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Delivery of doxorubicin using protein nanocarriers

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In this study, we compared two types of protein nanocarriers, bacteriophage λ and apoferritin, by their ability to encapsulate the chemotherapeutic drug doxorubicin. The successful encapsulation was proven by absorbance and fluorescence measurement of the nanocarrier after the removal of free doxorubicin by dialysis. Phage λ was able to encapsulate much higher (4 times) amount of doxorubicin than apoferritin. Also, more doxorubicin was released during the washing from apoferritin, which is undesired. Based on the obtained data, phage λ seems to be a better protein nanocarrier than apoferritin.

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1. Introduction

Anthracycline antibiotic drugs are widely used in treatment of many patients with cancer. First anthracycline drug, daunomycin (or daunorubicin) was found in a number of different wild type strains of Streptomyces. However, in cancer treatment the most commonly used anthracycline drug is doxorubicin (DOX) or one of its 2000 known analogs[1].

DOX is used in treatment of many different types of cancer such as neuroblastomas, leukaemia, lymphomas or breast, testicle, ovarian, lung, bladder, thyroid gland or head and neck carcinomas. Although it is so widely used, many side effects have been observed in patients such as sores in mouth and on lips, darkening of palms and nails, unusual bleeding and bruising, nausea and vomiting, and life-threatening cardiotoxicity[2].

To eliminate the negative side effects of cancer treatment, researchers are trying to find either new analogs of DOX which are non-toxic for healthy cells or new way to deliver DOX directly into the cancer cells. For targeted delivery, it is possible to administer the drug directly into solid tumor. However, non-solid tumors or tumors with unknown location in patient's body require encapsulation of DOX in suitable nanocarrier. Liposomal form of DOX is already being sold under the trade name Myocet[3]. For enhanced biocompatibility, the liposomes were modified with polyethylene glycol under the trade name Doxil[4].

Protein based natural nanocarriers in comparison with artificial nanocarriers seem to be more suitable for delivery of the drugs in patient's body because of their lower immune response. The protein nanocarriers are usually self-assembled and can either be protein cages, viral capsids or virus-like particles[5].

In this work, we compared two types of protein nanocarriers – phage λ and apoferritin, by their ability to encapsulate anthracycline drug doxorubicine. The encapsulation was verified by fluorescence of doxorubicin after the removal of free, non-encapsulated doxorubicin by dialysis.

2. Materials and Methods

2.1 Cultivation and purification of phage λ

Phage λ-producing strain of *Escherichia coli* was cultivated in Luria-Bertani broth (1 % tryptone, 0.05 % yeast extract and 1 % sodium chloride) with 0.2 % maltose for 24 h at 37 °C and 600 rpm. After cultivation, the culture was mixed with chloroform in 6:1 ratio and incubated for 1 h at 25 °C to kill the growing

E. coli. The samples were centrifuged at 5200 g and 4 °C for 10 min to remove *E. coli* and then at 10000 g and 4 °C for 6 min to remove remaining contaminants. Next, the supernatant containing phage was ultracentrifuged at 130000 g and 4 °C for 3 h. The pellet containing phage was resuspended in PBS at a concentration of 15µg/ml for protein and stored at 4 °C.

2.2 Encapsulation of doxorubicin in phage λ

The drug, doxorubicin was encapsulated in purified phage by infusion method. 80 µl of phage was mixed with 80 µl of doxorubicin at different concentrations (200; 100; 50; 25; 12.5 and 0 µg/ml). Incubation was conducted for 2 h at 25 °C in dark. Free doxorubicin was subsequently dialyzed using Amicon 3K (Merck-Millipore, MA, USA) for 15 min at 6000 g and 20 °C and the phage was rinsed twice with water. The volume was then made to original volume (160 µl).

2.3 Encapsulation of doxorubicin in apoferritin

Doxorubicin was encapsulated in apoferritin by opening and closing in various pH. 80 µl of apoferritin (15 µg/ml) was mixed with 80 µl of doxorubicin at different concentrations (200, 100, 50, 25, 12.5 and 0 µg/ml). 1 µl of 1M hydrochloric acid was added to sample to make the pH lower to 2 and open the apoferritin. The samples were mixed by centrifugation at 25 °C and 600 rpm for 15 min. 1 µl of 1M sodium hydroxide was added to make the pH higher to 7 and encapsulate doxorubicin in apoferritin. Free doxorubicin was subsequently filtered using Amicon 3K (Merck-Millipore, MA, USA) and centrifuged for 15 min at 6000 g and 20 °C and the phage was rinsed twice with water. The volume was then made to original volume (160 µl).

