The Use of Bacteriophages for Complex Treatment of Patients with Pancreatic Necrosis

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Abstract

Treatment of acute necrotizing pancreatitis has been challenged by rising number of opportunistic infections resistant to available antibiotic agents. Toxicity and immune supression are well known side-effects of antibioitic agents that alter their effectiveness in the treatment of acute necrotizing pancreatitis. Bacteriophage therapy has been known as alternative antiinfection modality which was utilized in several clinical studies.

This study was performed to determine clinical and bacteriological effect of piobakteriofag polyvalent purified (PPP) included into the complex treatment of acute necrotizing pancreatitis.

The study included 86 patients admitted to the Kiev Regional Clinical Hospital with acute destructive necrotizing pancreatitis. All patients underwent the standard therapy. In patients of group 1 (N=36), PPP was included into treatment from the first day after the operation. The comparison group (group 2, N=50) consisted of patients not receiving PPP in the course of treatment. The sensitivity, or the lack of sensitivity, of a specific microorganism, isolated from the biomaterial of a specific patient, to a particular bacteriophage, was determined. Comparison of the level of wound cleaning and duration of hospital stay was performed between two groups.

The most common microbial association was Enterococcus faecium with E.coli. 31 different cultures were obtained from the initial specimen collection, out of which 15 were found sensitive. Enterococcus faecium was only organism cultured 10-15 days following PPP administration. This appeared sensitive to PPP. Wounds of all other patients were cleared from the microorganisms.

Administration of PPP in the complex treatment of necrotizing pancreatitis lead to 1,49 times increase of the omentobursostomy wound and retroperitoneal space cleaning ($p<0,01$). It also lead to 1,24 times decrease of the duration of hospital stay ($p<0,05$).

In this study, we show that in patients with microbial associations, where there is sensitivity of a certain microorganism to the bacteriophage, the use of PPP causes clinical and bacteriological improvements.

Introduction

Interest in bacteriophages has increased due to the possibility of their use in numerous medication-resistant forms of pathogenic and opportunistic bacteria (1). Medications utilizing bacteriophages exist for the prevention and treatment of diseases caused by some cocci, enterobacteria, and pseudomonas. Bacteriophage therapy in combination with antibiotics, had proven effective (2). Bacteriophage therapy drugs are available as acid-resistant coated tablets, ointments, aerosols, suppositories, and liquid. They are applied to cuts, administered orally, sometimes used intravenously, etc. (2, 6, 8).

The use of bacteriophages to treat bacterial infections of surgical wounds is therefore quite practical, since phages are generally not susceptible to inhibitory effects once applied to wounds. Furthermore, in addition to the specific effectiveness of individual phages, bacteriophages are economical, harmless to humans, and do not cause dysbiotic violation.

Compared with antibiotics, bacteriophages have many advantages: phages are a natural way to combat bacteria; they do not cause side effects; they are compatible with all drugs; they are not allergens; they are not addictive; they have devastating effects on a particular type of bacteria while leaving other types of bacteria unharmed, meaning they do not cause dysbiosis.

The determination of the sensitivity of bacteria is an important step for treating bacterial infections, as this procedure ensures the choice of the most effective antibiotic or bacteriophage for treatment. Such a procedure determines which substances cause in-vitro bacterial colonies to slow in growth or die (3).

Aim of the study

Determine the nature of abdominal cavity microbial contamination during necrotizing pancreatitis, and the microbial sensitivity to bacteriophages, so as to select a rational therapy.

Materials and methods

In the period from 2013 to 2015, 86 patients diagnosed with acute necrotizing pancreatitis, including 53 men (61.6%) and 33 women (38.4%), were treated in the surgical department of the Kiev Regional Clinical Hospital (KRCH). The average age of men was 49.6 ± 11 years and the average age of women was 56 ± 12 years. Patients were divided into two groups.

The first group (the main group) consisted of 36 patients treated with Piobakteriofag polyvalent purified (PPP) — a mixture of sterile filtrate of fagolizatov staphylococci, streptococci, enterococci, Proteus, Klebsiella (pneumoniae and oxytoca), Pseudomonas, and Escherichia coli— during complex necrotic pancreatitis treatment.

In the second group (the comparison group), 50 patients underwent treatment of necrotic pancreatitis without the use of bacteriophages.

All patients received empirical antibiotic therapy before surgery.

Types of surgery are shown in Table 1.

Table 1

Type of Surgery	**Number of Patients**	
	Group 1	**Group 2**
Debridement of the necrotic pancreatic tissue+ Epigastric omentobursostomy	**14**	**18**
Debridement of the necrotic pancreatic tissue+ Epigastric omentobursostomy+ lumbostomy	**17**	**24**
Drainage of the pancreatic abscess	**2**	**5**
Drainage of the retroperitoneal abscess	**3**	**3**

Groups of patients are based on age, gender, and type of surgery.

