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·*Original Article*·

Endotoxins in the prostatic secretions of chronic prostatitis patients

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Abstract

Aim: To evaluate the clinical significance of the quantitative determinations of endotoxins in the expressed prostatic secretions (EPS) of chronic prostatitis (CP) patients. **Methods:** The EPS of 45 patients with CP and 15 normal volunteers were obtained for microscopic examination, bacterial culture and endotoxin determination. The level of endotoxins was determined by the Limulus-amebocyte-lysate test with chromogenic substrate. **Results:** Patients with CP had higher mean levels of endotoxins in EPS than normal volunteers [52.06 ± 32.83 EU·L^{-1} *vs*. 4.77 ± 4.14 EU·L^{-1} ($P < 0.05$)]. The levels of endotoxins in CP type II, type IIIa and type IIIb were 68.62 ± 34.78 EU·L^{-1}, 45.30 ± 23.33 EU·L^{-1} and 15.83 ± 5.31 EU·L^{-1}, respectively [type II *vs*. type IIIa ($P > 0.05$), type IIIb *vs*. normal controls ($P > 0.05$), type II/type IIIa *vs*. normal controls $P < 0.05$)]. **Conclusion:** CP patients have elevated levels of endotoxins in the EPS, which suggests that inflammation is a feature of this disease. EPS endotoxin determination is not only helpful in diagnostic confirmation, but also in evaluating the response to treatment in CP patients. *(Asian J Androl 2005 Mar; 7: 45–47)*

Keywords: endotoxin; chronic prostatitis; inflammation

1 Introduction

Chronic prostatitis (CP) is a disabling condition affecting 10 %–14 % of men of all ages and ethnic origins [1]. Endotoxins are lipopolysaccharides that form the outer cell membrane of gram-negative bacteria. They can cause a broad spectrum of disorders in many species by stimulating host macrophages to release inflammatory cytokines. Many microorganisms can release endotoxins when they grow and reproduce, such as gram-negative bacteria, gram-negative L-type bacteria and some strains of anaerobic bacteria.

The aim of this research was to determine the endotoxin level of expressed prostatic secretions (EPS) from CP patients to help elucidate the patients' status and the selection of appropriate antibiotics.

2 Materials and methods

2.1 Subjects

Forty-five CP patients who fulfilled the National Institutes of Health (NIH) diagnostic criteria for CP [2] were enrolled in the study. All patients had suffered from chronic pelvic pain (e.g. testicular pain, lower abdominal pain, urethral pain, perineal pain), urinary urgency and dysuria for more than 3 months. Patients underwent a standard evaluation, including routine microscopic examination of urine and expressed prostate secretion (EPS)

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with bacterial culture. Patients had not received any antimicrobial treatment before this evaluation. According to these criteria, CP patients were classified into three groups: 23 cases of type II (13 gram-negative and 10 gram-positive bacterial infections), type IIIa 11 cases and type IIIb 11 cases [2].

Fifteen normal male volunteers without any genitourinary symptoms were recruited as the controls. They were subjected to the same examinations as the CP patients. Table 1 lists the general characteristics of the subjects.

2.2 Examinations

Endotoxin determination was done with a chromogenic modification of the Limulus-amebocyte-lysate test [3], which is a two-step-micromethod using the following solutions:

Solution A: The lysate is solubilized in pyrogen-free water containing Ca^{2+} at a concentration of 2.0 mmol·L^{-1}, according to the recommendation of the manufacturer (Shanghai Medical Lab. Inc., Shanghai, China).

Solution B: The chromogenic substrate (*N*-acetyl-ile-glu-arg-p-nitro-anilin-hydrochloride) is solubilized in pyrogen-free water.

Solution C: The Tris/HCl buffer, 0.05 mol·L^{-1}, pH 9.0, containing 0.2 mol·L^{-1} NaCl.

Solution D: 20 % acetic acid.

