MINIREVIEW

The perspectives of the application of phage therapy in chronic bacterial prostatitis

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Abstract

Chronic bacterial prostatitis (CBP) is a long-lasting and crippling disease that strongly impacts the patient's quality of life. The diagnosis of CBP is difficult and the treatment regimens are not always successful. Poor penetration of antibiotics to the prostate tissue, the drug resistance of uropathogens, the adverse events associated with antibiotic treatment, the persistence of prostatic calculi, and biofilm formation in the prostate gland are factors that contribute toward decreasing the cure rate of CBP. The phenomenon of increasing antibiotic resistance, which has also been called a clinical super-challenge, has revived interest in therapy using bacterial viruses (bacteriophages or phages). Because of their mechanism of action, which is completely different from those of all antibiotics, phages are effective even against multidrug-resistant bacteria. Here, we describe the current perspectives on the possible application of phage therapy (PT) in treating CBP. The advantages of therapeutic phages, including their interactions with bacterial biofilm, as well as the safety of PT are discussed.

Introduction

Prostatitis is a serious clinical and social problem. It is the most frequently diagnosed illness in men under 50; one of every two men develops it at some time during his life (Naber, 2008). Patients with prostatitis are reported to make approximately 8 000 000 outpatient visits annually worldwide (Wiygul, 2005). They make 2 000 000 such visits annually in the United States (Schaeffer *et al.*, 2002). Thus, the healthcare system is burdened with the large costs of treating prostatitic patients (Benway & Moon, 2008; Clemens *et al.*, 2009).

Prostatitis is the common term for acute and chronic bacterial prostatitis (CBP), chronic pelvis pain syndrome, and asymptomatic inflammation of the prostate (Krieger *et al.*, 2008). Prostatitis syndromes are classified according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)/National Institutes of Health (NIH) classification (Krieger *et al.*, 1999). It distinguishes four types of prostatitis: acute bacterial prostatitis (ABP, NIH type I), CBP (NIH type II), chronic prostatitis or chronic pelvic pain syndrome, which includes the two subtypes, inflammatory (NIH type IIIa) and noninflammatory (NIH type IIIb), and asymptomatic inflammatory prostatitis (NIH type IV) (Nickel, 2000; Krieger *et al.*, 2008). CBP is present in 13% of cases of prostatitis (Shoskes *et al.*, 2003; Wiygul, 2005).

Chronic prostatitis (CP), which is defined as lasting longer than 3 months, is a crippling and difficult-to-treat disease (Benway & Moon, 2008; Wagenlehner *et al.*, 2008b; Clemens *et al.*, 2009). The frequency of occurrence of prostatitis symptoms is high and is similar to those of ischemic heart disease and diabetes. It was shown that prostatitis influences the quality of patients' lives similar to Crohn's disease, myocardial infarction, and angina (Naber *et al.*, 2000). It may manifest as various ailments and

disorders. The main symptoms of its bacterial form include pain in the area of the lower urinary tract connected to disturbances in ejaculation (its decrease or lack), hematospermia, disturbances in penile erection, disorders of the libido, and the emotional distress (Litwin *et al.*, 1999; Ghobish, 2002). In advanced cases, it can be a cause of sterility, epididymitis, orchitis, cystitis, nephritis, endocarditis, arthritis, septicemia, sexual dysfunction, and serious psychosomatic disorders as well. Prostatitis increases the risk of developing benign prostate hypertrophy and prostate cancer (Krieger *et al.*, 2008). The diagnosis of CBP is difficult and the treatment regimens are not always successful (Thin, 1991). Relapse and reinfection remain a major problem; therefore, novel forms of treatment are urgently needed (Wagenlehner *et al.*, 2008b).

The phenomenon of increasing antibiotic resistance, which has also been called a clinical super-challenge, has revived interest in phage therapy (PT) (Arias & Murray, 2009). Recently, a number of reviews have been published that address this issue in detail (Górski *et al.*, 2009). Here, we describe the current perspectives of the possible application of PT in treating CBP.

Etiology, pathogenesis, and treatment of CBP

The prostate is a tube–follicle gland whose ductules go to the semen cumulus, the rear wall of the urethra next to the semen cumulus, and also to the front wall of the urethra. In the infective forms of prostatitis, the pathogens most often penetrate by the ascending pathway. The common tracts of urine and semen facilitate the spread of the infection with urine from the posterior urethra to the prostate (Persson *et al.*, 1996; Domingue & Hellstrom, 1998). Prostate infection via the urethra is facilitated by phimosis, spasm of the external urinary sphincter, or urethral stricture, which raise the pressure in the posterior urethra and cause a swirl of the urine flow. The pathogenic microorganisms are transferred with the urine upwards from the posterior urethra through reflux to the prostate canaliculi (Moon, 2004; Nickel, 2005; Benway & Moon, 2008). The increased content of urates in the prostate may induce the formation of prostatic calculi, whose presence facilitates bacterial infection (Persson *et al.*, 1996). Disorders in the secretion of such antibacterial factors such as zinc-containing prostatic antibacterial factor, spermine, spermidine, and lysozyme may also play roles in the etiopathogenesis of CBP (Fair & Wehner, 1971).

