enterococcal strains to human PMP *in vitro* correlates with the diagnosis of CBP [10]. In a recent publication [11], we reported the detection of an extracellular staphylococcal product, designated secretory inhibitor of platelet microbicidal protein (SIPMP), that causes local inhibition of the bactericidal action of PMP in the fluid phase. We also demonstrated that SIPMP represents a hitherto unrecognized determinant of staphylococcal pathogenicity and SIPMP production is associated with prostatitis source.

The present study reports the detection *in vitro* of SIPMP phenotypes of urethral isolates along with a comparison with isolates from patients with or without CBP.

2 Materials and methods

Platelet microbicidal protein was prepared and standardized as described previously [10]. Well-characterized urethral isolates of microorganisms (n = 156) from patients with or without CBP were kindly provided by Serge Cherkasov (Orenburg State Medical Academy, Orenburg, Russia). The determination of patients and cases from which the isolates were initially obtained as CBP, and non-CBP were made by the contributing investigator, using standard clinical parameters, prior to knowledge of an isolate's SIPMP production. SIPMP production was tested according to the recently proposed procedures [11].

The mean values and standard mean errors were calculated. The proportion of CBP and non-CBP isolates with different levels of SIPMP was compared and differences between groups were assessed using unpaired t-test. $P < 0.05$ was considered significant.

3 Results

Exposure of 10^8 washed human platelets per mL to 5 mL of ice-cold 30% acetic acid resulted in mean supernatant protein concentrations of approximately 70 mg/mL. The bioactivity of such PMP preparations against *Bacillus subtilis* ranged from 0.5 μg/mL to 1.0 μg/mL. Control samples were found to possess no anti-*B. subtilis* bioactivity.

Among the 64 *Staphylococcus spp.* isolates studied, 39 urethral isolates were from patients with CBP, and 25 isolates were from patients without CBP (Table 1). Of the CBP-strains tested, 51.3% were found to produce SIPMP compared with only 20% ($P < 0.01$) of the non-CBP isolates. Of the 28 urethral diphtheroids isolates tested, 11 and 17 were from CBP and non-CBP cases, respectively. A significantly higher proportion of CBP strains of diphtheroids (63.6% *vs*.11.76%; $P < 0.001$) was SIPMP-positive compared with non-CBP strains. Among SIPMP-producing strains of micrococci tested, 20% of isolates were from the control group, whereas 40% of isolates were from patients with CBP. Of the 21 urethral streptococcal isolates studied, 10 were from patients without clinical symptoms of CBP, whereas 11 were from patients with CBP. We found no significant difference in the proportion of SIPMP-positive streptococcal isolates between the two groups.

Table 1. Secretory inhibitor of platelet microbicidal protein (SIPMP) production of urethral isolates. CBP, chronic bacterial prostatitis.

Organism	No. of SIPMP-producing strains/total	
	Healthy men	Patients with CBP
Staphylococcus spp.	5/25	20/39
Corynebacterium spp.	2/17	7/11
Micrococcus spp.	2/10	2/5
Streptococcus spp.	2/10	3/11
Enterobacteraceae	0/0	9/9
E. faecalis	0/4	11/15

The extracellular products of bacteria reduced the bioactivity of PMP (Tables 2 and 3). In contrast to bacteria isolated from the control group, the strains isolated from men with CBP demonstrated more intensive inhibition of PMP-induced killing of *B. subtilis*. The culture supernatants of staphylococci from the CBP group significantly more actively decreased bioactivity of PMP ($P < 0.05$). SIPMP-production of diphtheroids, micrococci and streptococci from men with CBP were not significantly different from strains of bacteria isolated from anterior urethra of healthy men ($P > 0.05$).

4 Discussion

Chronic bacterial prostatitis is characterized by recurrent urinary tract infections and persistence of bacteria in the prostatic secretory system, despite the presence of multiple antibacterial peptides in prostatic fluid [12, 13]. There is an urgent need to understand the virulence properties of bacteria that are associated with

Table 2. Distribution of secretory inhibitor of platelet microbicidal protein (SIPMP) positive cultures among CBP and non-CBP isolates, CBP, chronic bacterial prostatitis.

No. of organisms (healthy men/CBP)	No. of strains (healthy men/CBP) with different levels of SIPMP (%)			
	0	0.1–10.0	10.1–20.0	> 20
Staphylococcus spp. (25/39)	20/19	3/1	2/2	0/17
Corynebacterium spp. (17/11)	14/3	3/7	0/1	0/0
Micrococcus spp. (10/5)	8/3	1/0	1/2	0/0
Streptococcus spp. (10/11)	8/8	0/0	2/3	0/0
Enterobacteriaceae (0/9)	0/0	0/0	0/0	0/9
E. faecalis (4/15)	4/4	0/0	0/0	0/11

Table 3. Secretory inhibitor of platelet microbicidal protein (SIPMP) production of urethral isolates. CBP, chronic bacterial prostatitis; [b]$P < 0.05$, compared with healthy men.

Organism	Levels of SIPMP production (%) (Mean ± SEM)	
	Healthy men	Patients with CBP
Staphylococcus spp.	11.6 ± 2.2	27.5 ± 2.5[b]
Corynebacterium spp.	6.3 ± 0.7	9.3 ± 2.7
Micrococcus spp.	6.5 ± 3.7	14.5 ± 3.2
Streptococcus spp.	11.5 ± 0.4	13.7 ± 2.1
Enterobacteraceae	–	57.7 ± 1.5
E. faecalis	0	34.8 ± 2.9[b]

chronic infection of the prostate. Identifying such factors would be helpful in devising effective treatment strategies.

In the present work, we detected an extracellular bacterial product with remarkable anti-PMP potential. As PMP might play an important role in the killing of different bacterial pathogens and in preventing the onset of bacterial infection of the prostate, we speculated that SIPMP serves to protect invading bacteria by inducing local consumption of PMP in the fluid phase. The strategy underlying this process is straightforward and should be effective. We believe that SIPMP represents a widespread and hitherto unrecognized determinant of bacterial pathogenicity. Similarly, in a study of the distribution of streptococcal inhibitor of complement variants in pharyngitis and invasive isolates by Hoe *et al*. [14], 62% of group A *Streptococci* from patients with pharyngitis produced this extracellular protein. In addition, a recent study by Fernie-King *et al*. [15] shows that purified secretory inhibitor of complement could block two additional components of the immune system: lysozyme and secretory leukocyte proteinase inhibitor. Collectively, our study and the results of several studies [14–17] suggest that the inactivation of components of innate immunity might be important for bacterial pathogens to induce and perpetuate chronic infections of different localization by surviving or avoiding microbicidal protein-mediated clearance. If this hypothesis is correct, it suggests that organisms are capable of pronounced inhibition of PMP; further prolonged persistence in the urethra might alter the antimicrobial host defense of the genital tract in the presence of chronic infections, as demonstrated for persistent nonspecific urethritis [18]. Data from the present study might have significant implications for the understanding of the pathogenesis of CBP, as well as for future improvements in the prevention and therapy of CBP.

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