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The Role of Moderate and Virulent Endogenous Bacteriophages in the Treatment of Pyoinflammatory Infections in Intensive Care Unit Patients

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ABSTRACT In connection with the growth of resistance of pathogens of pyoinflammatory infections (PII) to antibiotics, physicians began to use bacteriophages, which are widespread where there are homologous bacteria. They are also found in the human body, possibly protecting against PII. It was found that mortality in patients with bacteriophages was lower than in patients without homologous endogenous bacteriophages. The most common were mild bacteriophages, which "protected" patients from infection, although it is believed that only virulent bacteriophages may do this.

AIM OF STUDY To study the effect of virulent and moderate endogenous bacteriophages on the course of pyoinflammatory infections in intensive care units (ICU) patients.

MATERIAL AND METHODS The study included 33 patients with positive blood culture who were treated in the ICU. Of these, 12 (36.4%) had endogenous bacteriophages (10 men, 2 women).

We isolated 16 strains of various bacteria. Bacteriological blood tests were performed using an automatic blood culture analyzer Bactec-9050. Identification of isolated microorganisms was carried out using an automatic microbiological analyzer WalkAway 40. Traditional virological methods on the basis of LLC SPC MicroMir were used for work with bacteriophages.

RESULTS AND DISCUSSION From 16 positive blood cultures, the following were isolated: *Klebsiella pneumoniae* — in 7 samples, *Acinetobacter* spp. — in 4 samples, *Staphylococcus* spp. — in 2 samples, and one strain in each sample: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Endogenous phages were isolated in 36.4% of patients, which were detected in all blood cultures. The exception was *K. pneumoniae* strains, for which in 42.9% of cases (3 cases out of 7) bacteriophages were absent.

Out of 12 patients with isolated bacteriophages, 6 had positive treatment outcomes.

Of 14 blood samples, 11 moderate bacteriophages were isolated, which amounted to 78.6%, virulent — 21.4%. Despite this, the protective effect of bacteriophages was noted.

CONCLUSION 1. All blood cultures contained homologous bacteriophages, except *K. pneumoniae* strains, which had no endogenous bacteriophages in 42.9% of cases. 2. Most of the isolated endogenous bacteriophages (78.6%) were moderate. 3. In the absence of homologous bacteriophages in the blood of ICU patients, the likelihood of death increases (66.7%) compared to patients with bacteriophages (33.3%).

Keywords: endogenous bacteriophages, microbiota, severe concomitant injury, ICU patients

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PII – purulent-inflammatory infections

ICU – Intensive Care Unit

Currently, the problem of antibiotic resistance of microorganisms that cause the development of pyo-inflammatory infections (PII) is especially acute. In this regard, there is a revival of interest in bacteriophages, which are widespread in nature where there are bacteria homologous to them. They are also found in the human body, possibly protecting it from PII. As our previous studies have shown, about a third of patients in intensive care units (ICU) of a surgical and burn profile have endogenous bacteriophages homologous to microorganisms isolated from blood. [1]. It was found that mortality in patients with bacteriophages was lower than in patients without homologous endogenous bacteriophages. It should be noted that the most common were moderate bacteriophages, which “protected” patients from infection, despite the fact that this role was believed to be inherent only in virulent bacteriophages. [2, 3]. Information from other researchers on this issue has not been found in the literature available to us, despite a rather large number of works devoted to a detailed study of the molecular biological basis of the transition of temperate phages to lysogenic ones and vice versa..

The aim of the work was to determine the frequency of isolation of moderate and virulent endogenous bacteriophages in positive blood cultures with PII in ICU patients.

MATERIAL AND METHODS

The study included 33 patients with positive blood culture who were treated in the ICU. Of these patients, 12 (36.4%) had endogenous bacteriophages (10 males, 2 females). The average age of the surveyed patients was 35.5 ± 1.9 years (from 27 to 48 years).

All patients received treatment in accordance with medical and economic standards.