CBP may be the outcome of an acute infection. It may appear during gonorrhea or purulent or nonspecific infections of the urethra (Domingue & Hellstrom, 1998). Most commonly, CBP is caused by bacteria that enter the prostate through the urethra, blood, the wall of the large intestine, or through urinary reflux to the prostate tissue. It is mostly generated by Gram-negative bacilli of the *Enterobacteriaceae* family, among which *Escherichia coli* predominates, as well as other bacteria such as *Pseudomonas aeruginosa* (Cox & Childs, 1991; Krieger *et al.*, 2003; Benway & Moon, 2008). Recent reports suggest a critical role of Gram-positive pathogens in CBP's etiology. The most common are *Enterococcus faecalis* and *Staphylococcus aureus* (Nickel & Costerton, 1992; Bergman, 1994; Bundrick *et al.*, 2003; Pronk *et al.*, 2006; Benway & Moon, 2008). The role of coagulase-negative staphylococci and *Corynebacterium* sp., so far recognized as nonpathogenic, is also discussed (Nickel & Costerton, 1992; Bergman, 1994; Riegel *et al.*, 1995; Nickel, 2000; Bundrick *et al.*, 2003; Pronk *et al.*, 2006; Benway & Moon, 2008). Atypical bacteria such as *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealiticum* were also isolated from the genitourinary tracts of CBP patients (Shurbaji *et al.*, 1988; Ohkawa *et al.*, 1993; Potts *et al.*, 2000; Krieger & Riley, 2002; Benway & Moon, 2008). Anaerobic bacteria can also cause prostatitis (Nielsen & Justesen, 1974; Golz & Mendling, 1991; Campbell *et al.*, 1992; Indudhara *et al.*, 1992; Nickel, 2000).

The main drugs used for the treatment of CBP are flouroquinolones, macrolides, and sulfonamides. Because of suitable pharmacokinetic characteristics and a wide antibacterial spectrum (which includes Gram-positive and Gram-negative bacteria as well as *Chlamydia* and *Mycoplasma*), flouroquinolones are the drugs of choice for prostatic patients (Wagenlehner *et al.*, 2008b). According to some reports, the cure rate after fluoroquinolone application ranges from 63% to 86% (Wagenlehner *et al.*, 2008b). The best results of the therapy are achieved in patients treated for a long term (4–6 weeks). Some authors recommend even a 3–6-month course of antibiotic treatment or prophylaxis (Wagenlehner *et al.*, 2009). Doxycycline and macrolides are the second-line drugs (Duclos *et al.*, 2007). Azithromycin and clarithromycin penetrate well into the prostate tissue, and they were effectively applied in CBP (Giannopoulos *et al.*, 2001; Škerk *et al.*, 2002, 2003, 2004). However, randomized clinical trials were not completed to confirm these clinical observations (Duclos *et al.*, 2007). Other drugs have not been proven to be highly effective, but in some cases, they may show some effects (Duclos *et al.*, 2007). Penicillins, which are used in the therapy of ABP, are not recommended for use in CBP (Benway & Moon, 2008). The first reports about linezolide application in CBP in combination with co-trimoxazole in the treatment of the *E. faecalis* infection were positive (Pronk *et al.*, 2006).

α-Blockers were recommended as adjuvant therapy to reduce the recurrence of CBP due to their properties of diminishing urinary bladder outlet obstruction, direct effect on pain, and reducing benign prostatic hyperplasia (Barbalias *et al.*, 1998; Nickel, 2006). Some clinical studies suggested that the application of terazosin or alfuzosin may

decrease the risk of recurrence of the clinical symptoms and infection; however, the use of α-blockers in the treatment of CP was recently questioned (Barbalias *et al*., 1998; Wagenlehner & Weidner, 2009).

Before the wide use of antimicrobials, prostatic massage was a strongly recommended treatment for patients with prostatitis (O'Conor, 1936; Duclos *et al*., 2007). It allows the drainage of blocked ducts and the penetration of antimicrobials into the gland or the disruption of bacterial biofilm (Hennenfent & Feliciano, 1998; Duclos *et al*., 2007). Some studies showed that frequent ejaculation as well as prostate massage connected with antibiotic treatment led to clinical benefits (Shoskes & Zeitlin, 1999; Yavascaoglu *et al*., 1999; Nickel *et al*., 1999). Surgical procedures such as transurethral resection of the prostate and radical prostatectomy are considered the last resort (Wagenlehner & Weidner, 2009).

