

Cytotoxicity of Liposomal Paclitaxel in Breast Cancer Cell Line MCF-7

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Abstract Regarding that the breast cancer is the most prevalent disease among women, paclitaxel, an anti-cancer drug, could be used in treatment of this disease. As paclitaxel has adverse effects, it was used of nanoliposome drug delivery technology in order to reduce adverse effects and improve drug efficacy. Certain ratios of phosphatidylcholine, cholesterol and paclitaxel were synthesized to prepare nanoliposomal paclitaxel. Using Zeta sizer device, the mean diameter of nanoliposomal paclitaxel was obtained 421.4 nm and its encapsulation efficiency was 91.3 %. By dialysis, drug release in nanoliposome paclitaxel formulation within 28 h was studied which was 5.53 %. This study showed that cytotoxicity effect of nanoliposomal paclitaxel is more than that of the standard form.

Keywords Breast cancer · Nano drug delivery · Paclitaxel · Nanoliposome

Introduction

Breast cancer is the most common cancer among women worldwide [1]. Annually one million cases of breast cancer are diagnosed around the world [2]. Breast cancer can be treated through chemotherapy; radiation and surgery [3]. Paclitaxel is of taxanes family that is used in the treatment

of breast cancer. This drug is used in the treatment of ovarian, breast, non-small cell lung, and head and neck cancer [4–6]. Paclitaxel drug with therapeutic effects in patients can cause side effects [7]. Injected carriers in nano scale are utilized to cross biological barriers, protect drug and release the optimize dosage of the drug [8]. Liposome is one of these carriers.

Liposomes are all phospholipid vesicles composed of one or more layers which are concentric aqueous chambers [9, 10]. Liposomes protect the drug against degradation and patients against side effects [11].

The aim of this study was to investigate the effect of improving drug efficacy and reducing its side effects using nanoliposomal paclitaxel.

Materials and Methods

Phosphatidylcholine, cholesterol, paclitaxel and MTT solution (0.5 mg/ml) were purchased from Sigma Chemical Co., USA. Ethanol and isopropanol and RPMI 1640 medium were purchased from Merck Co. and Invitrogen Co., respectively. MCF-7 cells were obtained from the Pasteur Institute Cell Bank of Iran.

Preparation of Nanoliposome and Drug Encapsulation

Phosphatidylcholine and cholesterol (15 to 1 ratio) was dissolved in 120 ml of 98 % ethanol (room temperature, 300 rpm) to obtain a transparent and yellow suspension. Then 13 mg of paclitaxel was added and mixed with a magnetic stirrer (ambient temperature, 30 min, 300 rpm). The solvent phase was evaporated by rotary evaporator (50 °C, 100 rpm) (Heidolph Co., Germany) and the resulted gels was dissolved in 13 ml of physiological serum.

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Then the obtained formulation was sonicated for 5 min (Bandelin Sonorex Digitec, 60 Hz).

Size Determination of Nanoliposomes

The mean diameter of the liposomes was determined using Zeta sizer device (Malvern Instruments Ltd.).

Encapsulation Efficiency

To evaluate the percentage of encapsulated drug, 2 ml (2 mg) of the formulation of liposomal paclitaxel was centrifuged at speed 13,000 rpm for 30 min at 4 °C. Then using a spectrophotometer (Model UV-1601PC company SHIMADZU), supernatant absorbance was removed at the wavelength of 227 nm. Then using the following Formula, encapsulation efficiency was calculated.

$$\text{Loading efficiency \%} = \frac{\text{encapsulated drug}}{\text{maximum encapsulated}} \times 100 \quad (1)$$

In order to plot the standard curve, the serial concentrations of the standard paclitaxel were prepared and their light absorbance was measured using a spectrophotometer at 227 nm wavelength.

Drug Release Examination

To study the release pattern of liposomal paclitaxel, 1 ml of the liposomal drug formulation was poured in the dialysis bag and placed in 100 ml phosphate buffered saline (PBS) pH 7.4 and then put on magnetic stirrer (28 h at 37 °C). To assess the pattern of drug release at various times, 1 ml of PBS buffer was removed and replaced with a 1 ml of fresh phosphate buffer. The light absorbance of the samples was measured at 227 nm wavelength at different times and then the concentration of the free drug in each period was calculated using the standard curve. Finally, the drug release profile versus time was plotted.

Toxicological Effects of Drugs

After culturing the cell line MCF-7, 100 µl of a suspension containing 10,000 cells was decanted in a 96 well plates at temperature of 37 °C and was incubated under CO₂ 5 %. After 24 h, the cell supernatant was removed and various concentrations of the nanoliposomal paclitaxel formulation and the control were poured on cells. Then they were incubated for 24 h at above conditions. After the supernatant was removed, 100 µl of a solution of MTT (0.5 mg/ml) was added and after 3 h incubation, the purple color (the Formazan formation) was solved in living cells in 100 µl of isopropanol. Light absorbance at 570 nm wavelength was

measured (Spectrophotometer Power Eave XS) and IC₅₀ was calculated using the pharm program.

