Using DNA nanotechnology to produce a drug delivery system*

Thi Huyen La, Thi Thu Thuy Nguyen, Van Phuc Pham, Thi Minh Huyen Nguyen and Quang Huan Le

Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

E-mail: huanlequang@gmail.com

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Abstract

Drug delivery to cancer cells in chemotherapy is one of the most advanced research topics. The effectiveness of the current cancer treatment drugs is limited because they are not capable of distinguishing between cancer cells and normal cells so that they kill not only cancer cells but also normal ones. To overcome this disadvantage by profiting from the differences in physical and chemical properties between cancer and normal cells, nanoparticles (NPs) delivering a drug are designed in a specific manner such that they can distinguish the cancer cells from the normal ones and are targeted only to the cancer cells. Currently, there are various drug delivery systems with many advantages, but sharing some common disadvantages such as difficulty with controlling the size, low encapsulation capacity and low stability. With the development and success of DNA nanotechnology, DNA strands are used to create effective drug delivery NPs with precisely controlled size and structure, safety and high stability. This article presents our study on drug encapsulation in DNA nanostructure which loaded docetaxel and curcumin in a desire to create a new and effective drug delivery system with high biological compatibility.

Keywords: nano DNA, drug delivery system, docetaxel, curcumin

Classification numbers: 4.02, 5.09

1. Introduction

Most traditional anticancer drugs have a variety of drawbacks including adverse side effects, poor selectivity and accumulation in tumors. Moreover, some of these drugs have been shown to induce multi-drug resistance in cancer cells, both in laboratory investigations and in clinical studies. In recent years, numerous nano-sized drug carriers such as micelles, nanoparticles (NPs), polymer–drug conjugates and stealth liposomes have been investigated in order to minimize side effects of anticancer drugs and enhance the antitumoral drug efficacy in cancer therapy [1–3].

Early clinical results suggest that NPs exhibit enhanced delivery efficiencies and reduced side effects due to more accurate localization in tumors and more active cellular uptake [2, 3]. The first generation of NP drug delivery systems is already in use; for example, a pegylated liposomal formulation for doxorubicin, has been used in a clinical setting for over two decades. Unfortunately, it causes only a modest increase in antitumor activity due to the slow release of doxorubicin from the liposomal sheaths [1, 4]. Metal NPs have also been targeted as promising drug delivery vehicles; gold NPs have already been used for the controlled release of chemotherapy drugs [5]. Although metal NPs are both biocompatible and chemically inert, they may be retained in the body long after administration of the drug, and accumulation of metal NPs can lead to toxicity [6, 7]. Thus, it remains a challenge to develop safe, biocompatible and effective nanocarriers for drug delivery.

With a high level of structural programmability and obvious biocompatibility, self-assembled DNA nanostructures are among the most promising candidates to serve as nanocarriers for drug delivery. It is relatively easy to construct complex DNA nanostructures with precisely defined shapes and dimensions using rational design systems.
principles [8–10]. In particular, the DNA origami method produces fully addressable structures in extremely high yields. In this method, a long single strand of DNA (scaffold strand, usually viral genomic DNA) is folded into arbitrary shapes by hundreds of short strands (staples, synthetic oligonucleotides). In addition to holding the scaffold in place, the staples provide addressable units for functionalization by various biomolecules and NPs. These modifications can be used to facilitate imaging, targeted delivery and controlled release of therapeutic compounds. Consequently, the potential of DNA origami structures for nanomedical applications has gained great interest [9,10]. Recent studies have shown that DNA macromolecules do not exhibit any obvious cytotoxicity or immunogenicity, both of which are important features of effective drug delivery vehicles [11,12]. Scaffolded DNA origami has an even greater potential to deliver therapeutic levels of drugs than polyhedral wireframe structures because the additional layers of tightly packed double-helices provide many more docking sites for intercalation. The high density of drugs can offset the effects of enzymatic degradation and the unintended release of the drug.

In this study we constructed six oligonucleotides to produce two three-dimensional drug-loaded DNA NPs: DNA NP complexes carrying docetaxel and curcumin. The complexes were administered to regular human lung cancer cells A549. The cytotoxicity of drug–DNA NPs and free drug to lung cancer cells was evaluated.

