The effects of HPMC concentration as an efficient pharmaceutical dosage form on phage release pattern from gels

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Abstract

Bacterial resistance to various antibiotics has been accelerated in recent years. Bacteriophages (phages) are natural particles that can attack bacteria. Phages can be used selectively for each particular strain. The best way for dealing with superficial infections is topical application of the drug. One of the best ways is the use of water-based gels, such as hydroxy propyl methyl cellulose (HPMC) based gels. In addition to sustained drug release properties of the gel base, HPMC itself has healing properties. The final composition of the gel should have the ability of maintaining its gel form for a suitable time period on the wound, while releasing its phage content. Isolation of selective phages of Klebsiella pneumoniae was done from waste water samples. After purification of phage, it has been trapped into HPMC gel. Gels with different concentrations were used to create plaques and their phage release pattern was studied. Finally, it was shown that the 2% HPMC possessed the most appropriate pharmaceutical features, in terms of the release and durability on the site of infection.

Keywords: HPMC hydrogel, Klebsiella pneumonia, Phage therapy, Superficial infections, Sustained release.

1. Introduction

Bacteriophages (phages) are natural particles that can attack bacteria (1). The intrinsic properties of phages made them suitable for the treatment of pathogenic bacteria in human infections (1-3). The resistance of bacteria to various antibiotics has been accelerated in recent years (4-5). The World Health Organization (WHO) has warned several times for bacterial antibiotic resistance (6). Increasing of antibiotic resistant infections has caused so many problems in modern society. One of the most striking examples of these problems is infected wounds and particularly burn wounds in hospitals (7). Strains such as
Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, and Staphylococcus aureus can infect burn wounds. In these infections and other superficial local infections, phages can be used selectively for each particular strain (8).

Choosing the best pharmaceutical form in any type of treatment is an important factor in the efficiency of the method (9). The best way to deal with superficial infections is topical application of the drug (7, 9). Among the various methods used to treat superficial infections, one and perhaps the best way is the use of water-based gels, such as hydroxy propyl methyl cellulose (HPMC) (10). In addition to the sustained drug release properties of the gel base (which is an important factor in the phage delivery), HPMC itself has healing properties (11-12). Finally, it can be noted that due to the ability of continuous phage release on the infected wound and the healing properties of the gel, phage delivery by HPMC pharmaceutical dosage form in the superficial infections can be an ideal route.

Two parameters of phage release rate at the wound site and the required time to infect certain number of bacteria in the wound are very important. The final composition of the gel should have the ability of keeping its gel form for suitable time on the wound while releasing its phage content. Phages can achieve higher doses after elimination of bacteria, based on their inherent properties. So, the most important factor in the regulation of the two items, depends on the concentration of the gel.

In this study, we aim to isolate phages and study their release rates from gels composed from different concentrations of HPMC.

2. Materials and methods

2.1. Bacteria and phage preparation

Klebsiella pneumoniae strain ATCC 13883 was purchased from “Scientific and Industrial Research Organization of Iran”.

Waste water samples, as good sources of phages, were collected from Vali Asr hospital of Fasa, Iran. Samples were centrifuged and the sediments were removed. The samples were filtered through 0.45 and 0.22 µm filters in two steps. These filtrates were used as phage sources. About 10 ml of bacteria in the exponential growth phase and 100 µl of filtrate were mixed and inoculated in 200 ml medium. After 24 hr of incubation in a shaking incubator in 120 rpm (Chest-Type GYRO-MAX 777 Incubator Shaker, Amerex Instruments, Inc.), the whole culture was centrifuged (7000 rpm for 7 minutes). The supernatant was removed and filtered. It was expected that phages were amplified in the supernatant liquid, if any phages existed in the sample in the first stage. So, the liquid was refrigerated and stored for plaque assay and spot assay.

2.2. Plaque assay

Plaque assay was performed by double layer agar method (13). 200 µl of bacteria and 100 µl of phage (in constant ratio at all stages) were mixed in 3 ml of soft agar (0.7% LB agar). The mixture was then poured on 1.5% LB agar plates. After 24 hr of incubation, plates were observed. Plaques should be seen if initial samples had any specific phages for K. pneumoniae.

2.3. Spot assay

Bacterial solution was mixed in 3 ml of soft agar and poured on the surface of of 1.5% LB agar plates. After about 30 minutes, 3 small drops of phage solution (amount of each one was about 10 µl) were applied on plate. After 24 hr of incubation, plates were examined. Phage sites should be lysed and look like a large plaque.

2.4. Phage purification

An isolated phage plaque was selected and plucked by a Pasteur pipette (both bottom and top of the agar) and dissolved in 2 ml suspension medium (SM) or saline. After filtration, 100 µl of this liquid was added to 10 ml of bacteria in the exponential growth phase. Following 24 hr of incubation, the culture media was centrifuged and the supernatant was removed and filtered. Plaque assay was performed again. This cycle was repeated for 3 times to achieve a pure phage source.