Of 12 patients with identified endogenous bacteriophages, 7 were examined once, 5 – twice, altogether 17 blood cultures were performed. In 16 of them, various bacteria were isolated, and in one case, no microbial growth was observed. Bacteriological blood tests were performed using an automatic blood culture analyzer Bactec-9050. The isolated microorganisms were identified using an automatic microbiological analyzer WalkAway 40.

To determine the presence of endogenous bacteriophages, vials with patient blood samples, Petri dishes with isolated microorganisms, as well as urine samples were sent to the laboratory of OOO NPTs "MicroMir". The work with bacteriophages was performed using traditional virological methods. Bacteriophages removed from the lysis zones after spot testing were examined using a JEOL-1011 electron microscope (Japan). In 7 patients, the isolation of endogenous bacteriophage was carried out without separating the material, and in 5 patients it was studied separately for each type of material (urine and blood). In total, 23 studies were carried out, bacteriophages were absent in 9 samples, and in 14 samples they were allocated.

Statistical analysis was performed using the Statistica 10 software package. Descriptive statistics of quantitative traits are presented by the mean and its standard error [4].

RESULTS AND DISCUSSION

From 16 positive blood cultures, the following were isolated: *Klebsiella pneumoniae* - in 7 samples, *Acinetobacter* spp. – in 4 samples, *Staphylococcus* spr. – in 2 samples, and one strain in each sample: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Endogenous bacteriophages were isolated in 14 samples during bacteremia. The results are presented in the table. Virulent phages were present in only three samples (21.4%): in 2 samples — bacteriophage to *K. pneumoniae* and in 1 sample — phage to *Acinetobacter* spp. The remaining 11 phages (78.6%) were moderate: to *Acinetobacter* spp. – 3 phages, 2 phages – to *K. pneumoniae* and *Staphylococcus* spp. and one each for *S. aureus* and *E. faecalis*. Phages to *P. aeruginosa* were isolated from one patient from blood and urine.

Table
Patients who had endogenous bacteriophages when isolated from various samples of biomaterials

#	Microbe	Date	The presence of an endogenous bacteriophage		Outcome
			Blood	Urine	
1	<i>Acinetobacter spp.</i>	4/VI – 15	Virulent		Discharged
	<i>Staphylococcus spp.</i>	11/VI – 15	Moderate		
2	<i>Klebsiella pneumoniae</i>	24/VI – 15	Virulent		Discharged
3	<i>Staphylococcus aureus</i>	13/I – 16	Moderate		Died
	<i>Klebsiella pneumoniae</i>	22/I – 16	Absent		
4	<i>Staphylococcus spp.</i>	13/I – 16	Moderate		Died
	<i>Klebsiella pneumoniae</i>	18/I – 16	Absent		
5	<i>Acinetobacter spp.</i>	7/IV – 16	Moderate		Discharged
6	<i>Klebsiella pneumoniae</i>	10/VIII – 16	Moderate		Discharged
	No growth	18/VIII – 16	Absent		
7	<i>Enterococcus faecalis</i>	19/IX – 16	Moderate		Discharged
8	<i>Acinetobacter spp.</i>	16/VIII – 17	Absent	Moderate	Died
9	<i>Klebsiella pneumoniae</i>	23/VIII – 17	Absent	Virulent	Died
10	<i>Klebsiella pneumoniae</i>	26/IX – 17	Moderate	Absent	Discharged
11	<i>Pseudomonas aeruginosa</i>	28/V – 17	Moderate	Moderate	Died
12	<i>Acinetobacter spp.</i>	16/VII – 18	Moderate	Absent	Died
	<i>Klebsiella pneumoniae</i>	18/VII – 18	Absent	Absent	

Endogenous bacteriophages were detected in all blood cultures, with the exception of *K. pneumoniae* strains, for which in 42.9% of cases (3 observations out of 7) bacteriophages were absent.

Of the 12 patients from whom bacteriophages were isolated, 6 (50%) had a positive treatment outcome and were discharged from the hospital.

Patient No. 1 was examined twice. The first study found *Acinetobacter spp.* and a virulent endogenous phage to it, at the second study, after 7 days, *Staphylococcus spp.* and a moderate bacteriophage homologous to it was found.