Causes of treatment failure

The most frequently reported problem that causes failure in the treatment of CBP is poor penetration of antibiotics into the prostate tissue. This is caused by the lack of fenestrations in the capillary beds in the gland and is complicated by an ion-trapping mechanism within the prostate parenchyma (Cunha, 1983; Charalabopoulos *et al*., 2003). As there is no active transport of the drugs within the gland, the antibiotic spectrum narrows to those that possess a strong property of passive penetration into the prostate (Stamey *et al*., 1970; Benway & Moon, 2008; Wagenlehner *et al*., 2008b). The degree of protein binding is also important because high plasma protein binding lowers the penetration of the drug into prostatic fluid (Charalabopoulos *et al*., 2003). The prostatic tissue is best penetrated by lipophilic drugs. Those antibiotics that have a high pKa reach a greater concentration in prostatic fluid because in chronic prostatitis the pH of human prostatic fluid increases from 6.5–6.7 to 7.0–8.3. From the group, whose isoelectric point lies in this range – the macrolides, the sulfonamides, and the fluoroquinolones – the latter have the best pharmacokinetic profile (high volume of distribution, long biological half-life, low serum protein binding) (Duclos *et al*., 2007; Wagenlehner *et al*., 2008b). Although new generations of fluoroquinolones (such as moxifloxacin) have much better prostate/plasma concentration ratios than older ones such as ciprofloxacin, there are still patients who do not respond to therapy (Wagenlehner *et al*., 2008b). To enhance the activity of antibiotics in the prostate, intraprostatic injections of antiobiotics were tested with some success by some authors (Yamamoto *et al*., 1996; Mayersak, 1998; Hu *et al*., 2002). These procedures are rather empirical and are not a standard of care (Wagenlehner *et al*., 2005). Interestingly, Shafik & Mohi-El-Din (1985) showed that the hemorrhoidal venous plexus, extending along the entire rectum, connects the hemorrhoidogenital veins with the prostatic venous plexus. This connection may play a role in genitourinary pathology and may also enable the drug to reach the prostate. There are a few reports on the application of transrectal infiltration of the prostate in the antibiotic treatment of CBP (Shafik, 1991; Yamamoto *et al*., 1996).

The increasing antibiotic resistance of uropathogens represents a rising dilemma (Wagenlehner *et al*., 2008a). It was shown that the resistance of *E. coli* isolates to norfloxacin was correlated with the prescription of quinolones due to a urinary tract infection (UTI) (Goettsch *et al*., 2000). Prior use of amoxicillin or trimetoprim (particularly given in lower doses or for longer than 6 days) increased the risk of ampicillin- and trimetoprim-resistant *E. coli* community-acquired UTIs (Hillier *et al*., 2007). Antibiotic misuse as well as their appropriate use may induce antibiotic resistance through a variety of mechanisms even against new antibiotics (Barbosa & Levy, 2000; Tenover, 2006; Arias & Murray, 2009). There is special concern about *Enterobacteriaceae*, in which the rate of extended-spectrum β-lactamase (ESBL) producers has increased to hazardous levels (Livermore, 2009). Fluoroquinolone-resistant organisms, which acquire resistance mainly by *gyrAB*/*parCE* mutations altering the fluoroquinolone binding to their targets – DNA gyrase and topoisomerase IV, respectively, and also overexpression of efflux pumps, are also a potential threat (Hooper, 2000; Hawkey & Jones, 2009; Livermore, 2009). The increased prevalence of Gram-positive pathogens in CBP patients also lowers the cure rate because of their low susceptibility to fluoroquinolones (Naber, 2008; Wagenlehner *et al*., 2008a). This then causes a lack of convenient (oral) options for the long-term therapy of CBP.

Adverse events associated with antibiotic use should also not be ignored (Meropol *et al*., 2008). In Bundrick's *et al*. (2003) study, a 28-day treatment with levofloxacin 500 mg once daily or ciprofloxacin 500 mg twice daily resulted in at least one treatment-emergent adverse event in 44.2% and 37.2% of cases, respectively. The most frequently reported side effects were those related to the gastrointestinal tract. It was shown that ciprofloxacin treatment may predispose to nosocomial *Clostridium difficile*-associated diarrhea (CAD) (Yip *et al*., 2001). An increased rate of CAD was also observed for gatifloxacin, a sixth-generation fluoroquinolone. The drug was withdrawn from sale in 2006 due to its association with severe hypoglycemia and hyperglycemia (Food & Drug Administration, 2008; Walbrown *et al*., 2008). Other side effects characteristic for fluoroquinolones include central nervous system symptoms (dizziness, headache, somnolence), and less common ones such as tendonitis and tendon rupture, QT interval prolongation, and alteration in glucose metabolism (Owens & Ambrose, 2005).

The persistence of prostatic calculi and biofilm formation in the prostate gland are other factors that contribute toward

a decrease in the cure rate of CBP (Wagenlehner *et al.*, 2008b).

Bacteriophages for PT

Bacteriophages (phages) are bacterial viruses and the most abundant life form on earth; they are estimated to be 10 times more numerous than bacteria (Ashelford *et al.*, 2000; Hendrix, 2002; Hanlon, 2007). They occupy all those habitats of the world where bacteria grow and can be found even in desert sand, ocean depths, and hot springs (Wichels *et al.*, 1998; Breitbart *et al.*, 2004; Prigent *et al.*, 2005; Säwström *et al.*, 2008). They are isolated from soil, water, and human or animal bodies (Dąbrowska *et al.*, 2005; Górski *et al.*, 2007). An efficient source of phages (including those used in PT) is sewage. Some therapeutic phages were also isolated from patients.

Phage virons have a different size and morphology: they are tailed (95% of all the phages), polyhedral, filamentous, or pleomorphic (Hendrix, 2002; Ackermann, 2003). Most phages contain double-stranded (ds)DNA, but there are groups with single-stranded (ss)DNA, ssRNA, or dsRNA, and their genetic diversity is remarkable (Hatfull, 2008). Research on phage genomics resulted in the most significant discoveries in biological sciences such as the identification of DNA as the genetic material (McAuliffe *et al.*, 2007). It has also provided a new strategy by revealing new molecular targets and peptides yielding novel antimicrobial drugs (Liu *et al.*, 2004).

