Evaluation of Apoptosis Rate of Pegylated Nanoliposomal Oxaliplatin in Breast Cancer MCF-7 and MDA-MB-231 Cells

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ABSTRACT

Cancer is an extremely dangerous disease among humans. Breast cancer is still most frequently form of cancer within women population. Oxaliplatin is a apoptotic effectual compound in order to treatment of cancer with cytotoxic side effects. Nanocarriers with specific physicochemical properties are able to reduce side effects of anticancer drugs. Nanoliposomes as carrier are the promising system for cancer treatmen due to stability, high targeting properties, better bioavailability, slow releasing and low systemic toxicity. By using polyethylene glycol (PEG) can increase blood-circulation time of the drug with decreased side effects and improved efficacy also PEG can increase penetration to the tumor tissue. In this investigation, oxaliplatin was loaded onto pegylated liposomes through reverse phase evaporation method. Profile of drug release were evaluated. Pegylated nanoliposomal oxaliplatin exhibited slow releasing potential in comparison to conventional formulations. The morphology of particles in each field was analyzed by scanning electron microscopy (SEM). Apoptosis rate was evaluated in breast cancer cell lines MCF-7 and MDA-MB-231 by flow cytometry. Pegylated nanoliposomal oxaliplatin showed a suitable impact on apoptosis of breast cancer cell lines MCF-7 and MDA-MB-231. Thus, pegylated nanoliposomal oxaliplatin can employ with low systemic toxicity in order to treatment of breast cancer.

Keywords: Breast cancer, Oxaliplatin, Liposome, Polyethylene glycol, Nano drug delivery.

INTRODUCTION

Cancerous diseases are considered as major causes of Deaths and mortality in the world and at present, breast cancer is one of the cancers causing death in women population. There are several ways for curing breast cancer and one of them is chemotherapy¹.

One of chemotherapy drugs based on platinum for treatment of cancer is oxaliplatin (Fig.1)². Oxaliplatin Anticancer drug is third generation
platinum (Pt). Oxaliplatin is highly potent which be employed to treat cancer. It inhibits DNA synthesis by involving DNA cross linking mechanisms.

Liposomes are vesicles of lipid closed bilayers phospholipid systems with an aqueous cavity. Liposomes can be used to vesiculize both hydrophilic and lipophilic drugs. Modes of liposome/cell intraction are consist of fusion and endocytosis. Polyethylene glycol attached to surface of a liposome can reduce interactions with reticuloendothelial system (RES) and increase blood – circulation time of the liposome (Figure 2).

Liposomes accumulate in tumor tissue due to the phenomenon of enhanced permeability and retention (EPR) effect.

Purpose of this investigation was improvment therapeutic index of breast cancer.

MATERIALS AND METHODS

Materials

MTT (3-(4,5-di-methylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), phosphatidylcholine, cholesterol, polyethylene glycol 2000 (PEG 2000), dextrose, fluorescent iso-thiocynate (FITC), propidium iodide (PI) were purchased from sigma company (SIGMA, USA). Chloroform, Ethanol and Isopropanol purchased from Merck Company (Merck, Germany). The RPMI-1640 culture medium was purchased from Invitrogen (Invitrogen, USA) and Oxaliplatin was prepared from Sobhan Ancology.
Company. Breast cancer cell lines (MCF-7, MDA-MB-231) were purchased from national cell bank of Pasteur institute of Iran.

**Preparation of nanoparticles pegylated liposomal oxaliplatin**

Pegylated liposomal oxaliplatin was prepared using the reverse phase evaporation technique. Phosphatidylcholine and cholesterol and polyethylene glycol 2000 (molar ratio 2:1:0.2) were dissolved in 15 ml chloroform and then 5 ml of oxaliplatin solution (1 mg/ml) in 5% (w/v) dextrose was added to resultant mixture. Sample no oxaliplatin-containing liposomes, 5% dextrose solution was dropped instead of oxaliplatin solution. The resultant solution was stirred (at 300 rpm, at room temperature, 1 h) to gain a transparent, yellow suspension. The solvent phase was removed by rotary evaporator (0.045 mpa, 2 h, 40° C) (Heidoiph, Germany) (pegylated nanoliposomal oxaliplatin and pegylated nano liposomal). The buffer phosphate saline (pH 7.4) (30 ml) was dropped into resultant liposomes. Resultant solution was stirred (150 rpm, room temperature, 24 h). The emulsion was sonicated (Bandelin Sonorex Digital, 60 Hz) for 10 min to reduce the size of liposomes. Then resultant solution was homogenised (7000 rpm, 5 min) (7000 rpm, 30 min) and then was extruded through a polycarbonate membrane (200 nm pore size) (pegylated nanoliposomal oxaliplatin and pegylated nano liposomal).

