Preparation of pegylated nanoliposomal oxaliplatin and investigation of its Efficacy on breast cancer cell lines MCF-7 and MDA-MB-231

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Cancer is an important issue and cause of death among human breast cancer is the most common form of cancer types within women population in world. Oxaliplatin is a chemotherapy compound for treatment of cancer with side effects. Drug delivery system (DDS) and using of nanocarriers are known as one of the effective methods for cancer treatment. In this study, nanotechnology was applied to decrease drug side effects and enhancement of therapeutic index. In order to improve chemotherapeutic effective of Oxaliplatin, Nanoliposomation is used in pharmacological as beneficial developments. To achieve this results polymers, such as polyethylene glycol can reduce interactions with reticuloendothelial system (RES) and increase the circulation lifetime of the drug. In this work, pegylated nanoliposomal oxaliplatin was synthesized by reverse phase evaporation technique. The particle size and zeta potential were confirmed with dynamic light scattering (DLS). The results showed that the size of pegylated nanoliposomal oxaliplatin was147.7 nm. The zeta potential of pegylated nanoliposomal oxaliplatin assessed -20.5 mv. Zeta potential exhibits stability of pegylated nanoliposomal preparation. Morphology of liposomal nanodrug were examined by scanning electron microscopy (SEM).Entrapment efficiency of pegylated nanoliposomal oxalipltin was determinated 40.2%. Cytotoxicity effects of drug-loaded nanoparticles and pure drug were evaluated for MCF-7 and MDA-MB-231 breast cancer cell lines by MTT assay. The IC50 values for pegylated nanoliposomal oxaliplatin and pure oxaliplatin were assessed 0.1046 mg/ml and 0. 2307 mg/ml respectively for MCF-7 and 0.05125 mg/ml and 0.1023 mg/ml respectively for MDA-MB-231, hence the IC50 values of pegylated nanoliposomal oxaliplatin was estimated as 1/2 the IC50 of pure oxaliplatin. This indicated cytotoxicity of oxaliplatin-loaded nanoparticle was decreased in comparison to pure drug. Finally, in this investigation, effect of pegylated nanoliposomal oxaliplatin on MDA-MB-231 showed 2-times decrease of (in) IC50 value in comparison with MCF-7 at 96 h incubation.

Keywords: Breast cancer, Oxaliplatin, Liposome, polyethylene glycol, Nano drug delivery

1. INTRODUCTION

Cancer represents one of the most common diseases in the world. As shown in figure 1 the breast cancer is the major cause of mortality among women world [1]. Breast cancer increases with age and all women are at risk. It is always caused by a genetic abnormality. However, only 5-10% of cancers are due to abnormality inherited from parents. About the 90% of breast cancers are due to genetic abnormalities that happen as a result of the aging process and the "wear and tear" of life in general. Treatment of breast cancer is consists of surgery, radiotherapy, hormonal therapy and chemotherapy [2]. Oxaliplatin is a chemotherapy drug based on platinum for curing breast cancer. Platinum compounds is include of cisplatin, carboplatin, oxaliplatin, satraplatin, nadaplatin, triplatin, etc [3]. Oxaliplatin antitumor compound is third generation platinum (Pt) [4]. The mechanism action of
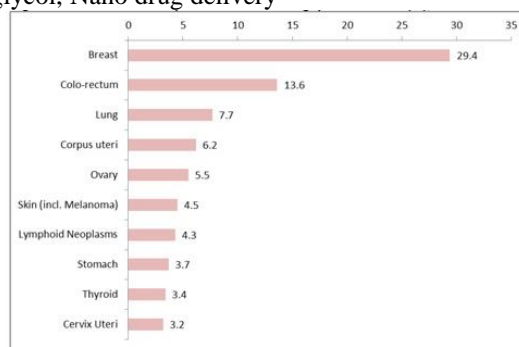
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Figure 1. The most common cancer in women.

