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Eradication of *Enterococcus faecalis* by Phage Therapy in Chronic Bacterial Prostatitis – *case report*

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ABSTRACT. The treatment of three patients suffering from chronic bacterial prostatitis who were qualified for an experimental phage therapy protocol managed at the *Phage Therapy Unit* in Wrocław is described. They had previously been treated unsuccessfully with long-term targeted antibiotics, autovaccines, and laser biostimulation. Rectal application of phage lysates targeted against *Enterococcus faecalis* cultured from the prostatic fluid gave encouraging results regarding bacterial eradication, abatement of clinical symptoms of prostatitis, and lack of early disease recurrence.

Abbreviations

CBP	chronic bacterial prostatitis	PFU	plaque-forming unit(s)
DRE	digital rectal examination	PSA	prostate-specific antigen
IIET	*Institute of Immunology and Experimental Therapy*	PT	phage therapy
NIH-CPSI	*National Institutes of Health Chronic Prostatitis Symptom Index*	Q_{max}	maximum urinary flow rate
		TRUS	transrectal ultrasound (method)

Enterococci are Gram-positive facultative anaerobic bacteria which are found in soil, water, food, and human and animal bodies (Nigutová *et al.* 2008; Drahovská *et al.* 2004; Dworniczek *et al.* 2003). They are regarded as commensals of the gastrointestinal tract but can also be the cause of dangerous infections, such as bacteremia, endocarditis, and intra-abdominal, wound, and urinary tract infections (Drahovská *et al.* 2004; Dworniczek *et al.* 2003). The most prevalent is *E. faecalis*, causing 80–90 % of such infections (Drahovská *et al.* 2004). Gram-positive and anaerobic bacteria are generally discussed as possible causes of CBP, which is most commonly evoked by *E. coli* but also by *Klebsiella* spp., *Proteus* spp., or *P. aeruginosa* (Wagenlehner *et al*. 2008; Benway and Moon 2008). Recently, however, prostate infection with *E. faecalis* has been more often considered to be a result of clinical challenge (Pronk *et al*. 2006; van Nieuwkoop *et al*. 2008). Despite the general progress in the diagnostics and pharmacotherapy of prostatitis (*e.g.*, the introduction of chinolones) the results of treatment of its chronic bacterial form are not satisfactory because of the low penetration of active registered antibiotics and chemotherapeutics into the prostate gland. In advanced cases, CBP can be the cause of infertility, epididymitis, orchitis, cystitis, nephritis, endocarditis, arthritis, septicemia, as well as serious psychosomatic disorders. Thus it is often regarded as a public health problem (Schaeffer 2003).

PT uses bacteriophages, which are bacterial viruses that selectively infect and kill bacterial cells, even those that have acquired resistance to antibiotics, such as vancomycin-resistant *E. faecium* (Biswas *et al.* 2002). The method has been known since the beginning of the 20th century; however, it subsequently gave way to antibiotics. The increasing drama of antibiotic resistance, recently referred to as a "clinical super-challenge", has revived interest in PT (Górski *et al.* 20079; Arias *et al*. 2009). The structure and genetics of bacteriophages, including enterobacteria phages, are now being intensively studied (Kurzępa *et al.* 2009; Nigutová *et al.* 2008). Recently published experimental findings reveal a large potential of phages in the treatment of urinary tract infections (Freitag *et al*. 2009; Nishikawa *et al*. 2009). A few phage preparations were registered recently for use in food processing and agriculture (Lang 2006; *OmniLytics* 2009). However, due to the lack of clinical trials, phage therapy is not yet considered as a standard treatment (Górski *et al.* 2007).

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Here we present the results of the treatment of three patients suffering from CBP caused by *E. faecalis* successfully cured with bacteriophage preparations.

METHODS

Subjects. The patients, sent from the *Urological and Andrological Clinic UROGEN*, were qualified for the experimental protocol "Experimental phage therapy of drug-resistant bacterial infections, including MRSA infections" (approved by an independent bioethics commission in accordance with the Helsinki Declaration of 1975 as revised in 1983) managed at the *Phage Therapy Unit of the Institute of Immunology and Experimental Therapy*. All the men were heterosexual and had physical sexual relations. In each case a microbiological examination of the prostate fluid was done to exclude infection with anaerobic bacteria, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and fungi and a urethral swab was taken to exclude *Chlamydia trachomatis* infection. They had not taken any chemotherapeutics or antibiotics for at least one month and had not ejaculated for at least 2 d before the investigation. The size of the prostate was defined by pelvic or TRUS and Q_{max} by uroflowmetry. Bone marrow, renal, pancreatic, hepatic, as well as immunological system functions were also monitored during PT. Each patient gave his written consent before beginning the treatment.