2.4 Verification of doxorubicin encapsulation in nanocarriers

Absorption spectra of the nanocarriers with encapsulated doxorubicin were measured from 200 to 800 nm. Emission spectra of the samples were measured with excitation at 480 nm (absorption maximum of doxorubicin) and emission from 515 to 815 nm.

3. Results and Discussion

3.1 Encapsulation of doxorubicin in phage λ

We used infusion method for encapsulation of cytostatic drug in apoferritin. This method does not require any surface modification of phage λ proteins, which can change its properties. Many drugs are able to enter the phage capsid using the pores between individual copies of major capsid protein E and they can bind either to viral nucleic acid or its proteins[6,7].

In present experiment, the phage λ protein (concentration 20 µg/ml) was mixed with doxorubicin of various concentrations (0, 12.5, 25, 50, 100 and 200 µg/ml) and incubated in dark to avoid quenching of doxorubicin. The phage was then dialyzed and washed to remove the excess molecules of doxorubicin.

Fig. 1A shows the absorbance spectrum of phage with encapsulated doxorubicin. The control sample with no addition of doxorubicin shows absorbance maxima at 260 and 280 nm, corresponding to the viral nucleic acid and its proteins. With higher applied concentrations of doxorubicin, we can see new absorbance maxima at 480 nm, which corresponds to the absorbance maximum of free doxorubicin. The absorbance at this maxima is linearly (determination coefficient 0.8013) increasing with the increasing applied concentration of doxorubicin.

Fig. 1B shows the measured fluorescent spectrum of these samples. The control sample exhibited no fluorescence under the conditions used. However, with the increasing concentration of applied doxorubicin, the fluorescence of whole phage was linearly (0.8855) increasing.

3.2 Encapsulation of doxorubicin in apoferritin

The apoferritin structure is dependent on the surrounding pH. In low pH, the apoferritin subunits are disassociated. After mixing with the desired drug, the pH can be made back to neutral, the subunits will associate again and the drug will be encapsulated in the nanocarrier[8,9].

The apoferritin was prepared with the same concentration as phage λ (20 µg/ml). The doxorubicin with various concentrations (0, 12.5, 25, 50, 100 and 200 µg/ml) was encapsulated in apoferritin by pH-changing method. The free doxorubicin was then removed by dialysis.

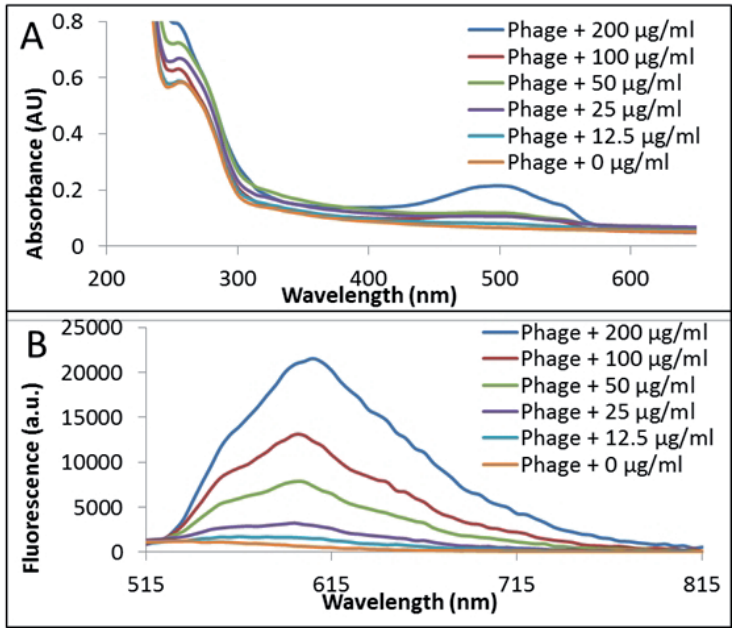

Figure 1. The influence of doxorubicin encapsulation on absorbance (1A) and emission (1B) spectra of phage λ.