Phages may choose a lytic or a lysogenic cycle to replicate in the host bacteria (Sandeep, 2006; Skurnik & Strauch, 2006; Hanlon, 2007). In the first case, they multiply within bacteria and release infective progeny after lysing the infected cell at the end of the cycle. Upon infection, the lysogenic phages may integrate their nucleic acid into the host's genomic DNA as prophage, which is then replicated as part of the bacterium's genome. Occasionally, it can be induced to initiate a phage lytic cycle, resulting in bacterial lysis and progeny release. The phage ability to kill the bacterial cells forms the basis of the idea of using phages as therapeutic agents. Although first applied for the treatment of infection almost 100 years ago, they were overlooked by the Western world after the discovery of antibiotics. However, PT and prophylaxis was continued extensively in Georgia and Poland (Ślopek *et al.*, 1983; Weber-Dąbrowska *et al.*, 2000; Sulakvelidze *et al.*, 2001; Sulakvelidze & Kutter, 2005; Fortuna *et al.*, 2008; Chanishvili, 2009; Górski *et al.*, 2009; Kutter *et al.*, 2009). Currently, a problem of the dry antibiotic pipeline (a very low rate of the development of new antibiotics) results in renaissance of interest in PT (Morel & Mossialos, 2010).

Because phages' mechanism of action is completely different from those of all antibiotics, they are even effective against multidrug-resistant bacteria (Hanlon, 2007). They can also be administered in patients in whom antibiotic use is contradicted. As the antibacterial spectrum of phages is much narrower than that of antibiotics, they can target pathogens without significantly affecting normal bacterial flora (Chibani-Chennoufi *et al.*, 2004; Kutter, 2005). For therapeutic purposes (e.g. the treatment of local nosocomial infections), new phages can be isolated from the environment or, in some cases, by selective passage of a set of phages presenting weak lytic activity in the target pathogenic bacteria. There are also methods that enable obtaining active phages by genetic modification (Kropinski, 2006; Górski *et al.*, 2009). Similar to antibiotics, bacteria may become resistant to phage, but in contrast, there are also mechanisms enabling the phage to adapt to overcome host limitations (Hyman & Abedon, 2010; Labrie *et al.*, 2010). Interestingly, the appearance of bacterial mutants resistant to a phage may be related to the loss of bacterial virulence (Levin & Bull, 2004). The density-dependent thresholds of phage growth are increasingly being discussed as being important for effective PT. Different models of phage growth should help to improve the treatment protocols according to acute or chronic infection, the simultaneous application of antibiotics, and local or general phage treatment (Weld *et al.*, 2004; Abedon, 2009; Cairns *et al.*, 2009; Gregory *et al.*, 2010). There are also some other limitations on PT that result from antiphage antibody formation and the stability of phage formulations (Górski *et al.*, 2007; Skurnik *et al.*, 2007). Interestingly, a study on bacteriophage interactions with mammalian cells demonstrates that they can also exert immunomodulatory and even antitumor activity directly (Górski & Weber-Dąbrowska, 2005; Kurzępa *et al.*, 2009; Budynek *et al.*, 2010). Some of these effects could be involved in the beneficial effect of PT.

Bacteriophages can penetrate our body. After an intravenous administration, they rapidly localize in the liver, spleen, lungs, kidney, and urine (Bogovazova *et al.*, 1991; Verma *et al.*, 2009), as is the case after oral administration (Reynaud *et al.*, 1992). However, it is important to protect them against gastric acid because therapeutic phages are rather sensitive to low pH (Smith *et al.*, 1987). There are data suggesting that phages can appear in the urine following their administration using different routes. Keller & Zatzman (1959) showed that dog kidney can concentrate phages following their intravenous or intra-arterial administration. More detailed studies were performed by Schultz & Neva (1965). According to their data, in mice and rats, phages appear in the urine when their serum concentration exceeds 10^5 PFU mL^{-1}. Moreover, phage administration did not cause any changes in urinalysis and no histological changes in the urinary tract were noted. Importantly, urine did not affect phage activity. Russell *et al.* (1976) confirmed these data in sharks, demonstrating that phages could be detected in their kidneys even 1 month after administration.

Data in humans are scarce, but they also confirm that both 'endogenous' and therapeutic phages may be present in patients' urine. Already in 1928, Caldwell (1928) showed phage presence in the urine in patients with UTI. Caroli *et al.* (1980) detected phages in routine urinalyses at a high concentration of 10^6 PFU mL^{-1}. Furthermore, Weber-Dąbrowska *et al.* (1987) described their presence in the urine of nine of 26 patients orally treated with phages.

Rectal administration may also be an efficient route of phage delivery. It was shown in rabbits and mice that it only takes a few minutes for phages to penetrate from the rectum through the intestinal wall into the circulation (Hoffmann, 1965; Sechter *et al.*, 1989). The blood phage level may be about two orders of magnitude higher than that with oral feeding (Hoffmann 1965). This may result from the lack of phage inactivation by gastric juice.