**Scanning Electron Microscopy (SEM)**

A drop of aqueous suspension of pegylated nanoliposomal oxaliplatin was spread on a slab and dried under vacuum. The diameter of particles in each sample (sample hemogenised at 7000 rpm, 5 min and sample hemogenised at 7000 rpm, 30 min) was analyzed by using JSM-5200 operation of scanning electron microscope (Tokyo, Japan) at 15 kV.

**In Vitro Release Study**

Oxaliplatin release rate was evaluated by membrane diffusion method. The liposome suspension equivalent to 1 mg of Oxaliplatin and pegylated nanoliposomal oxaliplatin was poured into dialysis bags (cut off 12000Da, sigma) separately. The dialysis bags were immersed inside a container containing 25 ml of phosphate buffer, pH 7.4, and left on the magnetic stirrer (37°C, 120 rpm, 24 h) separately. At certain intervals, 1.5 ml of phosphate buffer was taken and replaced with an equal volume of phephosphate buffer. The ODs samples were separately measured by spectrophotometr at wavelength of 210 nm.

**Evaluation of apoptosis rate**

Apoptose rate was evaluated on breast cancer cell lines MCF-7 and MDA-MB-231 by flow cytometry.

Cells cultured in 6-well plates were treated with pure oxaliplatin and pegylated nano liposomal...
oxaliplatin with the same amount of oxaliplatin and blank control (untreated cells were used as controls) for 72 h.

The cells were trypsinized, washed (by two washes with cold PBS) and re-suspended in 400 l of PBS (1 x 10^6 cells/well), and stained with Annexin V-FITC (5 l) then incubated in the dark for 15 min. Cells were subsequently stained with PI (10 l) and incubated in the dark for 5 min prior to evaluation by flow cytometry. (Annexin V-FITC at wavelength 525 nm) (PI at wavelength 631 nm)

**RESULT AND DISCUSSION**

**Morphology of nanoparticles**

Morphology of nanoparticles was analyzed by using scanning electron microscopy (SEM) (sample hemogenised at 7000 rpm, 5 min and sample hemogenised at 7000 rpm, 30 min).

The mean diameter of particles in sample hemogenised at 7000 rpm, 5 min was 147.7 nm (Fig. 3). The mean diameter of particles in sample hemogenised at 7000 rpm, 30 min was 60 nm (Figure 4).

**In Vitro Release Study**

The drug release profile indicated controlled release of Oxaliplatin from nanoparticles. The data of released oxaliplatin of pure oxaliplatin and pegylated nanoliposomal oxaliplatin in phosphate buffer were obtained at 24 h.

**Analysis of apoptosis rate**

Oxaliplatin-chitosan nanoparticles induced more apoptosis rate when compared with free Oxaliplatin and number of apoptotic cells increased in colorectal cancer.

Our results exhibited that pegylated nanoliposomal oxaliplatin induced suitable apoptosis rate in breast cancer cell lines MCF-7 and MDA-MB-231 at 72 h.

**Fig. 6: Apoptosis rate of pegylated nanoliposomal oxaliplatin and pure oxaliplatin was assessed for MCF-7 cell line by flow cytometry at 72h**

**Fig. 7: Apoptosis rate of pegylated nanoliposomal oxaliplatin and pure oxaliplatin was assessed for MDA-MB-231 cell line by flow cytometry at 72h**
CONCLUSION

In this article, novel formulation of oxaliplatin used as a promising method in order to improve the therapeutic index of breast cancer.

Profile of drug release indicated that pegylated nanoliposomal oxaliplatin had a slower release in comparison to pour oxaliplatin formulations therefore help to decrease the cytotoxicity of Oxaliplatin to the normal tissue. Reduction of the release rate of drug can be originated due to the presence of Poly ethylene glycol (PEG).

This study showed that pegylated nanoliposomal oxaliplatin induced suitable apoptosis rate in breast cancer MCF-7 and MDA-MB-231 cells.

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