EPS samples were diluted 1:10 with pyrogen-free water and heated for 10 min at 75 °C. Fifty μL of the sample were added to 50 μL of solution A and incubated for 33 min at 37 °C. After adding 100 μL of solution B diluted 1:2 with solution C, it was further incubated for 3 min at 37 °C. The chromogenic reaction was stopped by adding 200 μL of solution D. The extinction was read in a spectrophotometer at 405 nm. The endotoxin concentration of an unknown sample was determined according to a simultaneously established standard curve in a plasma pool from 15 healthy volunteers proven to be endotoxin-free.

2.3 Statistical analysis

Data were expressed in mean ± SD. Endotoxin levels of EPS were compared between the normal and the chronic prostatitis subjects, and also between different CP groups, using the Wilcoxon rank-sum (Mann–Whitney) test. $P < 0.05$ was considered excluding the null hypothesis. Commercial software SPSS 11.0 was used for statistical analysis.

3 Results

CP patients had significantly higher levels of endotoxins in their EPS than the controls, (52.06 ± 32.83 *vs*. 4.77 ± 4.14 EU·L^{-1}, respectively, $P < 0.05$).

There was no significant difference in the EPS endotoxin level between the type II (68.62 ± 34.78) and type IIIa patients (45.30 ± 23.33 EU·L^{-1}) ($P > 0.05$) and between the type IIIb (15.83 ± 5.31 EU·L^{-1}) and the normal controls (4.77 ± 4.14 EU·L^{-1}) ($P > 0.05$), but they are significantly higher in type II or type IIIa than those in the normal controls ($P < 0.05$). Table 2 summarizes the EPS endotoxin levels in different groups.

4 Discussion

Data derived from National Ambulatory Medical Care Surveys suggest that CP accounts for nearly 2 million visits to urologists and primary care physicians in the USA annually [4]. Infection is frequently believed to be the principal cause and antimicrobial treatment is widely used as the first-line treatment agents employed by most urologists and physicians [5, 6]. However, it was indi-

Table 1. Characteristics of study subjects.

	Type II	Type IIIa	Type IIIb	Controls
Age (years)	28	27	29	26
Number of leucocytes in EPS	>25 HP^{-1}	>25 HP^{-1}	Negative	Negative
Urine microscopy	Negative	Negative	Negative	Negative
EPS culture	Positive	Negative	Negative	Negative

Table 2. EPS endotoxin levels in different groups. [b]$P < 0.05$, compared with control.

	CP in general	Type II	Type IIIa	Type IIIb	Controls
Endotoxin (EU·L^{-1})	52.06 ± 32.83	68.62 ± 34.78[b]	45.30 ± 23.33[b]	15.83 ± 5.31	4.77 ± 4.14

cated that only 10 %–20 % of patients have been documented to have a bacterial infection [7]. Most men do not know the cause of their disease. Some scholars think there is a close relationship between CP and some atypical microorganisms, such as gram-negative L-type bacteria and anaerobic bacteria [8], not easily discovered by routine methods. Nickel *et al.* [9] found that antibiotics improved the symptoms in 30 % of men with II/IIIa prostatitis, but the improvement occurred regardless of the presence of bacterial organisms in their EPS. The differentiation between inflammatory and non-inflammatory CP is crucial in order to evaluate an evidence-based use of antibiotic therapy.

In this study it was shown that CP patients have elevated levels of the proinflammatory factor endotoxin in their EPS ($P < 0.05$). It appears to indicate a biologically relevant inflammatory process in the prostate of CP patients, as would be expected in an ongoing inflammatory response. This proinflammatory factor in CP patients may be able to stratify them into useful subgroups.

The present findings represent a measureable EPS parameter that can be readily assessed clinically. In bacterial infection, including infection with the above mentioned atypical bacteria, endotoxins are released and their level is increased in the EPS. The method is a faster, cheaper and more easily attainable approach than culture and some other special examinations.

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