Our experiments on phage penetration to prostate tissue confirmed that phages (*E. coli* T4 phage and one of our therapeutic enterococcal phages) can penetrate rat prostate tissue after their intravenous administration (Międzybrodzki *et al.*, 2008). Moreover, we could also isolate therapeutic phages from rodent urine and prostate after rectal application (R. Międzybrodzki, M. Kłak, B. Weber-Dąbrowska & A. Górski, unpublished data).

Bacterial biofilm, prostatitis, and phages

The majority of bacteria grow in biofilms, which is an agglomeration of microorganisms (forming microcolonies) and their extracellular polysaccharide matrix products (Sutherland *et al.*, 2004; Tenke *et al.*, 2004). The microcolonies embedded in the matrix material are intersected by branching water channels that carry huge amounts of fluid into the community (Costerton *et al.*, 2003). There are single-species and mixed-species biofilms developed from Gram-negative or Gram-positive bacteria (Sutherland *et al.*, 2004; Donlan, 2009). Biofilms are found in natural and artificial environments where a surface is exposed to adequate moisture. A biofilm is formed after the initial contact of planktonic bacteria with a foreign surface such as mucosa, dental plaque, medical devices, or water pipes (Arakawa *et al.*, 1999; Tenke *et al.*, 2004; Trautner & Darouiche, 2004). Microorganisms comprising the microbial communities of the human skin, oral cavity, genitourinary tract, and gastrointestinal tract can form biofilms as well (Donlan & Costerton, 2002).

There is evidence that biofilms may play a role in infectious diseases, but the processes by which biofilm-associated organisms elicit disease are not fully understood. The suggested mechanisms include detachment of cells or cell aggregates from the biofilm on a medical device, the production of endotoxins, resistance to the host immune system, and providing a proper environment for the generation of resistant organisms (Donlan & Costerton, 2002). Bacteria within a biofilm are phenotypically different from their planktonic counterparts. They activate many genes that change their surfaces and other molecular targets, increasing their resistance to antimicrobial agents and host defenses. They may survive the application of antibacterial agents at concentrations 1000–1500 times higher than those needed to eradicate planktonic bacteria of the same species (Tenke *et al.*, 2004). The formation of a biofilm allows bacteria to persist for a long time in the genitourinary tract and interfere with bacterial eradication. Biofilms may grow over months or even years before causing symptoms. Their formation may result in an increased ability of bacteria initiating acute prostatitis to persist in the prostatic secretory system, which leads to a chronic infection (Soto *et al.*, 2007).

The formation of a biofilm causes a problem in detecting chronic infection because cultures from patients who show many signs of bacterial infection may be negative sometimes (Costerton *et al.*, 2003). The pathogens exist both as biofilms and as planktonic cells. The planktonic cells should be killed by circulating antibiotics and activated phagocytes. Because the planktonic cells are killed and the biofilm cells are not released, no colonies develop on plates. Turbulent urethral flow or intraprostatic ductal reflux may be a cause of ascending infection from the urethra to the prostate. Thus, the planktonic bacteria can form a biofilm adherent to the epithelium of the prostate ductal system and produce protective envelopes (exopolysaccharide or glycocalyx), which leads to persistent immunological stimulation, chronic inflammation, and pain (Choong & Whitfield, 2000). The diagnosis of CBP can be difficult because antimicrobial therapy eliminates the planktonic bacteria, but not the adherent bacterial biofilm deep within the prostate gland, as was shown in patients with CBP confirmed by biopsy and culture of the prostate gland, but who had negative cultures from prostatic secretion (Choong & Whitfield, 2000). Biopsies collected from men with prostatitis helped in understanding the role of biofilm in CBP. Nickel & Costerton (1993) showed bacterial attachment to the ductal walls, especially for *P. aeruginosa*. Arakawa *et al.* (1999) observed, using electron microscopy, bacteria and their biofilm formation in prostatic tissues from patients with intractable chronic prostatitis.

Bacterial biofilm is difficult to eradicate because of its resistance to antimicrobial treatment and removal by the host immune system (Lu & Collins, 2007; Donlan, 2009). Of note, bacteriophages can produce polysaccharide depolymerases that degrade the biofilm's extracellular polysaccharide matrix (Hughes *et al.*, 1998; Donlan, 2009). Biofilm polysaccharide normally protects the bacteria against the majority of phages. However, if phages produce the specific polysaccharide depolymerase, they may be able to degrade the biofilm's extracellular polysaccharide matrix and gain access to the bacterial surfaces (Hughes *et al.*, 1998). This is

why bacteriophage treatment has been proposed as a method to control biofilm (Donlan, 2009). Numerous phages have been isolated that induce enzymes capable of degrading the extracellular polysaccharide matrix. Hughes *et al.* (1998) isolated a bacteriophage specific to *Enterobacter agglomerans* and demonstrated that its ability to control *Enterobacter* biofilm was due to the production of polysaccharide depolymerase. Doolittle *et al.* (1995) observed that the extracellular matrix of the *E. coli* biofilms did not protect the bacterial cells from infection with phage T4. Phage T4 can infect and replicate within *E. coli* biofilms and disrupt the morphology of the biofilm by killing bacterial cells. It is also possible to construct genetically engineered phages with greater efficacy against biofilm. Lu & Collins (2007) showed that their T7 phage expressing dispersin B, an enzyme that hydrolyzes β-1,6-*N*-acetyl-D-glucosamine (a bacterial integrin crucial for biofilm formation), reduced *E. coli* biofilm levels more efficiently than the nonenzymatic phage.