Patient No. 2 had *K. pneumoniae* and homologous virulent phage in the blood.

In patient No. 6, examined in dynamics, *K. pneumoniae* and an endogenous homologous bacteriophage were initially isolated from the blood, but after 8 days the blood sample became sterile and the phage was absent.

In 2 patients (No. 5 and No. 7), *Acinetobacter spp.* was found in the blood and *E. faecalis*, as well as moderate bacteriophages to them.

In patient No. 10, the presence of endogenous phage was studied separately – in blood and urine. The *K. pneumoniae* strain and a bacteriophage homologous to it were isolated from the blood; the phage was absent in the urine.

Six patients died: №№ 3, 4, 8, 9, 11 и 12.

In 3 patients, a change in pathogens was found (nos. 3, 4 and 12). During the initial study, *S. aureus* (No. 3), *Staphylococcus spp.* (No. 4), *Acinetobacter sp.* (No. 12) and bacteriophages homologous to them. During the first 9 days, we also isolated from their blood *K. pneumoniae*.

In 2 more patients, from blood were isolated *Acinetobacter spp.* (№ 8) and *K. pneumoniae* (№ 9), at the same time, their phages were present only in urine: in patient No. 8 - moderate, in patient No. 9 – virulent phage.

Of interest is patient No. 11, from whose blood the *P. aeruginosa* strain was isolated, and moderate bacteriophages were found in the blood and urine, but they did not lyse microbial cells.

Two deceased patients (No. 11 and No. 12) were diagnosed with sepsis. In patient No. 11, death occurred in the presence of a moderate phage to *P. aeruginosa* in blood and urine, which suggests the absence of its lytic effect. In patient No. 12, a change in pathogens occurred, and upon repeated examination, the phage to *K. pneumoniae* was not isolated.

Taking into account the change of pathogens, homologous bacteriophages were isolated from the blood of 9 patients, of whom 3 (33.3%) died.

Of the 6 patients who died, 4 (66.7%) had no bacteriophage by the time of death, and 2 (33.3%) had endogenous homologous bacteriophages isolated only from urine. In this regard, it can be assumed that only bacteriophages in the blood provide antimicrobial protection of patients.

Thus, due to the small number of observations, we note only a tendency, and not the statistical significance of the results obtained, concerning lethality in the isolation of bacteriophages from the blood or their absence in it (33.3% of 9 patients and 66.7% of 6, respectively $t=1,4$, $p=0,31$).

CONCLUSION

Our results indicate that in patients of the intensive care unit with the development of bacteremia and sepsis, Gram-negative microorganisms were the leading pathogens (75% of the total number of strains), which coincides with the data obtained in a multicenter study in Russia (72,7%) [5].

In the present study, *K. pneumoniae* was the causative agent of bacteremia in 43.75% of cases, which is slightly higher than the indicators of the multicenter study (34%). The proportion of non-fermenting Gram-negative bacteria identified in this work was 31.25%.

This work shows that moderate bacteriophages can protect the human body from the development of a purulent-inflammatory infection. But the mechanism of such a protective action of moderate bacteriophages has not yet been clarified. Possibility of direct lytic action of moderate bacteriophages is assumed. In addition, the majority of moderate bacteriophages under the influence of various factors can transform into a virulent form [6, 7]. The presence of endogenous bacteriophages, in itself, does not guarantee the success of the treatment of critically ill patients in the intensive care units, however, apparently, it is a positive factor in the protection of the body.

FINDINGS

1. All blood cultures of the examined patients of the intensive care units contained bacteriophages homologous to them, with the exception of *K. pneumoniae* strains, in which endogenous bacteriophages were absent in 42.9% of cases.

2. Most of the isolated endogenous bacteriophages (78.6%) were moderate ones.

3. In the absence of homologous bacteriophages in the blood of patients in the intensive care unit, the likelihood of death increases (66.7%) compared to the outcomes in patients with bacteriophages (33.3%).

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