Phage preparations. PT was based on lytic phages from the IIET bacteriophage collection. All enterococcal phages were isolated from 134 samples of city sewage, environmental, or drinking water samples from different sources. Phage suspensions (bacterial lysates) active against *E. faecalis* were prepared at the *Bacteriophage Laboratory of IIET* according to the modified method of Ślopek *et al.* (1983). Briefly, phages and the appropriate live bacteria were added to LB medium and incubated at 37 °C until complete lysis occurred (≈4–6 h). Then the suspension was filtered through a 0.22-μm *Millipore* filter. The phage titer in the preparation was between 10^7 to 10^9 PFU/mL (Table I).

To examine phage-lytic activity, the bacterial strains isolated from the patients were inoculated in broth and after 4 h of incubation (37 °C), 3 mL of the suspension was smeared on dried Petri dishes with Wahl medium (Ślopek *et al.* 1983). One drop of each bacteriophage preparation was applied on the plate (with a maximum of six different suspensions on one plate). The plates were incubated at 37 °C for 4–5 h and, then, transferred to a refrigerator. The results were read on the following day. Phages were regarded as active when they caused complete lysis of the tested strain. For each patient, only specific phage preparations that were active against the *E. faecalis* strain isolated from the patient were used for the therapy.

RESULTS

The results of the phage therapy are summarized in Table I.

The *first patient* (case *1*), a 32-year-old man, suffered from phimosis and pollakiuria and complained of chronic pain in the lower abdomen and crotch. He underwent preputioplasty. Because his main symptoms remained after the surgery, he underwent further diagnostic investigation. Microbiological testing of the prostatic fluid showed the presence of *E. faecalis*. On DRE the prostate was enlarged, symmetrical, and mushy. Its volume was 50 mL and Q_{max} was 18 mL/s. The total PSA level in the serum was 0.78 ng/mL. The diagnosis of CBP was made. Because of the failure of earlier antibiotic therapies, the patient was administered a three-month course of oral ciprofloxacin (500 mg twice daily) supported by an oral autovaccine against *E. faecalis* (1 capsule daily) prepared by the *Center of Microbiological Research and Autovaccines in Cracow* (Poland) and transrectal laser biostimulation (twice a week). Nevertheless, after this treatment the prostatic fluid cultures still showed *E. faecalis*. On DRE, the prostate was still enlarged, symmetrical, and mushy, its volume being 42 mL and Q_{max} 20 mL/s. On the NIH-CPSI, which is a standard evaluation tool that assesses pain, urinary symptoms, and impact on quality of life, based on which the severity of CBP can be classified as mild (0–14 points), moderate (15–29 points), or severe (30–43 points), his symptoms score was 23 (Litwin *et al.* 1999). The patient was qualified for PT. He applied 10 mL of the phage preparation against *E. faecalis* rectally (twice daily) for 28 d. After PT his prostate gland was normal, rubbery, smooth, and symmetrical on rectal examination. Its volume decreased to 28 mL and Q_{max} increased to 26 mL/s; NIH-CPSI decreased to 3 points. Control microbiological cultures of the prostatic fluid taken one and eight weeks after PT were negative.

The *second patient* (case *2*), a 41-year-old man, had been admitted to the Clinic because of infertility. During diagnostic testing, CBP was suspected. Culture of the prostatic fluid yielded *E. faecalis*. On DRE his prostate was enlarged, symmetrical, mushy, and painful. Its volume was 37 mL and Q_{max} was 12 mL/s.

Total PSA level in the serum was 0.76 ng/mL. Because of the resistance of *E. faecalis* to fluorochinolones and the patient's refusal to take nitrofurans due to possible adverse effects on spermatogenesis, he underwent treatment only with oral autovaccine against *E. faecalis* (1 capsule daily) and transrectal laser biostimulation (twice a week) for three months (Schlegel *et al.* 1991). Bacterial cultures of prostatic fluid done three months after this therapy again showed *E. faecalis*. In TRUS its volume was 35 mL and its image was nonhomogeneous. Q_{max} was 13 mL/s. The patient was qualified for PT. He applied 10 mL of the phage preparation against *E. faecalis* rectally (two times daily) for 33 d. After PT his prostate gland was normal, rubbery, smooth, and symmetrical on rectal examination. Its volume decreased to 15.3 mL, the TRUS image was homogeneous, and Q_{max} increased to 22 mL/s. Control microbiological cultures of prostatic fluid taken 8 and 25 weeks after PT were negative.