2. Materials and methods

2.1. Materials

All oligonucleotides were designed and synthesized from Macrogen (Korea) having the following sequence:

**MicelT1**: 5’-CTCAGTGACAGCGCTTCTGGAGCGT-3’

**MicelT2**: 5’-CAGAACGGCTGTGGCTAAACAGTAACC

DNA origami structures were also found to be very stable in cell lysate, a prerequisite for controlled release of a drug to subcellular targets. One of the first demonstrations of a DNA nanostructure-based drug delivery platform was reported by Chang et al [13], who developed doxorubicin aptamer conjugated DNA icosahedra that demonstrated efficient killing of cancer cells [10,12]. Scaffolded DNA origami has even greater potential to deliver therapeutic levels of drugs than polyhedral wireframe structures because the additional layers of tightly packed double-helices provide many more docking sites for intercalation. The high density of drugs can offset the effects of enzymatic degradation and the unintended release of the drug.
Table 2. Loading efficiency of curcumin into DNA NP.

| Sample | Initial curcumin concentration (mM) | Unloading curcumin (mM) | Loading efficiency of curcumin into DNA NP (%) |
|--------|-------------------------------------|-------------------------|-----------------------------------------------|
| 1      | 0.4                                 | 0.0115                  | 97.13                                         |
| 2      | 0.8                                 | 0.0233                  | 97.08                                         |
| 3      | 1.6                                 | 0.1156                  | 92.56                                         |
| 4      | 3.2                                 | 0.2375                  | 92.75                                         |

Figure 4. SEM images of DNA nanostructure: (a) in 300 nm and (b) in 500 nm.

Figure 5. SEM images of DNA–curcumin complexes: (a) in 200 nm and (b) in 500 nm.

Figure 6. SEM images of DNA–docetaxel complexes: (a) in 200 nm and (b) in 500 nm.

**MicelT3:** 5′-AGTTTCGTGGTCACTCGTTTGGGTGGTGTTGGGTGGTGGG-3′;

**MicelD1:** 5′-CGATGACCTGCTTCGGTTACTG-TTAGCCTGCTCTAC-3′;

**MicelD2:** 5′-AATGCGTAGAGCACCACTG-AGGCATT-3′;

**MicelD3:** 5′-TTTGGTGGTGGTTGGTGTTGGG-3′. Docetaxel and curcumin purchased from Sigma (St Louis, MO) were used without further purification. Anhydrous dimethyl sulfoxide (DMSO) was obtained from Merck (Darmstadt, Germany). All other chemicals of analytical grade were used without further purification.
2.2. Constructing the model of DNA NPs

The six above oligonucleotides (5 nM) were diluted in 1× TAE/Mg$_2$+ buffer (Tris, 40 mM; acetic acid, 20 mM; ethylenediaminetetraacetic acid (EDTA), 2 mM and magnesium acetate, 12.5 mM; pH 8.0) in one eppendorf with the same molar ratio. They were self-assembled to NP construction after heating to 90 °C in 5 min, then slowly cooled to room temperature.
2.3. Drug loading

Docetaxel and curcumin solution (2 mM) was incubated with the DNA NP structures (2.5 nM) for 24 h and then centrifuged at 10 000 rpm at room temperature for 10 min. After centrifuging, the dark red precipitate (drug-loaded DNA NP) and the free doxorubicin in the supernatant were isolated and quantified by measuring the absorption of doxorubicin at 480 nm with a microplate reader (TECAN, Infinite M200, Switzerland). The docetaxel (or curcumin) loading content in the DNA NP is calculated according to the following formula:

\[ C_f = C_i - C_u, \]

where \( C_f \) and \( C_i \) are the final and initial loading contents of docetaxel/curcumin in DNA NPs, respectively, \( C_u \) is unloaded docetaxel/curcumin and the loading efficiency of docetaxel/curcumin in DNA NPs is

\[ \eta = \frac{C_f}{C_i} \times 100\%. \]

2.4. Transmission electron microscopy (TEM)

TEM was performed with an JEM1010-JEOL, operated at an acceleration voltage of 100 kV. For the preparation of samples in 0.5% DMSO solution, a drop of sample solution was placed onto a 200-mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with filter paper to remove surface water, followed by air drying. Negative staining was performed by using a droplet of a 5 wt% uranyl acetate solution. The samples were air dried before measurement.

2.5. Scanning electron microscopy (SEM) morphological analysis

NP suspensions were diluted to 50 \( \mu \)g ml\(^{-1} \) and dried overnight on viewing stubs. Morphology was observed by SEM (S-4800: \( M: \times 25-\times 800.000, \delta = 1 \) nm, \( U = 0.5-30 \) kV).

2.6. Particle size/zeta potential determination

NP suspensions were diluted to 0.5 mg ml\(^{-1} \) and transferred into fold capillary cells for both zeta potential and particle size determination. Samples were analyzed using a Malvern ZetaSizer Ver.6.20 (Malvern Instruments).

2.7. Cellular uptake of DNA–drug complexes

Human lung cancer cells A549 were seeded in six well plates in standard growth medium at a density of \( 10^5 \) cells per well. After incubation overnight for 24 h, the cells were treated with DNA NPs and at different concentrations (0.4, 0.6, 0.8, 1.6 and 3.2 mM) in 0.5% DMSO solution and incubated for 48 h. Then cells were centrifuged, washed with phosphate buffered saline (PBS) solution and observed under fluorescent microscope.

3. Results and discussion

3.1. DNA NP design

All single-stranded DNA were self-assembled after heating to 90 °C in 5 min then slowly cooled to room temperature forming the construction given in figure 1.

