Improvement of in vitro Anticancer Activity of Doxorubicin by Sodium Alginate Nanoparticles Delivery

ABSTRACT

Nanoparticles (NPs) have been widely applied in drug delivery of anticancer drugs to reduce their toxicity to normal tissue. Sodium alginate nanoparticles (ALG-NPs) were prepared in our previous study to deliver doxorubicin (DOX) to expect to increase the efficacy of DOX, thus to decrease the dosage and lessen the toxicity. The cytotoxicity of blank ALG-NPs and DOX loading ALG-NPs (DOX–ALG-NPs) were investigated in Hela, MCF-7, MDA-MB-231, A549 and NCI-H1299 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide staining method. When the concentration of ALG-NPs was not more than 125 μg/mL, ALG-NPs generally had no inhibition to the growth of cancer cells with a cell viability higher than 90%. DOX–ALG-NPs demonstrated stronger anticancer activity than free DOX solution at most tested concentrations after 24, 48 and 72 h treatment in the tested cell lines. The IC_{50} value of DOX–ALG-NPs was less than DOX solution after different treatment time in different tested cells. Higher intracellular uptake of DOX was observed in DOX–ALG-NPs group than in free DOX solution group under inverted fluorescence microscope. The results indicated that DOX–ALG-NPs might achieved the required anticancer efficacy at low concentration, thus to be possible to reduce the toxic and side effect of drug.

KEYWORDS sodium alginate nanoparticles, doxorubicin, anticancer activity in vitro

INTRODUCTION

Cancer is the second killer worldwide, and with its incidence and mortality higher and higher, effective prevention and treatment of cancer has become a worldwide problem. At present, clinical treatment of cancer chiefly depends on radiotherapy, chemotherapy and surgery treatment. Although chemical therapy is the main treatment for patients with advanced cancer, the poor selectivity of chemotherapeutic drugs cause kinds of toxic and side effect even dangerous to life. Doxorubicin (DOX) is a wide-spectrum anticancer agent of good efficacy. However, its clinical use is limited by the toxicity to many organs such as heart, kidney and lung. Many researches tried to improve its toxic and side effect and microparticle drug delivery system (DDS) has been regarded as a good choice. Development of microparticle DDS, characterized by enhancing drug efficacy as well as lowering toxic and side effect, has become one of the hot spots in pharmaceutics field.

Nanoparticles (NPs), a kind of microparticle generally in size of 10−1,000 nm, have the advantages of controlling drug release, changing the circulation time and distribution of drug in vivo, improving drug stability and bioavailability. The advantages enable NPs to play an important role in researches and application of cancer treatment. Sodium alginate (ALG) is a natural poly-anionic polysaccharide extracted from brown seaweed or kelp. It is a low-cost biodegradable polymer carrier material of rich source, good adhesive property, no toxicity and good biocompatibility. ALG was applied extensively in studies of NPs, liposomes, pellets, matrix tablets and intragastric floating DDS. In our previous work, we used ALG as carrier material to prepare DOX loading NPs, DOX–ALG-NPs. The prepared DOX–ALG-NPs have high drug loading rate about 20% and generated a sustained release of drug. In this work, we investigated the anticancer activity of DOX–ALG-NPs in different

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cancer cells to figure out its potential in chemotherapeutic drug delivery.

MATERIALS AND METHODS

Materials

Doxorubicin loading sodium alginate nanoparticles (DOX–ALG-NPs) were prepared as previously reported. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Dulbecco’s Modified Eagle medium (DMEM) were provided by Sigma Company (St Louis, MO, USA). Fetal bovine serum (FBS) were provided by Gemini Bio-Products (West Sacramento, CA, USA). Penicillin and streptomycin solution was provided by Beyotime Biotechnology Ltd. (Shanghai, China). All other chemicals and reagents used were analytical grade.

Cell culture

All human cancer cells used in this study, including human breast adenocarcinoma cell line MCF-7 and MDA-MB-231, human lung cancer cell line A549 and NCI-H1299, and human cervical cancer cell line Hela, were provided by the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM containing 10% (v/v) FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated at 37°C under 5% CO₂.

Influence of ALG-NPs on growth of cancer cells

The MCF-7, MDA-MB-231, A549, NCI-H1299 and Hela cells were seeded on a 96-well plate at a density of 5 × 10³ cells/well and cultured as mentioned above. Then the cells were treated with ALG-NPs (16 μg/mL), DOX solution and DOX–ALG-NPs for 24, 48 and 72 h, respectively. In DOX solution group and DOX-ALG-NPs group, the cells were treated with different concentrations of DOX (0.25, 0.50, 1.0, 2.0, 4.0 μg/mL). The cell viability was measured using MTT staining method described above. The inhibition rate (IR%) and the 50% inhibitory concentration (IC₅₀) was calculated. Five replicates were tested.