Oxaliplatin is similar to mechanism of cisplatin, involving DNA cross linking mechanisms [5]. Oxaliplatin is one of potent compounds be used in treatment of cancer, which inhibits DNA replication and transcription [6].

In order to improve chemotherapeutic effective of oxaliplatin, nanoliposomation is used in pharmacological as beneficial developments. Liposomes are colloidal, spherically shape vesicles of one or more lipid closed bilayers phospholipid systems and were used as drug delivery systems [7]. To achieve this results polymers, such as polyethylene glycol can reduce interactions with

reticuloendothelial system (RES) and increase the circulation lifetime of the drug. Liposomes are able to pass successfully through biological barriers protection of drugs against destructive conditions and compounds, delivering the drug to the target tissue [8]. Polyethylene glycol used to increase resistance, applicability and solubility and extending the circulation lifetime of liposoms [9, 10]. Polyethylene glycolis used to reduce interactions with reticuloendothelial cells and not to be taken up by them [11]. Liposomal drug nanoparticles can self-load the drug as a carrier and prevents damage to non-cancerous tissues. Moreover, they can increase the rate of drug delivery to target cells (figure (2)). Indeed, liposome uptake by tumors relies primarily on the enhanced permeability and retention (EPR) effect [12, 13].
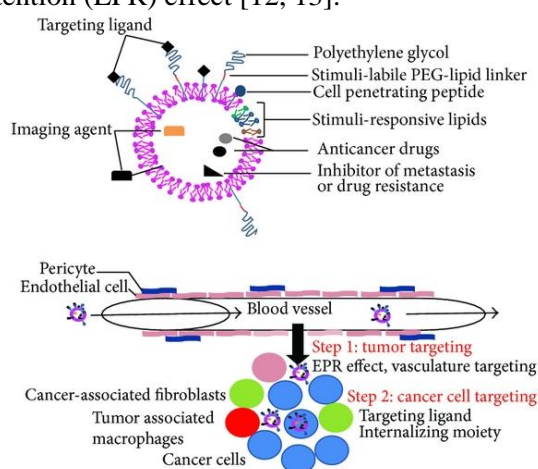

Figure 2. Schematic picture of a liposomal nanocarrier and its effect on cancer cell [13].

This article aimed to improve the drug therapeutic index and decreasing its side effects by preparation of pegylated nanoliposomal of oxaliplatin.

2. MATERIALS AND METHODS

2.1 Materials

Phosphatidylcholine, cholesterol, polyethylene glycol 2000 (PEG 2000) and MTT (3-(4,5-di-methylthiazolyl-2)-2, 5-di-phenyltetrazolium bromide), were purchased from sigma company (SIGMA, USA). 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) were purchased from cell bank of Pasteur institute Iran.

2.2 Preparation of pegylated nanoliposomal oxaliplatin

In order to produce pegylated liposomal oxaliplatin, phosphatidylcholine (50mg) and cholesterol (10mg)

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and polyethylene glycol 2000 (15mg) were dissolved in 15 ml chloroform and then 5 ml of oxaliplatin solution (1 mg/ml) added to resultant mixture. The resultant solution was stirred (at 300 rpm, at room temperature, 1h) to gain a transparent, yellow suspension. The solvent phase was removed by rotary evaporator (Heidoiph, Germany). The buffer phosphate saline (pH 7.4) (30 ml) was added to resultant film. Resultant solution was stirred (150 rpm, room temperature, 24 h). Also control solution was prepared without oxaliplatin. The emulsion was sonicated (Bandelin Sonorex Digital, 60 HZ) for 10 min to reduce the size of liposomes. [14] Then resultant solution was hemogenised (7000 rpm, 5 min)and Then was extruded through a polycarbonate membrane (200 nm pore size) (pegylated nanoliposomal oxaliplatin and pegylated nano liposomal) .

2.3 The particle size and zeta potential measurement

The particle size and zeta potential were measured by Zeta sizer (Malvern, Instruments Ltd, Worcestershire, Zen 3600 UK) [15].
Charges present on the surface of the vesicles indicate in vivo performance of the liposomes. Thus the zeta potential demonstrates the degree of repulsion between adjacent vesicles.