3.2. Loading efficiency of docetaxel and curcumin in DNA NP

Dilute curcumin with concentration of 0.1 mg ml\(^{-1} \) in 0.5% DMSO solution, then measure the absorbance at a wavelength of 300–700 nm. The results showed the highest absorption of curcumin at 430 nm (figure 2).

To determine the correlation between the concentration of curcumin and the absorption of curcumin, curcumin was diluted with concentrations of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg ml\(^{-1} \). Then the optical density (OD) was measured at 430 nm. By this method we have received data of OD values and the standard curve as in table 1 and figure 3. So the curcumin used in this experiment has maximum absorption at 430 nm.

We have observed the linear correlation with the equation \( Y = 33.7X + 0.151 \), where \( X \) is the concentration of curcumin in solution (mg ml\(^{-1} \)), \( Y \) is the measured absorbance (OD). The standard curves are used in order to determine the effect of curcumin packing in complex nano-DNA. After incubating curcumin with DNA NPs, the mixture was centrifuged to separate unloaded curcumin from curcumin–DNA complexes. Unloaded curcumin in super layer is determined based on the standard curve and used to calculate the packing efficiency of curcumin on DNA NPs. Results of determining the encapsulation efficiency of curcumin into the DNA NP are shown in table 2.

By this method we obtained the values of the loading efficiency of curcumin in DNA NPs in the range 92–97%. Moreover, the results showed almost no leftovers after packing at low concentrations of curcumin but the highest encapsulation efficiency for curcumin at a concentration of 0.8 mM (294.704 mg ml\(^{-1} \)).

By the same way, loading efficiency of docetaxel in DNA NPs was 87% (for docetaxel, maximum absorption at wavelength of 234 nm).

Figure 8. Uptake of DNA–curcumin complexes by lung cancer A549 under fluorescent microscope.
3.3. SEM characterization of DNA–drug complexes

SEM images showed DNA–drug complexes having size range between 100 and 200 nm (figures 4–6).

SEM showed that the size of DNA NP without drug packaging is not clear; it sticks together. However, when linked to the drug, such as curcumin and docetaxel, DNA NPs are clearly visible. Furthermore, the structure of DNA–curcumin NPs have larger size in comparison with that of DNA–docetaxel ones.

3.4. Particle size/zeta potential determination

NP suspensions were diluted to 0.5 mg ml\(^{-1}\) and transferred into fold capillary cells for both zeta potential and particle size determination. Results of the determination of the size of the DNA NPs and DNA–drug complexes (by ZetaSizer Ver.6.20, Malvern Instruments) are presented in figure 7.

The zeta potential (mV) of DNA NPs was $-30.8$ while that of DNA–docetaxel complexes was $-5.14$. DNA NPs have high negative charge and DNA–docetaxel complexes have lower negative charge in comparison with DNA NPs, which means that DNA attracts docetaxel by electrostatic force.

3.5. Cellular uptake of DNA–drug complexes by human lung cancer cells A549

The cytotoxicity test results of the DNA NP complexes on human lung cancer cells A549 are presented in figures 8–9.

4. Discussion

One of the advantages of the drug based on the DNA molecule is the high capability to encapsulate drug complexes. In our experiment the ability to package curcumin in nano-DNA complexes reached 97%, while for docetaxel it is 87%. This is a very high packaging efficiency, while the package performance of other complexes is usually very low. For example, doxorubicin packaging efficiency of core–shell NPs is only $58.1 \pm 4.7\%$, as reported by Manaspon et al [14].

The obtained results are only initial ones. We do hope that by optimizing the conditions of the experiment as well as the ratio between the portions of the drug and the complex, it is possible to further improve the drug encapsulation efficiency of the complex. Furthermore, in order to increase the stability of the complexes, it is necessary to protect DNA–drug complexes by using natural or synthetic polymer such as polyethylene glycerol (PEG) or human serum albumin, depending on the drug absorption type: oral or intravenous. The result of the structure determination of the complexes by SEM showed that after a certain time there appears the agglutination phenomenon.

The study of the cytotoxicity on human lung cancer cell line A549 has shown that after the addition of DNA particles, cancer cells could still normally live for 48 h. The addition of docetaxel alone kills many more cells in comparison with DNA–docetaxel complex after 24 h, but after 48 h the numbers of dead cells are the same in both cases. This result shows that docetaxel kills cancer cells, but if it is packed with DNA, the concentration of docetaxel in the environment at early period is low. This drug is slowly released into the environment and its concentration reaches the maximum after 48 h.

5. Conclusion

A protocol was described for the preparation of DNA–docetaxel/curcumin using specific oligonucleotide which has
loaded drug and targeted cancer cell properties. Encapsulation efficiency of nanocomplexes reached 97% for curcumin and 87% for docetaxel. Docetaxel in the complex of DNA-docetaxel has the ability to kill cancer cells in the slow drug release mechanism.

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