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IN VITRO EVALUATION OF LOADED PACLITAXEL ON THE NIOSOME NANOPARTICLES AND ITS CYTOTOXIC EFFECTS ON THE CELL LINE OF OVARIAN CANCER A2780S

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ABSTRACT

Today, cancer is one the most important challenges in modern medicine. Meanwhile, ovarian cancer is one of the most common causes of mortality among cancers. The initial response to the treatment and then becoming resistant to the paclitaxel is one of basal challenges of treatment of ovarian cancer. Recently, using nanotechnology including drug nanocarrier named niosome can decrease adverse effects and increase the efficiency of treatment. The aim of this research is using niosome nanoparticles containing paclitaxel and investigation of their lethal effect on ovarian cancer cell line. Niosome nanoparticle is prepared by injection ether method. The research found that by using of niosome nanoparticles can be provided a suitable formulation of paclitaxel drug. Therefore, the results show the efficiency of nanoniosome paclitaxel is more than free drug. This

decreases used dose and therefore the damage of other tissues.

KEYWORDS: Nanoniosome, Paclitaxel, ovarian cancer, Iran.

INTRODUCTION

Cancer starts when cell mutates in the growth controlling genes. In the natural modes, if cell mutates irreparably, it will kill himself but if it can't kill himself, these cells and/or their progeny and lineage may divide uncontrollably with wrong genetic information.[1] Ovarian cancer is fifth cause of mortality of cancer among women. The symptoms of this disease are non-specific and most women are diagnosed in advanced stages (stages 3 & 4) of disease. Released peritoneal, lymphatic, or bloody metastases by peritoneum are the most common metastases.[2] Prevalent treatment of advanced ovarian cancer is surgery and then chemotherapy by using of drugs on the base of platinum. Unfortunately, unlike initial response to the chemotherapy, there is significant risk about relapse and resistance against chemotherapy. This decreases life span. Only 5-15% diagnosed patients in advanced stages live 10 years. Therefore, studying for finding new chemotherapy drugs or bearing down drug resistant mechanisms for treatment of this disease are very important.[3] In this study formulation was prepared by reverse phase evaporation employing Span 60. Taxanes (Paclitaxel) is an effective Chemotherapy agent which is extracted is from the bark of Taxus brevifolia.In clinics, paclitaxel is used For tumor treatment ovarian.[4] Recently, nanotechnology allows targeted treatment to reduce adverse effects of drug and increase of efficiency. Nano-scale drug carriers are used for passing of biological barriers and drug release. Niosome is one of these carriers.[5] Niosomes are non-ionic surfactant vesicles that their vesicle system can be used as carriers of lipophilic and amphiphilic. Theirs non-ionic property leads to less toxicity and theirs limited reaction with cell that increase therapeutic index of encapsulated drug.[6] To date, different carriers have been used for delivery of paclitaxel, but researchers have failed to produce a suitable nanoparticle formulation from paclitaxel.

METHODS AND MATERIALS

Materials

Paclitaxel, span 60, cholesterol, polyethylene glycol 6000, Culture medium RPMI 16-40, ethanol, isopropanol and diethyl ether were purchased from Sigma Company. A2780S cell was provided from cell bank of Iran Pasteur Institute.

Preparation of nanoparticles containing drug

At first 150 mg span 60, 75 mg cholesterol and 14 mg polyethylene glycol 6000 were mixed in 14 ml diethyl ether. Then 2 ml ethanol 96% containing 30 mg paclitaxel was added during two steps. The mixture was put on the stirrer for 1h at 37ºC and 300 rpm to mix completely. After full dissolution, obtained solution was dropped to the 8 ml phosphate buffer (pH 7.2) that has been put on the stirrer at 70ºC. With slow injection of lipid solution to the watery phase, temperature difference leads to quick evaporation of ether and then formation of niosomes. Vesicles were put on the sonicator device for 3 min at ambient temperature to homogenize. And then the homogenizer 10000 rpm for 4 minutes more size of the vesicles were uniform.

Determination of size of nanoniosomes

For determination of mean diameter of nanoparticles, paclitaxel nanoniosome formulation with PBS with ratio 1:100 and pH 7.2 was prepared. After measurement of absorption at 633 nm, nanoparticles size and zeta potential were investigated by zetasizer machine (Nano ZS3600, Malvern Instruments and UK).

Encapsulation efficiency

For determination of the amount of loaded drug, the resulting suspension was centrifuged for 15 min at 4ºC and 30000 rpm and separated supernatant. Centrifugation was done again to remove not-connected drug to the nanoparticle. Afterwards, drug encapsulation and loading rate were calculated using formula 1 & 2.

$$encapsulation\ percent = \frac{prime\ paclitaxel\left(\frac{mg}{ml}\right) - available\ paclitaxel\ in\ the\ supernatant\left(\frac{mg}{ml}\right)}{prime\ paclitaxel\left(\frac{mg}{ml}\right)} * 100$$

$$loading\ percent = \frac{the\ amount\ of\ available\ drug\ in\ the\ nanoparticle\ (mg/ml)}{weight\ of\ nanoparticle\ (mg/ml)} * 100$$

drug release of niosome nanoparticles containing paclitaxel

Paclitaxel release rate of niosome vesicles was investigated by dialysis method (cut off 10000 D). 6 ml drug and control niosome formulation was poured in dialysis bag. The suspended bag in 15 ml phosphate buffer (pH 7.2, 1 M) was put on the stirrer for 48h at lab temperature. In different timescales, 1.5 ml buffer was replaced with fresh buffer. Optical absorption of collected detachments was studied at 227 nm.