Bacteriophages for the treatment of antibiotic-resistant uropathogens

The therapeutic effectiveness of bacteriophages against multidrug-resistant bacteria of uropathogenic potential, such as ESBL-producing *E. coli*, imipenem-resistant or multidrug-resistant strains of *P. aeruginosa*, and vancomycin-resistant *Enterococcus faecium*, was presented in animal models of bacteremia (Biswas *et al.*, 2002; Wang *et al.*, 2006a, b; Vinodkumar *et al.*, 2008). In all those experiments, a single intraperitoneal dose of the specific phage administered 40–45 min after bacterial challenge could rescue 100% of mice. In a classic study by Smith & Huggins (1982), one dose of intramuscularly injected phage rescued more mice against a potentially lethal intramuscular inoculation of *E. coli* than multiple doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim plus sulfafurazole. Interestingly, a synergistic antibacterial effect was observed *in vitro* for T4-like phages (the majority of therapeutic phages belong to this family) and such antibiotics as β-lactams and quinolones (Comeau *et al.*, 2007). This effect was also confirmed *in vivo* for filamentous *P. aeruginosa* phages and low doses of gentamycin (Hagens *et al.*, 2006).

A few reports presented the potential benefit of phage applications in urology. Boratyńska *et al.* (1994) summarized the results of treatment with phage lysates of 15 patients (24–77 years old) with recurrent UTI (a few acute exacerbations per year) during the course of chronic pyelocystitis, nephrolithiasis, vesicoureteral reflux, or floating kidney and UTI after kidney transplantation (KT). UTI was caused by *E. coli* ($n = 9$), *P. aeruginosa* ($n = 2$), *Klebsiella pneumoniae* ($n = 2$), *Proteus vulgaris* ($n = 2$), *Enterobacter aerogenes* ($n = 2$), and *S. aureus* ($n = 2$), which were all resistant to the available chemotherapeutics. The patients received 10 mL of specific phage orally three to four times daily after neutralization of gastric juice. The patients who had KT ($n = 3$) were simultaneously treated with antibiotics. The treatment lasted 3–11 months (mean: 5.4 months). Long-lasting (12–36 months) abatement of symptoms of UTI and pathogen eradication were achieved in five patients (including one with KT). In three cases, remission was observed after 3–6 months. Phages were present in the urine of some patients. No changes were observed in blood morphology, serum proteins, electrolytes, or renal and liver function.

Researchers from a leading medical institute in Bucharest reported the results of phage treatment of a much larger group (87 patients) with UTI (Zilişteanu *et al.*, 1971). The phages, applied as the only antibacterial treatment in the case of infection with multidrug-resistant bacteria, exerted remarkable effects in acute UTI, resulting in rapid temperature decline and retreat of leukocyturia. A synergistic effect with simultaneous antibiotic treatment was observed. Similar results were obtained by Russian physicians (Perepanova *et al.*, 1995). In a group of 46 patients with acute or chronic urogenital inflammation, treated both locally (direct administration into the urinary bladder) and orally with phages targeting *P. aeruginosa*, *Proteus* sp., *Staphylocccus* sp., or *E. coli* or with combined pyobacteriophage (in monotherapy and in combination with antibiotics), a clinical improvement was observed in 92% of the cases. According to some authors, extending the time between micturitions and alkalization of the urine may increase the effectiveness of PT in UTIs (Krueger *et al.*, 1930; Lityński, 1950).

Safety of PT

Bacteriophages are generally regarded as safe; there is no evidence in the literature that they can cause any serious side effects, induce disease, or cause any harm in mammalian cells (Kutter, 2005; Górski *et al.*, 2009). A special concern related to therapeutic phages is the possibility of bacteriophage-mediated transfer of genes involved in bacterial pathogenicity (Górski *et al.*, 2009). Thus, generally, only the obligately lytic phages are considered a suitable substrate for therapeutic phage preparations (Sandeep, 2006). Moreover, lysogenic phages are inadequate for PT because they may not destroy the bacterial cell immediately. Some authors have suggested that, in theory, a rapid release of bacterial endotoxins during phage-mediated bacterial lysis (a phenomenon observed with some antibiotics) may cause serious side effects (Lepper *et al.*, 2002; Dixon, 2004). So far, our own observations in patients and the results of current clinical trials do not support these assumptions. Moreover, in our *in vitro* experiments, a protective phage activity, expressed by decreasing the formation of free oxygen species released by human neutrophils stimulated by live bacteria, was observed (Międzybrodzki *et al.*, 2008).

The first phage preparations that were studied extensively for genetic safety and oral toxicity have been registered for use in the food and agriculture industries and introduced into the market. LMP-102 and Listex P100 contain phages specific to various *Listeria monocytogenes* strains and are used for the prevention of *Listeria* infection of food (Carlton *et al.*, 2005; Food & Drug Administration, 2006; EBI Food Safety, 2009). AgriPhage acts to prevent and control the infection of pepper and tomato plants with *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* (OmniLytics, 2008). The company offers an updated formulation for each grower based on an analysis of field samples. This approach is similar to some PT protocols in humans, in which only specific phages identified by the phage-typing procedure are applied in the treatment (Górski *et al.*, 2009).