Table I. Clinical results of the phage therapy (PT)

Clinical data		Case *1*	Case *2*	Case *3*
Patient's age		32 years	41 years	45 years
Application of the phage preparation against *Enterococcus faecalis*		rectal (10 mL twice daily)	rectal (10 mL twice daily)	rectal (10 mL twice daily)
Phage titer in preparation		2.8×10^8 PFU/mL	7.5×10^7 PFU/mL	4.5×10^7 PFU/mL
Duration of the PT		28 d	33 d	30 d
Microbiological culture of the prostatic fluid	before PT	*Enterococcus faecalis*	*Enterococcus faecalis*	*Enterococcus faecalis*
	after PT	negative after 1 and 8 weeks	negative after 8 and 25 weeks	negative after 3 and 10 weeks
Prostate volume defined by ultrasound (normal = 20–25 mL)	before PT	42 mL	35 mL	40 mL
	after PT	28 mL	15.3 mL	22 mL
Prostate gland on digital rectal examination (DRE)	before PT	enlarged, symmetrical, mushy	enlarged, symmetrical, mushy, painful	enlarged, symmetrical, mushy
	after PT	normal, symmetrical, smooth, rubbery	normal, symmetrical, smooth, rubbery	normal, symmetrical, smooth, rubbery
Maximum urinary flow rate ($Q_{max(norm)} = \approx 25$ mL/s)	before PT	20 mL/s	13 mL/s	16 mL/s
	after PT	26 mL/s	22 mL/s	23 mL/s

The *third patient* (case *3*), a 45-year old man, had been admitted one year previously because of pollakiuria and complaints of chronic pain in the lower abdomen. *E. faecalis* was cultured from his prostatic fluid. On DRE the prostate was enlarged, symmetrical, and mushy. Its volume was 45 mL and Q_{max} was 16 mL/s. Serum PSA concentration was 0.751 ng/mL. CBP was diagnosed. The patient was administered a three-month course of oral amoxicillin and clavulanic acid (1000 mg twice daily) and nitrofurantoin (100 mg three times daily) supported by an oral autovaccine against *E. faecalis* (1 capsule daily) and transrectal laser biostimulation (twice a week). Bacterial cultures of the prostatic fluid done 6 weeks after this therapy still showed *E. faecalis*. In TRUS the prostate volume was 40 mL and its image was nonhomogeneous, Q_{max} was 16 mL/s, and NIH-CPSI was 20 points. Complaints of pollakiuria and chronic pain in the lower abdomen remained. He was qualified for PT and applied 10 mL of the phage preparation against *E. faecalis* rectally (twice daily) for 30 d. After PT his prostate gland was normal, rubbery, smooth, and symmetrical on rectal examination. Its volume decreased to 22 mL, the TRUS image was homogeneous, and Q_{max} increased to 23 mL/s. Control microbiological cultures of prostatic fluid taken 3 and 10 weeks after PT were negative. NIH-CPSI decreased to 3 points and all complaints receded after treatment.

DISCUSSION

The application of phage preparations produced encouraging results in all the patients regarding bacterial eradication, abatement of symptoms, and lack of early disease recurrence. The improvement in

NIH-CPSI (presented only for the first and third cases because the second patient did not complain of urinary symptoms, although his complaints were typical during clinical examination), reductions in prostate size and pain, and the significant increases in Q_{max} after PT should also be emphasized (Table I). It is essential that changes in the function of liver, pancreas, kidney, and bone marrow were not observed in the patients during PT. All patients had depressed immune function prior to the phage therapy (diminished T- and B-lymphocyte proliferative responses to *in vitro* activation with OKT3, PHA, and *S. aureus* Cowan) and these responses did not change significantly in response to the therapy.

Our first patients were originally treated orally with phages according to the Ślopek *et al*. (1983) but we recently switched to rectal application. It was shown that the hemorrhoidal venous plexus, extending along the whole rectum, connects *via* the hemorrhoidogenital veins with the prostatic venous plexus, the veins of which are unidirectional (Shafik and Mohi-El-Din 1985). This feature may play a role in genitourinary pathology and may also enable drugs to reach the prostate. That is why the rectal approach may be attractive in the treatment of prostatitis. There are a few reports on the application of submucosal anal injection in the antibiotic treatment of CBP (Hu *et al*. 2002). Moreover, bacteriophages can be detected in the circulation of rabbits and mice only a few minutes after rectal introduction and the blood phage level may be about two orders of magnitude higher than with oral feeding (Hoffmann 1965).

Our *in vitro* investigations suggest that phages can inhibit the formation of free oxygen radicals by endotoxin-stimulated neutrophils (Międzybrodzki *et al.* 2008). Such activity, aside from the antibacterial effect, may also play a role in the reduction of oxidative stress which accompanies the chronic inflammatory process in the prostate gland (Zhou *et al*. 2006). Interestingly, we have also observed a significant decrease in C-reactive protein level in patients treated with phage preparations, suggesting their anti-inflammatory properties (Międzybrodzki *et al*. 2009). It cannot be excluded that, although completed at least 4 months before PT, some of the elements of the previously applied therapeutic methods, such as the administration of autovaccines or laser biostimulation, could have influenced the success of PT. However, compared with the normal values, the decreased reactivity of the patients' immunological systems observed before beginning PT would rather not confirm these suggestions.

The possibility of eradicating bacterial pathogens thanks to PT could give hope to men with CBP in whom antibiotic therapy failed or is contradicted. One should of course be aware that the general use of phages will be possible when their efficiency and safety have been verified in large-scale controlled clinical trials.

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