During surgery, tissue specimen was obtained for culture and sensitivity to bacteriophages analysis.

Patients from the first group were exposed to the 15-20 mL PPP (1:5) during regular sessions of wound dressing and lavage of the infective cavity in the postoperative period. PPP solution was administered through transcutaneous drains placed in the omental and retroperitoneal space to irrigate infective cavity. The exposure time was 15-20 minutes and was regulated by means of clamping the drains.

Quantitative and qualitative identification of microorganisms was carried out by plating material on selective and differential nutrient media (agar): blood agar, egg-yolk-colored agar, enterococcus agar, endo agar, sugar broth produced by HiMedia (India), and St**.** Petersburg Pasteur Institute of Epidemiology and Microbiology, in accordance with existing regulations and guidance documents (5, 7). The identification of isolated bacteria was performed by Berdey's technique (4).

Research was conducted in a bacteriological laboratory to measure the lytic activity of virulent bacteriophages using Otto technique (5).

Based on the results of the study, the sensitivity, or the lack of sensitivity, of a specific microorganism, isolated from the biomaterial of a specific patient, to a particular bacteriophage, was determined. The duration of omentobursostomy wound and retroperitoneal space cleaning, the duration of hospital stay between two groups were compared using Mann-Whitney test.

Results and Discussion

Piobakteriofag polyvalent purified was diluted with a saline solution at concentrations of 1: 2, 1: 3, 1: 4, 1: 5, 1: 6, 1: 7, 1: 8, 1: 9, 1:10, 1:11, and the sensitivity of microorganisms, both in association and in isolated form, was determined (table 2).

Table 2

Dilution and sensitivity to Piobakteriofag polyvalent purified

Dilution	***1:2***	***1:3***	***1:4***	***1:5***	***1:6***	***1:7***	***1:8***	***1:9***	***1:10***	***1:11***
Sensitivity	82,7 %	82,7 %	82,7 %	82,7 %	80,7 %	77,7%	52,7 %	43,2 %	17,3%	0,7%

Piobakteriofag polyvalent purified, in a dilution of 1:5, results in a constant sensitivity. Some sensitivity is present in the 1:10 dilution of PPP, and at greater dilution, there is no sensitivity.

The study involved 86 patients, and 119 microbial cultures were isolated. Microorganisms were both in monoculture and in microbial associations (Table 3).

Table 3

Sensitivity of isolated microorganisms to Piobakteriofag polyvalent purified

№	Isolated Microorganism	Quantity Sensitive		Percent Sensitive, %		
		Group 1	Group 2	Group 1	Group 2	Overall Sensitivity
1	Enterococcus faecium	13	16	38,3	47,0	42,65
2	E.coli	11	14	37,9	48,3	43,1
3	St.epidermidis	12	10	42,9	35,7	39,3
4	St.aureus	10	5	55,5	27,8	41,65
5	Kl.pneumoniae	5	3	50	30	40

Enterococcus faecium was isolated in 34 cases— from 13 patients in the first group and from 21 in the second. Sensitivity to PPP was noticed in 85.3% of the cases— 38.3% in the first group and 47.0% in the second. 14.7% of Enterococcus faecium was found to be resistant to PPP. E.coli was detected in 29 cases— in 11 patients of the first group, and in 18 of the second. Sensitivity to PPP was noticed in 86.2% of the cases— 37.9% in the first group and 48.3% in the second. 13.8% of E. coli was found to be resistant to PPP. St.epidermidis was found in 28 cases— in 12 patients of the first group, and in 10 patients of the second. Sensitivity to PPP was noticed in 78.6% of the cases— 42.9% in the first group and 35.7% in the second. 21.4% of St.epidermidis was found to be resistant to PPP. St.aureus was found in 18 cases— in 10 patients from the first group, and in 5 patients from the second. Sensitivity to PPP was noticed in 83.3% of the cases— 55.5% in the first group and 27.8% in the second. 16.7% of St.aureus was found to be resistant to PPP. Kl.pneumoniae was found in 10 cases— in 5 patients from the first group, and in 3 patients from the second. Sensitivity to PPP was noticed in 80.0% of the cases— 50.0% in the first group and 30.0% in the second. 20.0% of Kl.pneumoniae was found to be resistant to PPP.

Gram-positive flora (Enterococcus faecium, St.epidermidis, St. aureus) and gram-negative flora (E.coli, Kl.pneumoniae) proved sensitive to polyvalent piobakteriofah purified in 82.7% of cases; resistant in 17.3% of cases.

The degree of initial contamination of wounds ranged from 10^5 (St. aureus, St.epidermidis, Enterococcus faecium), to 10^7 (Kl. Pneumoniae, E. coli).

Sensitivity to PPP appears to be independent of the initial level of bacterial contamination in the wound, based on bacteriological findings.