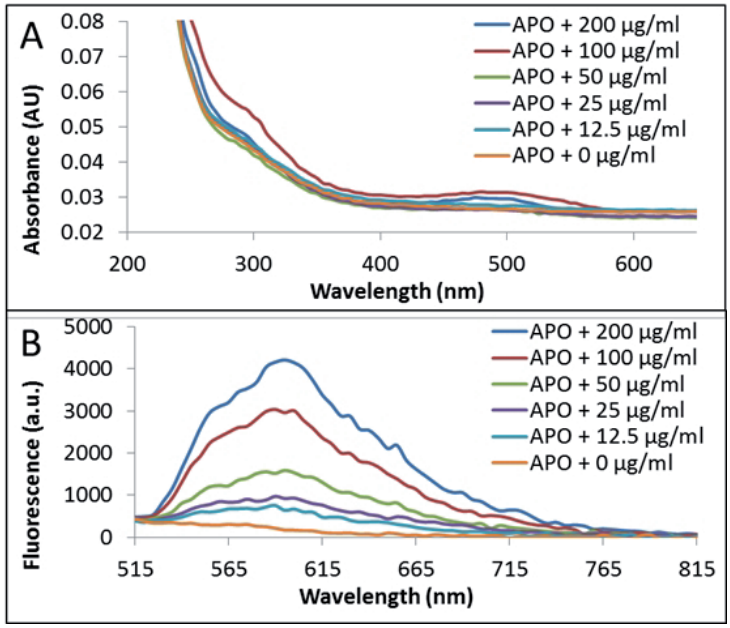

Figure 2. The influence of doxorubicin encapsulation on absorbance (2A) and emission (2B) spectra of apoferritin.

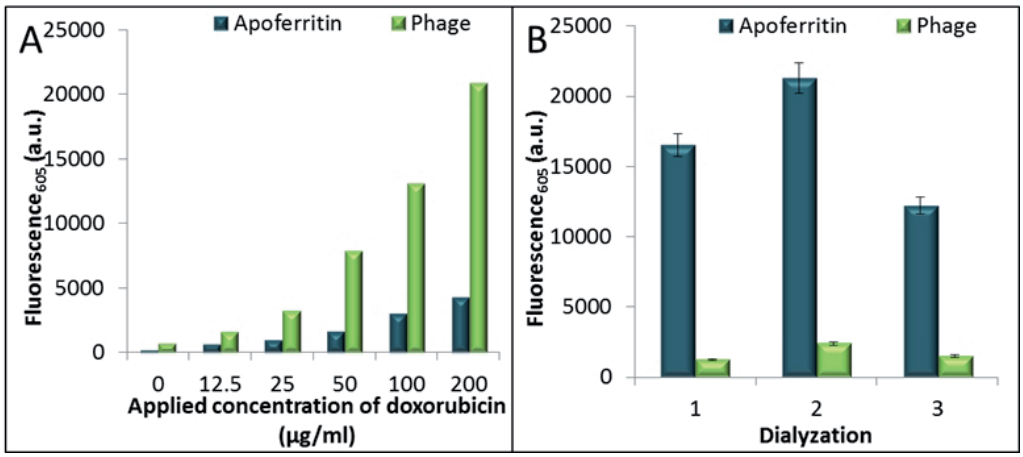

Figure 3. The comparison of phage and apoferritin as nanocarriers. Fluorescence of doxorubicin encapsulated in phage and apoferritin (3A) and undesired release of doxorubicin from nanocarriers during dialyzation measured by fluorescence (3B).

Fig. 2A shows the absorbance spectrum of apoferritin with encapsulated doxorubicin. Control apoferritin with no added doxorubicin shows only the absorbance maximum at 280 nm, corresponding to absorbance maximum of proteins. Similar as with phage λ, the increasing concentration of doxorubicin caused linearly (determination coefficient 0.788) increasing absorbance at 480 nm.

We also measured a fluorescent spectrum of the apoferritin with encapsulated doxorubicin (**Fig. 2B**). The fluorescence was also linearly (determination coefficient 0.925) dependent on the applied concentration of doxorubicin.

However, the fluorescence was 4 times higher in the case of encapsulation in phage λ than in apoferritin (**Fig 3A**). Also, we studied the undesired release of doxorubicin from both nanocarriers during dialysis. The doxorubicin was 10 times more released from apoferritin than from phage λ (**Fig. 3B**).

4. Conclusion

In this work, well-studied coliphage λ was used as a nanocarrier for doxorubicin, using the infusion method of encapsulation. The successful encapsulation was proven by absorbance and fluorescence measurement of the whole phage. The absorbance of phage λ at 480 nm increased from 0.06 to 0.22 after encapsulation of 200 µg/ml doxorubicin. The fluorescence of phage λ at 600 nm (the emission maximum of doxorubicin) increased from 1000 to 22000 after encapsulation of 200 µg/ml doxorubicin.

Encapsulation by phage λ was compared with protein nanocarrier apoferritin. At the same concentration, apoferritin was able to encapsulate 4 times lower amount of doxorubicin than phage. Undesired release of doxorubicin from apoferritin was 10 times higher in comparison with phage. Based on the obtained data, phage λ seems to be a better protein nanocarrier than apoferritin.

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The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study.

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