Results

Size Determination of Nanoliposomes

The mean diameter of nanoliposomal Paclitaxel was obtained 421.4 nm.

Encapsulation Efficiency

Encapsulation efficiency (EE %) of the formulation were determined 91.3 % according to the Formula 1.

Evaluation of Drug Release

Paclitaxel released from the nanoliposomal paclitaxel formulation in PBS buffer was calculated during time intervals of 30 min, 1 h, 2, 3, 24, and 28 h using the standard curve of paclitaxel (Fig. 1). The following chart is based on the drug release pattern. This chart shows the maximum amount of drug released from liposomes within 28 h, about 5.53 %.

Investigation of Drug Cytotoxicity

Cytotoxicity of the drug in different concentrations was evaluated based on MTT method. The results are given in Fig. 2.

Discussion

Drug delivery is one of the main challenges facing biotech [11]. It has been proven that nanocarriers have the ability to deliver drugs to the targeted cells. Liposome is one of lipid

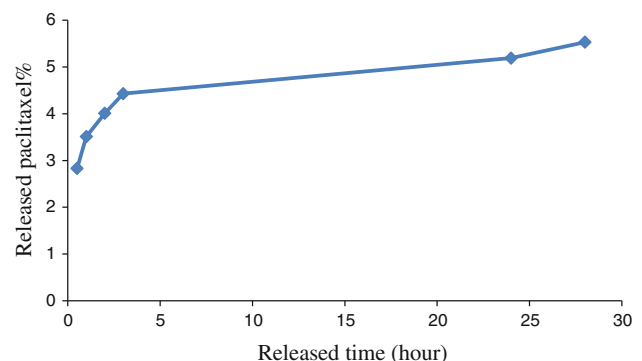


Fig. 1 Paclitaxel release from formulations liposomal paclitaxel in PBS buffer

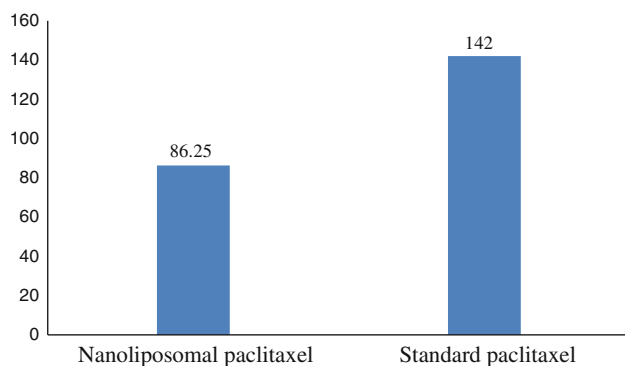


Fig. 2 The values of IC₅₀ (µl/ml) for two formulations nanoliposomal paclitaxel and standard paclitaxel

nanocarriers. Liposomes protect the drug against degradation and the patient against side effects of encapsulated drug [8].

In 2005, Chen et al. [12] investigated the effect of adriamycin encapsulated in the liposome prescribed for spreaded models of the breast cancer by different ways. The results indicate a high percentage of adriamycin encapsulated in liposome absorbance by tumor cells and the results showed the increment of toxicity of the drug. In 2007, Giotta et al. [13] studied the doxorubicin drug encapsulated in the liposome in cyclophosphamide combination as the first-line treatment of breast cancer. Results of the survey indicated an increase in cytotoxic drug encapsulated in liposomes compared to free form of doxorubicin.

In this study, the influence of the nanoliposomal paclitaxel formulation on breast cancer cell line was studied which shows that a significant amount of drug efficiency encapsulation is located inside the vesicles and this can be largely controlled by the ratio of phospholipid, cholesterol, drugs and methods (the reverse-phase evaporation). The study of nanoliposomal drug release pattern indicates that the drug release is relatively slow and as expected, using liposomes as a drug delivery factor has a significant role in slowing the drug release. This is because it takes more time for the drug to pass through the layers of phospholipid layers of the liposome.

The comparison between toxicological effects of the nanoliposomal paclitaxel formulation with standard

paclitaxel indicates the more effect of nanoliposomal paclitaxel formulation in destroying cancer cells. To justify this it can be said that the nanoliposomal paclitaxel formulation because of owing phospholipid structures similar to the bilayer structure of MCF-7 cell membranes could better interact with these cells and by welding them together and release the drug directly into the target cell cause the death increment of these cells.

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