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Immunobiological Safety Profile and Therapeutic Effectiveness of Klebsiella pneumonia Bacteriophages Using Acute and Sub-Chronic Animal Toxicity Study

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Abstract

Recently, numerous pre-clinical and clinical studies have shown their significant phage therapy efficacy against many antibiotic resistant pathogens and proved to be one of the best alternatives to the antibiotics. Bacteriophages can also be used as biocontrol agents in agriculture and petroleum industries. However, only few researchers have focused to monitor the phage-mediated acute immune reactions during phage therapy. Besides, phage safety was evaluated by determining toxicity after acute and sub-chronic oral administration of low and high dose of bacteriophages in male and female rodents. Here, in this study, we had orally administered the bacteriophages against Klebsiella pneumonia XDR strain in low (1015 PFU) and high dose (1020 PFU) quantities to rats in acute (single dose) and chronic toxicity (daily dose for 28 days) model. No significant clinical sign was observed in all the experimental groups as well as in the control animals. Besides, no significant change in feed intake and body weight was observed throughout the study period. After 28 days of phage dosing, blood was collected for estimation of hematology, biochemistry, and cytokines assay. The data suggested no alterations in the haematological profile, clinical biochemical parameters, relative organ weights, and immune biomarkers. Also, the gross pathological examinations of all the major organs were found to be normal across the treatment groups. Cytokines i.e. interlukin-1 beta (IL-1β), IL-4, IL-6, and INF-gamma were observed within normal range for rat regardless of treatment. The results suggested no acute and sub-chronic toxicity in oral administration of low (105 PFU) and high dose (107PFU) of isolated bacteriophages. Thus, in conclusion these results support the long term oral bacteriophage therapy without having any strong acute immune response.

Keywords—Bacteriophages, XDR, Viral immune mechanism, Toxicity

Abstract:

Klebsiella pneumoniae (K. pneumoniae) is one of the most common gram-negative bacteria that is responsible for hospital-acquired bacterial infections, including pneumonia, bacteremia, urinary tract infections, liver abscess, and wound infections in immune compromised patients [1, 2]. Pneumonia that is caused by K. pneumoniae is usually associated with a high mortality rate. In fact, despite the development of broad-spectrum antibiotics, mortality rates greater than 50% have been reported in those infected with Klebsiella pneumonia [3]. In addition, treating Klebsiella pneumoniae has become more difficult due to the worldwide increase in multidrug resistant strains, leaving only limited clinical treatment options [4–8]. In effect, about 80% of the nosocomial infections caused by K. pneumoniae are due to these multidrug-resistant strains; the incidence of ESBLs (extended-spectrum beta-lactamase) isolates ranges from 8% to 44% [9, 10].

Bacteriophages, or phages, are viruses that specifically attack and kill their host bacteria, a process that is regarded as a possible treatment method for combating bacterial infectious diseases. Bacteriophage treatment is particularly desirable because of the side effects and inefficacy associated with antibiotics and the emergence of new antibiotic-resistant strains. The bacteriophages have garnered increasing attention as an alternative to controlling bacterial infectious diseases with antibiotics since avoiding an antibiotic treatment would avoid the spread of multiresistant bacteria [11–14]. In comparison with antibiotics, phages have many advantageous qualities, such as a high abundance, host specificity, rapid exponential replication, and a decline in numbers when the target bacteria decrease. Furthermore, phage therapy has already been tested in a variety of bacterial species and has accomplished

some achievements [15–17]. For example, Chhibber et al. delivered phage via an intraperitoneal route to treat experimental lobar pneumonia that was induced by K. pneumoniae in mice and had a great efficacy [9]. However, an intraperitoneal administration of a phage would not be either practical or efficient for treating pneumonia in humans. In the present study, we evaluated the therapeutic efficacy of phage 1513 which was delivered intranasally in order to protect against pneumonia that is induced by a strain of multidrug resistant K. pneumoniae.