3. Entrapment efficiency

For evaluation of encapsulated oxaliplatin, 1 ml from formulation was centrifuged at 13,000 rpm for 2 h at 4°C and the optical density (OD) of the upper phase of solutions were measured at 210 nm wavelength by spectrophotometer (UV-160IPC, Shimadzu, Japan). Calculations were performed by using the standard curve. The standard curve of oxaliplatin was obtained by different concentration of pure oxaliplatin and their optical density(OD) were determinated (measured) at 210 nm 254 nm wavelength by spectrophotometer. Encapsulation efficiencies were calculated as follows [16]:

$$\eta_{OX} = \frac{OX_a}{OX_t} \times 100 \qquad (1)$$

Where, η is the oxaliplatin encapsulation efficiency, OX_a and OX_t are actual and theory amount of oxaliplatin loaded in nanoparticles respectively.

3.1. In vitro release study

In order to determine of the oxaliplatin release rate from liposomes employed membrane diffusion method, thus 1 ml from oxaliplatin and pegylated nanoliposomal oxaliplatin added to the dialysis bags(cut off 12000Da, sigma) separately and dialysis bags were transported to 25 ml phosphate buffer, pH 7.4, and left on the magnetic stirrer (37 ºC, 120 rpm,

24 h) separately. At certain intervals, 1.5 ml of phosphate buffer around dialysis bags was taken and replaced with an equal volume of the phosphate buffer. The optical density samples were separately measured by spectrophotomer at 210 nm wavelength. Then the amounts of released oxaliplatin and pegylated nanoliposomal oxaliplatin in phosphate buffer were assessed by spectrophotometer and standard curve [17].

3.2 Evaluation of cellular cytotoxicity

Cellular cytotoxicity was evaluated on breast cancer cell lines MCF-7 and MDA-MB-231 by MTT assay.

A sample of 100 µL of cells suspension contained 10,000 cells were seeded onto 96-well plates in 1 ml of RPMI 1640 medium containing 10% fetal bovine serum (FBS) and incubated for 24 hours under 10% carbon dioxide at 37° C. Subsequent to 24 hours of cells culturing, the supernatant was removed and various concentrations of formulations of pure oxaliplatin and pegylated nano liposomal oxaliplatin and its control was substituted and then incubated for 96 hours at 37°C. After 96 h of incubation again culture supernatants were poured off and 100 µL the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium Bromide (MTT) solution with pH equal to 4.7 was added to each well and incubated for 3 h. MTT solution was replaced with 100 µL Isopropanol and stirred to dissolve the formed Formazan crystals. The optical absorption was determined at 570 nm by a spectrophotometer. The cell viability rate was calculated from the ratio of treated cells absorption with different formulations of the drug to the absorption of control cells, and the results were assessed by the Pharm program. IC50 values (IC50 values expressed drug concentrations that cause 50% cell death) was determined for each of the samples. The experiment was performed in triplicates. Cytotoxicity effects of drug nanoparticles and pure drug were evaluated for breast cancer cell lines MCF-7 and MDA-MB-231 by MTT assay.

4. RESULT AND DISCUSSION

Polyethylene glycol- coated liposomes can reduce interactions with reticuloendothelial cells and increase the circulation lifetime for the drug [17].

Zeta potential increases repulsive forces, while electrostatic repulsion of particles with the same electric charge prevents aggregation of the spheres particles [18].

Oxaliplatin is a third generation of platinum (Pt) anti-tumor compound and has minimum ototoxicity and nephro-toxicity in comparison to cisplatin and carboplatin platinum compounds [19, 20].