2.5. HPMC preparation

HPMC powder was dispersed in water and stirred about 2 hr to 12 hr at a temperature of 85 °C. Different concentrations of gel were prepared in sterile conditions (2%, 4%, 8% and 16% W/V). Then phage stocks were prepared in two
different concentrations ($2 \times 10^2$ and $2 \times 10^3$ pfu/ml). Each concentration of phage and gel were mixed in equal volume ratios (1:1). Finally, eight pharmaceutical dosage forms ($2 \times 10^2$ and $2 \times 10^3$ pfu/ml of phage in 1, 2, 4 and 8 % gel based carrier) were obtained.

2.6. Phage release pattern test

For evaluation of phage release pattern from HPMC gel, single layer agar method was used. In this method, a plate of 1.5% LB agar was placed in incubator for 12 hr to become dry. Then 100 µl of bacteria as a layer was poured on the plate. After 30 minutes, 2 ml of the prepared gel was distributed on the surface of the plate. The number of created plaques in 24 and 48 hr were counted. To measure the release pattern of phages from HPMC gels through a specific time, after culture of the bacteria on the surface of the plates, they were incubated for 4 hr for an initial growth. After addition of the bacteriophage containing gel, the results were reported based on the number of plaques after 72 hr of incubation. The method was more like a single layer plaque assay.

2.7. TEM analysis

Ten µl of phage lysate with high titer was placed on carbon grids and stained with 1% uranyl acetate. The used TEM device was CM-10 by Philips.

3. Results

Isolated and purified phages were evaluated and determined using transmission electron microscopy (TEM). TEM analysis of phages from sewage samples that infected K. pneumoniae, showed KLPN types that belong to the siphoviridae family (Figure 1).
The 1% HPMC gel released the highest level of phage particles after 24 hr in both cases of higher and lower phage titers. This formulation released 98% (2×10^2 pfu/ml) and 97% (2×10^3 pfu/ml) of its loaded phages in 24 hr into the plate. Phage release from 2% HPMC gel was reduced to about 93% (2×10^2 pfu/ml) and 91% (2×10^3 pfu/ml). Moreover, in 4% and 8% gels, the release rate was reduced to 82 and 71% (2×10^2 pfu/ml) and 79 and 51% (2×10^3 pfu/ml), respectively.

During 48 hr, 4% and 8% HPMC gel carriers released about 84% and 77% (2×10^2 pfu/ml) and 81 and 63% (2×10^3 pfu/ml) of their stored phage. Since the ratio between the number of phages and bacteria at the infected site must be in a balance for plaque formation, plaques created with low concentrations of gel carriers were clearer and more abundant (Table 1).

### 4. Discussion

Increasing bacterial resistance rate through the world has surged an interest to find an alternative approach to counter bacterial infections. Phage therapy is a novel and accepted way to cope with infections (14). Some types of skin and soft tissue infections are difficult to treat (15). Using new pharmaceutical formulations such as sustained release hydrogels can be very beneficial in these situations. HPMC hydrogels are able to deliver active compounds, chemicals, and biologic elements across the gel to the skin surface. So, it can be a good pharmaceutical form of drug delivery in cases of skin burns, injuries, and infections (16). The release of phage in phage therapy using gels, is one of the most important parameters. After evaluation of phage release patterns in different concentrations of HPMC, 1% and 2% HPMC gels were identified as the most effective forms (Figure 2). As it can be seen, the abundance and plaque shapes are the same in plates of phage solution and 1% HPMC gel (Figure 2).

Carrier viscosity grew with increase of HPMC concentrations in gels, proportionally. So, as an advantage, carrier durability on the scars and infections may be longer. In low viscosities (1 and 2%), HPMC hydrogels quickly release their contents on the plate, which is not very favorable; because enough exposure time is necessary for phages to infect bacteria. Moreover, the continuous release of phages at the site of infection seems necessary. The durability for phage release was more than 24 hr in 4% and 8% HPMC gels, and their surveillance was about 2 days on the applied location, but because of phage trapping in the gel and low rates of phage release, their effects were less desirable on the action site. On the other hand, the ratio of the durability and phage release was desirable in 2% HPMC gel. The gel shelf life was about 12 hr on application site. The drug remained for enough favorable time on the action site and released enough phages for phage therapy. How-
ever, in some studies on burn wounds infected by *K. pneumonia*, the best results have been observed with 3% HPMC gels (17-18).

5. Conclusion
Since the chemical treatment by antibiotics actually is reaching its final deadlock, a critical need to replace them is strongly sensed. Usage of phages for eradication of resistant infections especially in recent years is under development (19). Application of hydrogels like HPMC as a sustained release drug carrier can be very suit-

6. References