Discussion

Hospital-acquired pneumonia that is caused by Klebsiella pneumoniae is always a threat as well as a fastidious public, human health problem [19]. Despite advances in antimicrobial therapy, the morbidity and mortality remain high and out of control. Furthermore, the emergence of multidrug resistance aggravates this situation. An increasing number of extended-spectrum-β-lactamase-producing and KPC-type carbapenemases-producing K. pneumoniae nosocomial isolates have been reported [4, 7, 20–22]. Since antibiotic treatment has associated restrictions and shortcomings, phage therapy is now more frequently being considered as a potential treatment and prevention for bacterial infections [23]. In the present study, we isolated a new bacteriophage (phage 1513) that could effectively control pneumonia that is caused by a clinical multidrug resistance K. pneumoniae in vitro and in vivo.

The in vitro study showed phage 1513's effectiveness against K. pneumoniae with a short latent time and a large burst size. In addition, the phage possessed stability within physiological ranges of temperature and pH. It formed plagues on 5 of 10 clinical Klebsiella strains tested, notably KP 1513. Plaques did not exist on Pseudomonas aeruginosa (ATCC27853), Staphylococcus aureus (Newman), and E. coli (CGMCC 1.797). It was consistent with previous documents that bacteriophage are highly specific and can only infect a single species of bacterium, usually a subset of strains within that species [13, 24]. Therefore, phage 1513 has a narrow spectrum and is potentially active against the MRKP strains.

However, it can be found that an early trend of phage resistance emerged after 11 hours of incubation (Figure 3). Mutants that are resistant to phage infections are a critical problem for the application of phage therapy [25]. However, some studies have demonstrated that the resistance is partially due to receptor molecule variation, which acts as virulence factor of pathogens. As a result, bacteria have to attenuate their virulence in order to be resistant to the phage lysis [26]. On the other hand, a phage can also mutate to adapt to the change of pathogens [27]. Further investigations are required to determine whether the phage-resistant K. pneumoniae cells that have emerged through phage therapy have reduced the pathogenic ability. Perhaps a cocktail of several phage strains will be necessary for controlling bacterial variation.

Chhibber et al. have demonstrated that a single intraperitoneal administration of a high bacteriophage dose, which was 100 times the infectious bacterial dose, administered immediately after the intranasal infection has been shown to rescue 100% of animals [9]. As we know, the delivery route has a critical influence on phage therapy. People rarely deliver intraperitoneally in clinical treatments; however, they take drugs by nebulisation, which is similar to intranasal administration. Compared with intranasal injection, intraperitoneally administering the phage is more suitable for systemic infections but not for local infections, like pneumonia. Moreover, if phages were delivered intraperitoneally, they would be detected and cleared out more quickly by the immune system. In this study, we demonstrated that intranasal administration can also effectively treat pneumonia that is caused by K. pneumoniae in mice. We gave a various dose of phage (2 × 109 PFU/mouse, 2 × 108 PFU/mouse, 2 × 107 PFU/mouse) 2 h after infection and, as a result, the survival rate was affected. This is probably because the increasing dose of bacteriophages results in more rapid bacterial killing, thereby increasing the survival rate [28]. Besides, phage 1513 cannot provide significant protection 24 h prior to infection (data not shown). It is speculated that the phage has been eliminated before it attached to the host and cracked it. The clearance rate of the phage particles from body fluids by the reticuloendothelial system is a critical parameter for phage therapy [26]. It may be more effective to increase the dose of phage or give it less than 24 h before infection. But phage 1513 has obvious advantages on account of the significant effect in the treatment experiment.

In addition, from Figure 6, we can see that bacteria numbers significantly decreased in the phage-treated mice, which suggests that the phage effectively killed K. pneumoniae in vivo. Consequently, in the phage-treated mice, lung lesions and an inflammatory response were clearly less prominent in comparison to the control. These were all concordant with the survival study results. Moreover, many reports have indicated that a cytokine storm results in lung injury and poor clinical outcomes [29, 30]. Therefore, the phage may be beneficial to the lung by decreasing the host's inflammatory response, as demonstrated by the levels of IL-6 and TNF-α.

In conclusion, the results of this study suggest that a phage treatment that is administered intranasally has great potential for treating pneumonia and other infections caused by K. pneumoniae. The safety of phage 1513 and its activity against biofilm formation will be investigated in further study.

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