Recently, the results of three placebo-controlled double-blinded clinical trials confirming the safety of phage application were published. Bruttin & Brüssow (2005) administered a T4 bacteriophage formulation orally to 15 healthy adult volunteers. The partially purified phages were applied three times daily for 2 days in 150 mL of mineral water in two doses of 10^3 and 10^5 PFU mL^{-1}. No adverse events related to the administration of the phage, including normal indices of hepatic function, were observed for the two doses compared with the placebo. The safety of the topical application of a preparation of eight natural lytic bacteriophages against *P. aeruginosa*, *S. aureus*, and *E. coli*, called WPP-201, was tested in patients with chronic venous leg ulcers (Rhoads *et al.*, 2009). The phages, suspended in sterile phosphate-buffered saline (PBS) (each phage at a final concentration of approximately 1×10^8 PFU mL^{-1}), were applied to the wounds once a week via an ultrasonic debridement device for 12 weeks or until wound healing (they were not specifically directed against the microbiological flora of the wounds). Combined dressings with Promogran, bovine lactoferin, xylitol, Acticoat, and Allevyn were used and the administration of antibiotics was allowed if clinical signs of infection were observed. Twenty of the 22 patients in the test group and 19 of the 20 in the control group completed the study. No significant differences were observed in the frequency of adverse events and wound healing between the study groups. A phase I/II trial of a therapeutic bacteriophage preparation in patients with chronic ear infection exclusively or predominantly with antibiotic-resistant *P. aeruginosa* was completed in the United Kingdom (Wright *et al.*, 2009). A mixture (0.2 mL) of six phages in 10% glycerol in PBS (10^5 of each phage) was applied in a single dose to the ear after individual confirmation of *Pseudomonas* susceptibility to any of the phages in the preparation. The placebo group received only the glycerol–saline vehicle. No serious adverse events were reported. Mild-to-moderate treatment-related adverse events were reported in 50% of the phage-treated patients ($n = 12$) and in 42% of the patients of the placebo group ($n = 12$), but they were considered unrelated to the phage application. It should be emphasized that this trial showed not only the safety of the phage preparation, but, more importantly, its efficacy in the treatment of chronic otitis, indicated by the significantly improved clinical outcomes and decreased bacterial counts.

These results confirm earlier observations of the results of a phage lysate application. In Ślopek's study of 138 patients treated orally and/or topically, only in three cases were adverse events reported, involving abdominal pain between days 3 and 5 of oral therapy in two patients and one case of a local allergic reaction to the topical phage preparation. It is noteworthy that intravenous phage administration has been used without deleterious effects as a diagnostic tool to test the immunological response in patients with immunodeficiencies (Borysowski & Górski, 2008).

Phage application in the treatment of CBP

Although the history of PT is over 90 years old, there are no data in the literature available on the PT of prostatitis. We could find only two Russian reports in which phages were used in CP treatment (http://medi.ru/doc/6280613.htm; http://ru-patent.info/21/25-29/2128052.html). Furthermore, a recently published summary of the Georgian and Russian experience also does not mention this condition (Chanishvili, 2009).

At our Phage Therapy Unit of the Institute of Immunology and Experimental Therapy, we conduct the experimental treatment 'Experimental phage therapy of drug-resistant bacterial infections, including MRSA infections' (approved by an independent bioethics commission in accordance with the Helsinki Declaration of World Medical Association) (Górski *et al.*, 2007). According to this protocol, only those patients in whom a potential bacterial pathogen was isolated and previous appropriate antibiotic treatments had failed or the use of the targeted drug was contradicted could qualify for therapy with phage formulations prepared according to the modified Ślopek *et al.* (1983) procedure (Międzybrodzki *et al.*, 2008). Our first patients suffering from CBP were treated orally with phages according to the Ślopek procedures, but we then switched to rectal administration, which may be more efficacious in the treatment of prostatitis (better local penetration, no possible detrimental effects of gastric acid on phages). We recently described in detail three cases of prostatic patients treated with phages in whom successful pathogen eradication was achieved (Letkiewicz *et al.*, 2009). In all the patients, *E. faecalis* was cultured from expressed prostate fluid (EPS) and the presence of unusual pathogens was excluded. Two patients were previously treated orally with targeted antibiotics for 3 months; in another patient, the antibiotics could not be used because of bacterial resistance to fluorochinolones and the patient's

refusal to take other antibiotics due to their possible adverse effects. In all of the patients, an oral autovaccine against *E. faecalis* and transrectal laser biostimulation were applied as adjuvant treatment. Despite the treatment, bacterial infection and clinical symptoms persisted. Specific phage preparations active against *E. faecalis* isolated from the patients (10^7–10^9 PFU mL^{-1}) were prepared and applied rectally, 10 mL two times daily, for 28–33 days. The treatment caused bacterial eradication (confirmed by two negative cultures of EPS conducted 7–17 weeks apart), improvement in the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI; analyzed in two cases), reduction in prostate size and pain, and significant increases in the maximum urinary flow rate in all cases.