MTT test

MTT test was used to investigate cytotoxicity of the formulation containing paclitaxel and compared its effect than standard drug. A2780S cell (10000 cell) was cultured in DMEM medium with 1*104 number per each well of 96-well plate. Culture medium containing 10% fetal bovine serum and 1% penicillin/streptomycin antibiotic was put at 37ºC and 10% carbon dioxide condition. After 24h of cells culture and clinging them, the supernatant was removed. The cells with same concentration (nanoniosome containing drug, free drug and control) 37, 75, 150, 300 and 600 Micro-Molar were placed. After 48h incubation, the mediums containing drug formulation were taken and 100µl MTT solution (0.5 mg/ml PBS with pH 7.2) was added to each well. Incubation was done for 1h at 37ºC. Therefore, MTT solution was taken and 100µl isopropanol 100% added to each well to dissolve formed formazan crystals. Finally, the amount of absorption at 570 nm was read by ELISA reader instrument (Bio Tek Instruments, VT and U.S.A.).

stability of noisome nanoparticles containing paclitaxel

Resultant nanoparticles of this method were again converted to the suspension after lyophilization, studied their size and size distribution by zetasizer and compared than before lyophilization.

Statistical analysis

Obtained data were analyzed by SPSS software version 11. In addition, all stages of toxicity were analyzed by Pharm software.

RESULTS

Characterization of nanoparticles

In this research, we succeed to prepare noisome nanoparticles containing paclitaxel by using of ether injection method. Results of size and zeta potential were calculated at 217 nm and -23.7 mV, respectively. Encapsulation and loading percent obtained 83±3.3 and 4.1±1.1, respectively.

Drug release rate

The results of drug release showed formulation has a suitable retention power. As after 48h, it was calculated about 3.9±0.22. As shown in Fig. 1, drug release rate is continuously and ascending.

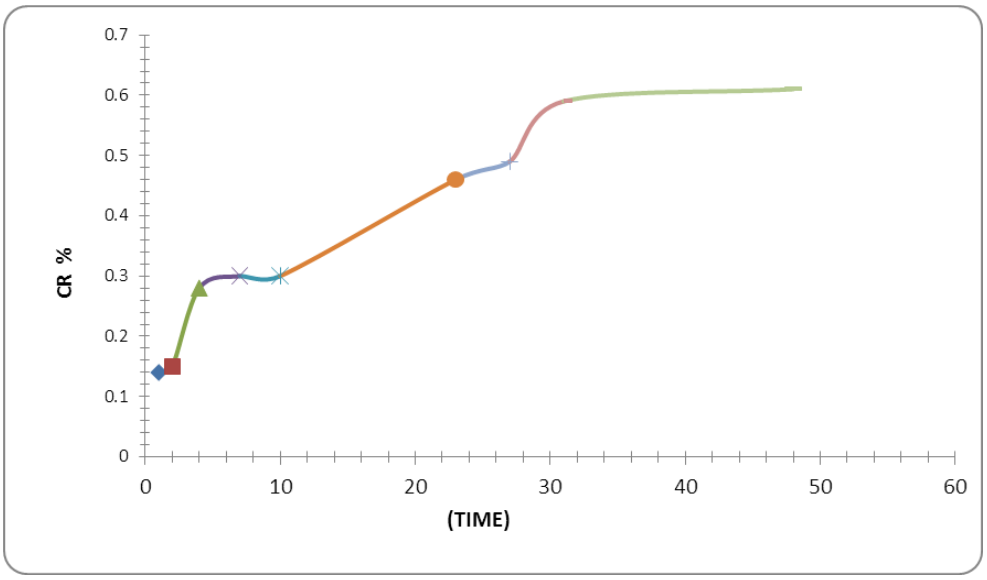

Fig. 1: the pattern of paclitaxel release of noisome nanoparticle.

Stability of resultant nanoparticles

Results of zetasizer showed lyophilized nanoparticles are well stable in laboratory condition. The variation of lyophilized nanoparticles size and zeta potential were insignificant than before lyophilization (Table 1).

Table 1.