Intracellular DOX uptake test in cancer cells

The MCF-7, MDA-MB-231, A549, NCI-H1299 and Hela cells were seeded on a 6-well plate at a density of 1 × 10⁴ cells/well in 3 mL of DMEM containing 10% (v/v) FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated at 37°C in a humidified incubator for 24 h. The cells were treated with DOX solution and DOX-ALG-NPs for 4 h, respectively, and the concentration of DOX was 1 μg/mL. Then the cells were washed and observed under an inverted fluorescence microscope (TE2000-s, Nikon). The excitation and emission wavelength was 488 and 575 nm, respectively.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). The statistical analysis was performed with SPSS 19.0 software using the Student’s t-test. A P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Influence of ALG-NPs on growth of cancer cells

With the prolongation of treatment time, the blank ALG-NPs showed little inhibition to the growth of the tested cancer cells when concentration was not more than 125 μg/mL, but demonstrated obviously increasing inhibition at higher concentration (Fig. 1). Generally, the growth inhibition increased as the concentration of ALG-NPs increased. When the concentration was not more than 125 μg/mL, ALG-NPs had no obvious toxicity in MCF-7, MDA-MB-231, A549, NCI-H1299 and Hela cells, and the cell viability was higher than 90%. The results indicated that ALG-NPs had no interference to the anticancer activity of DOX–ALG-NPs in the tested cell lines when the concentration of ALG-NPs was not very high. Calculated by the drug loading rate, the ALG-NPs concentration was not more than 20 μg/mL in the subsequent assays.

In vitro anticancer activity of DOX–ALG-NPs in cancer cells by MTT assay

The results of cytotoxicity of DOX and DOX–ALG-NPs were illustrated in Fig. 2 and the IC₅₀ value was listed in Table 1. DOX and DOX–ALG-NPs showed distinct inhibi-
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Inhibition to the cell growth in the tested five cancer cells when drug concentration was 0.25 μg/mL and higher. The inhibitory rate varied in different cell lines. After treatment of 24 h, DOX and DOX–ALG-NPs showed relatively high toxicity in NCI-H1299 cells and Hela cells. After 48 and 72 h treatment, the inhibition rate was much great in A549, NCI-H1299 and Hela cells. The inhibition rate enhanced with the increase of drug concentration and the prolongation of treatment time. In MCF-7 and MDA-MB-231 cells, the inhibition rate of DOX–ALG-NPs was significantly higher than that of DOX solution at almost all concentrations and treatment times tested. In A549 and Hela cells, the significant difference between the cytotoxicity of DOX–ALG-NPs and DOX solution was mainly observed after treatment of 24 and 48 h, and only slight difference was observed at low concentration after 72 h treatment. In NCI-H1299 cells, the difference of cytotoxicity between DOX–ALG-NPs and DOX solution was observed after short treatment time and at low drug concentration. After different treatment time, the IC_{50} value of DOX–ALG-NPs was lower than that of DOX solution. The results suggested that loading DOX in ALG-NPs promoted the anticancer activity of DOX at appropriate concentration and treatment time. In this work, the improvement was more distinct after short treatment than long treatment as well as at low concentration than high concentration in most tested cell lines. The results may provide supporting evidence for reducing dosage of DOX when used as dosage form of ALG-NPs, which might subsequently ameliorate toxic and side effect of drug.

**Intracellular DOX uptake test in cancer cells**

Doxorubicin emits red fluorescence when excited by light source of certain wavelength. In the tested five cancer cells, stronger fluorescence was observed in DOX–ALG-NPs group when compared with DOX solution group (Fig. 3). It indicated that DOX uptake was enhanced by ALG-NPs delivery, which enabled higher drug concentration in cancer cells to result in a stronger anticancer activity.

It has been reported that there are two ways for NPs to enter cells, one is diffusion and the other is...
endocytosis. The size of NPs entering cell by diffusion were generally less than 100 nm. Most of NPs enter cell by endocytosis, which can effectively avoid efflux of membrane pump to increase the intracellular drug concentration, thus to enhance efficacy. The DOX–ALG-NPs prepared in this work were about 270 nm in spherical shape. The uptake of DOX–ALG-NPs in cancer cells was chiefly through endocytosis, while that of DOX was mainly through diffusion caused by drug concentration gradient. More researches are expected to investigate the effect of ALG-NPs on efflux of DOX.

CONCLUSION

In this work, the influence of ALG-NPs and DOX–ALG-NPs on cell growth was investigated in MCF-7, MDA-MB-231, A549, NCI-H1299 and Hela cells. ALG-NPs affected the cell viability little at the tested concentration. ALG-NPs delivery of DOX promoted the anticancer activity of DOX and reduced the IC50 value in different cancer cell lines. The higher intracellular uptake of DOX mediated by ALG-NPs may account for the enhancement of efficacy. ALG-NPs may be a potential delivery vehicle of anticancer drugs to enhance therapeutic effect and reduce toxic and side effect.

REFERENCES

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