Our most recent analysis, which can be considered similar to a small case–control study (Letkiewicz *et al.*, 2010), summarizes the results of PT in patients with CBP in whom the results of bacterial culture of EPS before and during or after PT were available ($n = 22$). *Enterococcus faecalis* ($n = 16$), *E. coli* ($n = 5$), *K. pneumoniae* ($n = 2$), *P. aeruginosa* ($n = 1$), and *Staphylococcus haemoliticus* ($n = 1$) were confirmed as the target pathogens (four patients had a mixed infection). The patients received specific phage lysates rectally ($n = 20$), orally ($n = 5$) and/or topically on the glans penis ($n = 2$). The PT duration was 22–99 days (average: 47 days). Eradication of the pathogen, as confirmed by two consecutive EPS cultures (an interval of at least 2 weeks) during or after PT, was observed in 50% of the cases. In six patients, bacterial eradication was confirmed in one EPS culture. A significant decrease in the EPS leukocyte count (available in patients in whom a full Stamey–Meares test (Meares & Stamey, 1968) before and after therapy test was performed) was observed from 16.8 ± 3.3 to 7.9 ± 3.3 cells hpf^{-1} ($n = 6$, $P < 0.05$) as well as a significant reduction of the prostate volume from 38.3 ± 2.5 to 20.7 ± 1.4 mL ($n = 11$, $P < 0.05$) and an increase in the maximum urinary flow rate from 20.7 ± 2.1 to 26.3 ± 0.8 mL s^{-1} ($n = 9$, $P < 0.05$). The NIH-CPSI, which standardizes the assessment of pain, urinary symptoms, and the impact of the disease on the patient's quality of life, decreased from 18.1 ± 4.6 to 10.3 ± 3.0 points ($n = 11$), but this result was not significant. Changes in the function of liver, pancreas, kidney, and bone marrow were not observed. No side effects were reported in the study group. There were no complaints regarding the frequency and duration of application of the phage preparations by the rectal route.

The phage lysates that are used for PT may not only have direct antibacterial action but also immunomodulating effects mediated by the phages themselves as well as by bacterial antigens. Although bacteriophages are not pathogenic, they can elicit an immune response in the human host (e.g. antibody production or the induction of interferon) (Górski *et al.*, 2007). Bacterial extracts used orally can activate the immune system in humans (Ruedl *et al.*, 1994). A potential clinical effect of immunotherapy in CBP patients was observed for a multicomponent vaccine containing *S. aureus*, *K. pneumoniae*, *P. vulgaris*, and *E. coli* antigens (Bondarenko *et al.*, 2004). It was also shown in rats that serosal exposure of killed *E. coli* increased specific antibacterial immunoglobulin titers and bacteria coated with specific immunoglobulin G (IgG) and IgA were found in the prostate of immunized animals infected previously with *E. coli*, although it did not protect the animals against acute bacterial prostatitis (Ceri *et al.*, 1999). Theoretically, several weeks of rectal application of phage lysate in our patients could also influence bacterial adherence to the rectal mucosa, resulting in a decrease in their influx into the prostate. However, we were able to demonstrate successful eradication in a patient treated only with an oral phage preparation.

In addition, our experimental data strongly suggest that purified phages may have anti-inflammatory properties. *In vitro*, they can modulate phagocyte function, decrease the activation of nuclear factor-κB induced in human mononuclear cells by human herpesvirus-1, and reduce platelet and T-cell adhesion to the extracellular matrix and fibrinogen, a reaction that contributes toward the development of an inflammatory reaction (Kniotek *et al.*, 2004; Przerwa *et al.*, 2006; Gorczyca *et al.*, 2007). *In vivo*, it was observed that purified phages tend to diminish the migration of mononuclear cells and neutrophils to the site of a skin graft (Górski *et al.*, 2006). Our experiments showed that phages can inhibit the formation of reactive oxygen species by neutrophils stimulated both by live bacteria and by their endotoxins, even independent of the phage's capability to lyse the bacterial strain (Międzybrodzki *et al.*, 2008). This activity may play a role in reducing the oxidative stress that accompanies the chronic inflammatory process in the prostate gland induced by bacterial pathogens and may be a part of the pathophysiology of chronic pelvic pain in prostatitis (Shahed & Shoskes, 2000; Zhou *et al.*, 2006). Interestingly, we have also observed a significant decrease in the level of C-reactive protein, an inflammatory marker, during the first 2–5 weeks of oral (10 mL of the phage lysate three times daily after neutralization of gastric juice with 10 mL of dihydroxyaluminum sodium carbonate) and/or local (two times daily for wet compresses or irrigation of a fistula) phage treatment (Międzybrodzki *et al.*, 2009). This could be a cause of the reduction of some CP symptoms such as pelvic pain because some studies indicate a role of inflammatory cytokines in CBP (He *et al.*, 2009).

Conclusions

CBP remains a difficult clinical dilemma affecting millions of patients worldwide, and increasing microbial resistance further aggravates this challenge. The current greatly revived

interest in PT suggests that this approach may also be considered in the treatment of CBP, and our initial observations seem to be very encouraging. Further studies are urgently needed to confirm the therapeutic value of PT of bacterial prostatitis.

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Authors' contribution

S.L. and R.M. contributed equally to this work.

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