Formulation	Size (nm)	Zeta potential
Suspension of noisome nanoparticle containing paclitaxel before lyophilization	217	-23.7
Suspension of noisome nanoparticle containing paclitaxel after lyophilization	232	-25.5

Cytotoxicity and viability percent

The results of MTT test indicated toxic effect of nanoniosome paclitaxel on the aforementioned cell line is more than free drug. As, the amount of IC50 of nanodrug and free drug on the A2780S cell line obtained 120.3 ±11.8 and 180.4±18.3 Micro-Molar, respectively. Also, it was observed cytotoxicity of nanodrug increased with increasing of concentration than standard drug (Diagram 1).

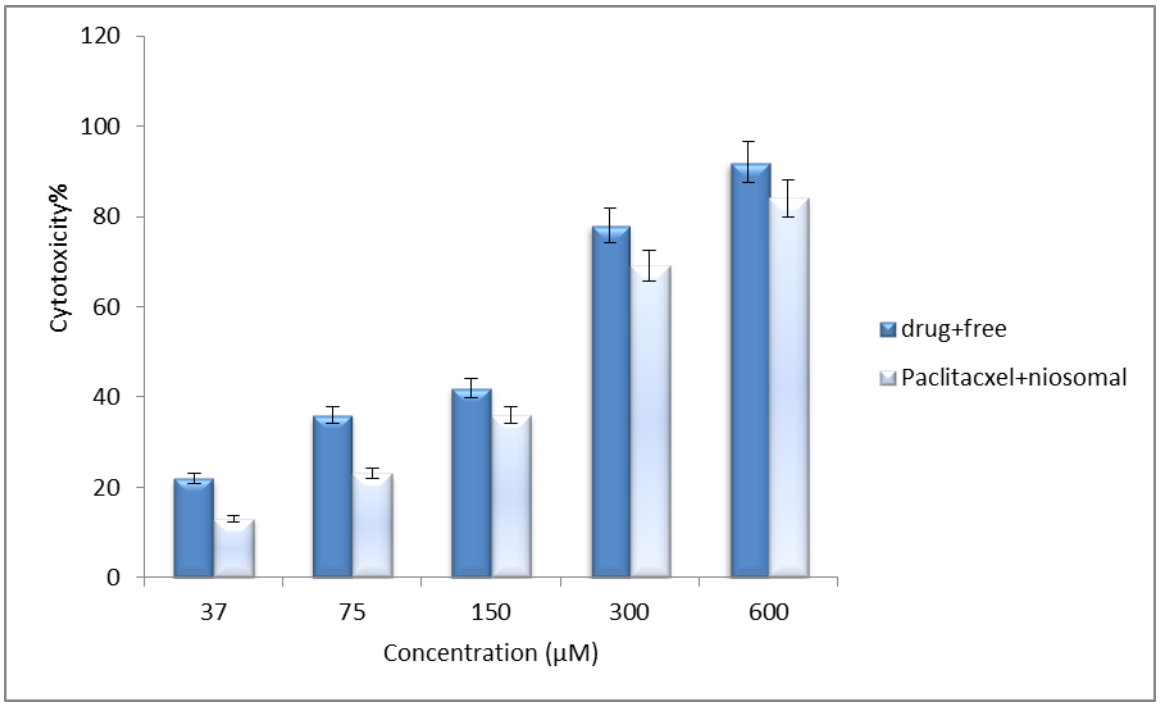

Diagram 1: Diagram of cytotoxicity on A2780S line.

DISCUSSION

This study was conducted to optimize and assess the toxic effects of Paclitaxel nano-niosomes on ovarian cancer cell lines. Results of the research showed an increase in cytotoxic effects of Paclitaxel encapsulated in niosomes compared with the Standard form of paclitaxel. Overall, nano-niosomes synthesis techniques have proved beneficial in improving therapeutic drugs.[7] when nano-niosomes are used for pharmaceutical functions, their toxicity and fate are crucial aspects that need to be evaluated.[8] Mr Zare and his colleagues in 2013 (9) niosomes nano-paclitaxel in breast cancer lines studied. In this study, the amount of drug release were estimated at around 8% in 48 hours. Their results showed that the cytotoxicity of paclitaxel enjoyed niosomes than free drug. In the present study drug release within 48 hours of the release of paclitaxel from niosomes about 3.9 ± 0.22 was reported that the slow release over 48 hours and enjoyed an upward trend. In this research, paclitaxel niosome nanoparticles were prepared by ether injection method. Pegylated niosomes containing drug and without drug were provided that their stability and solubility of paclitaxel was increased by polyethylene glycol. Cytotoxicity effect was investigated in two pegylated formulation (containing drug and without drug) by MTT test. The results exhibited non-toxicity of testator nanoparticle. The results indicated loaded nanoparticles with drug have the fewest IC50 or in the other words, the most toxicity than standard drug. This shows increase of drug efficiency, and therefore use of drug nanocarriers. In addition, the results confirmed the use of

nanotechnology and niosome nanoparticle can provide a suitable formulation of paclitaxel drug would have more efficiency than standard drug. As a result, this decreases used dose and adverse effects. Finally, it is concluded this paclitaxel formulation is as a suitable replacement for chemotherapy.

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CONFLICT OF INTEREST

None declared.

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