4.1. Morphology and determination of particle sizes and zeta potential

The mean diameter of pegylated nano-liposomal oxaliplatin was determined as 147/7 nm. The zeta potential of pegylated nanoliposomal oxaliplatin was assessed -20.5 mv. Zeta potential exhibit stability of pegylated nano liposomal preparation.
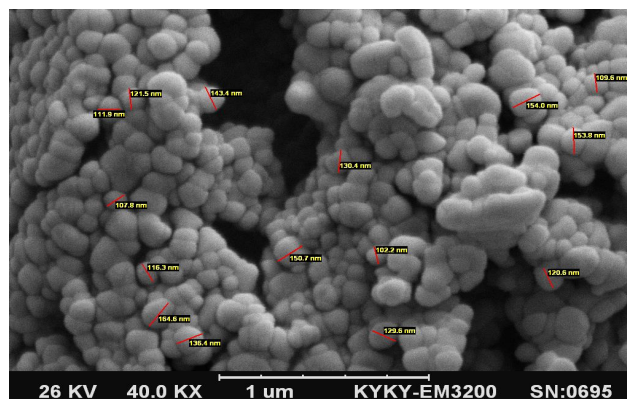

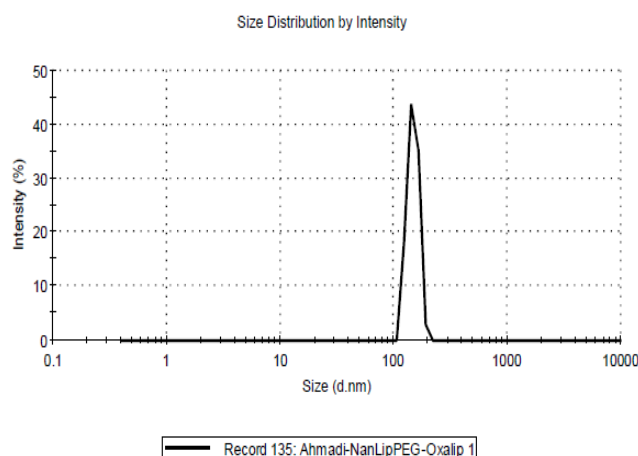**Figure 3.** Representative scanning electron microscopy (SEM) image of nanodrug.

Figure 4. Dynamic light scattering (DLS) examination for the particle size distribution.

4.2. Entrapment efficiency

The entrapment efficiency was calculated according to the standard curve and using the encapsulation formula. The percent of encapsulation was 40.2%.

4.3. In vitro release study

The values of released oxaliplatin from two formulations of pure oxaliplatin and pegylated nanoliposomal oxaliplatin in phosphate buffer was assessed at 1, 2, 3, 4, 5, 6, 17, 18, 20, 22, 24, 48, 96, 120 hours. Our results demonstrate the release of oxaliplatin from pegylated nanoliposomal was gradually increased over time at 6 hours about 38% and at 120 h there was an increase of over 80% and pure oxaliplatin exhibited the highest level at 2,3,4 hours about 90% .

4.4. Cytotoxicity assay

In our investigation, pegylated nanoliposomal oxaliplatin exhibited slightly decrease of cytotoxicity when cell viability was evaluated (determined) at 24 and 48 h and it showed increase of cytotoxicity when cell viability was evaluated (determined) at 96 h. This could be due to retard release of oxaliplatin from pegylated nanoliposomal oxaliplatin in compared to pure oxaliplatin. Cause of this observation is reduction of oxaliplatin release rate from liposomes at 48 h and a drastic increase of oxaliplatin release rate from liposomes at 96 h.

IC50 values express (represent) drug concentrations that cause 50% cell death for the pure oxaliplatin and pegylated nano liposomal oxaliplatin. Results demonstrated the higher effectiveness of the pegylated nano liposomal oxaliplatin formulation in comparison to pure oxaliplatin. Also, it depicted that the cytotoxicity of the empty pegylated nano liposomal was low.

In MTT assay, the IC50 of the pegylated nano liposomal oxaliplatin formulation was about 1/2 the IC50 of pure oxaliplatin at 96 h incubation. (MCF-7 and MDA-MB-231 breast cancer cell lines).