Although group 2 was not subjected to PPP treatment, sensitivity to PPP was studied in the group regardless. Mean sensitivity to PPP in the group 1 was 44,9%, while mean sensitivity in the group 2 was 37,8%. The most common organism cultured in both groups was Enterococcus faecium. At the same time Enterococcus faecium has shown the highest sensitivity comparing to other organisms.

Table 4
The sensitivity of microorganisms to PPP over time

N	Isolated Microorganisms	First Sample		After 5-10 days		After 10-15 days	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
1.	Enterococcus faecium E.coli	3	3	2	-	1	-
		3	3	1	-	-	-
2.	E.coli St.aureus	3	1	1	-	-	-
		1	3	1	-	-	-
3.	Enterococcus faecium E.coli St.epidermidis	1	2	1	-	-	-
		1	2	1	-	-	-
		2	1	-	-	-	-
4.	St.aureus Kl.pneumoniae	1	1	-	-	-	-
			2	-	1	1	-

In the first sample, microbial association is evident in 15 patients (Table 4). A combination of Enterococcus faecium and E.coli was detected in 6 patients. In 3 patients, Enterococcus faecium was sensitive to PPP while E. coli was resistant; in 3 other patients, the reverse was true. In 4 patients, a combination of E. coli and St. aureus was found. Of these, 3 patients had E. coli sensitive to PPP and St. aureus resistant to PPP, while 1 had E. coli resistant to PPP and St. aureus sensitive to PPP. In 3 necrotizing pancreatitis patients, a microbial association of Enterococcus faecium, E.coli, and St.epidermidis was detected. Of these patients, 1 patient had Enterococcus faecium and E.coli sensitive to PPP, and St.epidermidis resistant to PPP; and 2 patients, in the first sample, showed St.epidermidis sensitive to PPP, and Enterococcus faecium and E.coli resistant to PPP. In 2 patients, a combination of St. aureus and Kl.pneumoniae was found. Of these, 1 patient had St. aureus sensitive to PPP and Kl.pneumoniae resistant to PPP; and 1 patient had both St.aureus and Kl.pneumoniae resistant to PPP.

Five to ten days after the application of PPP on wounds where the association of Enterococcus faecium and E.coli was detected, it was noticed that patients who initially had bacteria resistant to PPP, had a change in microorganism sensitivity. In the first patient, the detected Enterococcus faecium is sensitive to PPP; in the second patient, both Enterococcus faecium and E.coli are sensitive to PPP. In 4 patients, after the application of PPP, microorganisms were not found. Of the patients with E.coli and St.aureus association in the wound, 5-10 days after the application of PPP, 1 patient had a change of microorganism sensitivity and 3 had no microflora growth. Of the 3 patients infected by Enterococcus faecium, E.coli, and St.epidermidis, 5-10 days after the use of PPP, 1 patient experienced a change in microorganism sensitivity, and the rest had no microflora growth. Of the 2 patients with determined St.aureus and Kl.pneumoniae association, 5-10 days after PPP application, 1 patient still had bacteria resistant to the bacteriophage.

Ten to fifteen days after the application of PPP, one patient with a detected association of Enterococcus faecium and E.coli, who previously had Enterococcus faecium resistant to PPP, was noted to now have Enterococcus faecium sensitive to PPP. Therefore, with time, the microorganism changed its

sensitivity to PPP. One patient with a detected association of St.aureus and Kl.pneumoniae, also experienced a change in sensitivity of the previously resistant microorganism.

The most common microbial association was Enterococcus faecium with E.coli. 31 different cultures were obtained from the initial specimen collection, out of which 15 were found sensitive. Enterococcus faecium was only organism cultured 10-15 days following PPP administration. This appeared sensitive to PPP. Wounds of all other patients were cleared from the microorganisms.

Table 5

The comparison of wound healing and duration of hospital stay between two groups.

	Omentobursostomy wound and retroperitoneal space healing (days)	Average hospital stay (days)
Group 1	12,5±2,1	21,6±1,1
Group 2	18,7±2,5	26,8±2,1

Administration of PPP in the complex treatment of necrotizing pancreatitis lead to 1,49 times increase of the omentobursostomy wound and retroperitoneal space cleaning ($p<0,01$). It also lead to 1,24 times decrease of the duration of hospital stay ($p<0,05$).

In this study, we show that in patients with microbial associations, where there is sensitivity of a certain microorganism to the bacteriophage, the use of PPP causes clinical and bacteriological improvements.

Conclusions

1. In a dilution of 1: 5 PPP, microorganisms are sensitive to Piobakteriofag polyvalent purified; in a dilution of 1:10, they are somewhat sensitive; however, at higher dilution, they are resistant.
2. In 82.7% of patients with necrotizing pancreatitis, microorganisms were found to be sensitive to Piobakteriofag polyvalent purified; in 17.3% of cases, microorganisms were resistant.
3. In patients with microbial associations, one microorganism may be sensitive to the bacteriophage; using the bacteriophage results in clinical and bacteriological improvement.

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