Effect of pegylated nanoliposomal oxaliplatin on MDA-MB-231 showed about 2-times decrease in IC50 value in comparison to MCF-7 at 96 h incubation. This could originate from increasing of cellular uptake by MDA-MB-231 cell line in comparison to MCF-7 cell line.
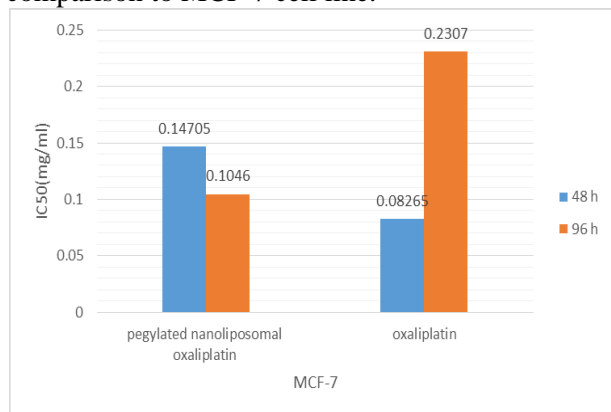
Figure 5. The IC50 of pegylated nanoliposomal oxaliplatin and pure oxaliplatin was assessed for MCF-7 cell line by using MTT assay at 48h and 96h.
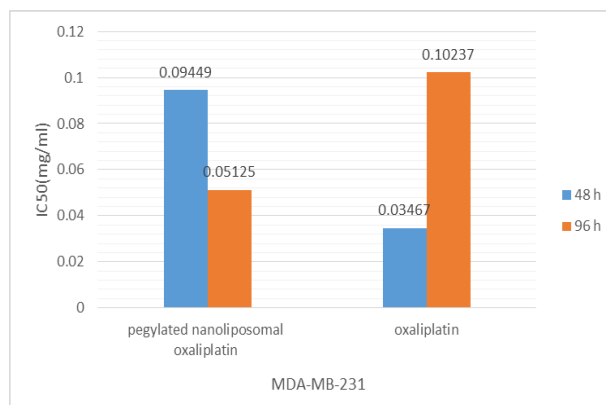
Figure 6. The IC50 of pegylated nanoliposomal oxaliplatin and pure oxaliplatin was assessed for MDA-MB-231 cell line by using MTT assay at 48h and 96h.
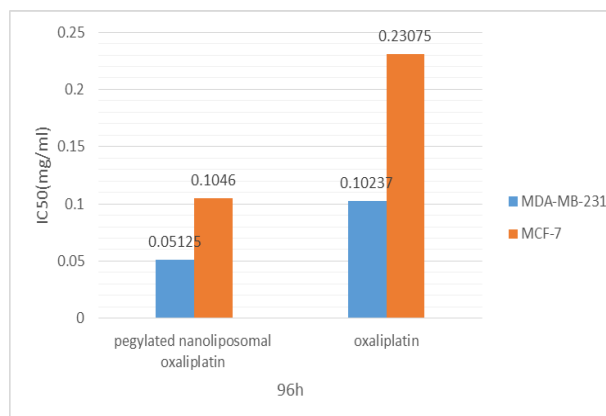
Figure 7. The IC50 values of pegylated nanoliposomal oxaliplatin and pure oxaliplatin were evaluated on breast cancer cell lines MCF-7 and MDA-MB-231 by MTT assay.

5. CONCLUSION

In this paper, a novel method was proposed that Pegylated liposomes at nano scale carry oxaliplatin in order to increase the effectiveness and decrease the side effects of the drug. Pegylated nanoliposomal oxaliplatin formulation was successfully prepared by reverse phase evaporation method. The results indicated that cytotoxicity of pegylated nanoliposomal oxaliplatin formulations was better. In this approach, it was found that pegylated nanoliposomal oxaliplatin had a slower release of oxaliplatin to prevent drug dispertion before reaching the cancer cells. The in vitro release study showed that pegylated nanoliposomal oxaliplatin on MDA-MB-231 showed about 2-times decrease in IC50 value compared with MCF-7 at 96 h incubation.

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