

Original Article

Cytotoxicity assessment of nanoliposomal Paclitaxel and nanoliposomal Hydroxyurea in MCF-7 cells

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Abstract

Nanotechnology has revolutionized diagnosis and treatment of cancers. Breast cancer is one of the most prevalent cancers among women. Paclitaxel and hydroxyurea are important drugs in treatment of breast cancer but their use is limited due to a series of significant side effects. On the other hand, nanoliposomes are widely used as nanocarriers for targeted drug delivery. In this study, cytotoxic effects of nanoliposomal hydroxyurea and nanoliposomal Paclitaxel were investigated. Liposomes were prepared using reversed phase evaporation. Cytotoxicity of liposomal formulations of the drugs was determined using Methylthiazolyldiphenyl-tetrazolium bromide (MTT) method. Results showed an IC₅₀ level of 86.25 μ l/ml for nanoliposomal Hydroxyurea and 43.78 μ l/ml for nanoliposomal Paclitaxel. In general, it can be concluded that that cytotoxicity of nanoliposomal drugs are higher than that of standard formulations.

Keywords: Nanotechnology, Breast cancer, Paclitaxel, Hydroxyurea, Liposome, Cytotoxicity

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Introduction

Cancer is one of the most prevalent illnesses in the world. Approximately 11.3 million people suffered from this illness until 2007 (Vasen et al., 1998). One of the most prevalent cancers among women is Breast Cancer (BC) which is also highly prevalent among Iranian women (Warner, 2011; Sajadi et al., 2005; Harirchi et al., 2004).

Use of nanocarriers in drug delivery has started to revolved treatment of several cancers including BC. Among nanocarriers, lipids have attracted more attention in recent years (Woodley, 1985). In addition to therapeutic advantages, nanocarrier-based drug delivery systems also provides the advantage of enhancing drug economic life (Costantino et al., 2011). One particular form of nanocarriers is liposome which is consistent of vesicles of lipid. Liposomes are considered to be promising carriers for targeted delivery of breast cancer drugs such as Paclitaxel and hydroxyurea (Torchilin, 2006).

The aim of this study was to examine the cytotoxicity of nanoliposomal Hydroxyurea (HU) and nanoliposomal Paclitaxel and in comparison to standard formulations.

Materials and Methods

Materials

Hydroxyurea, Paclitaxel, phosphatidylcholine, cholesterol, PEG 2000 and Methylthiazolyldiphenyl-tetrazolium bromide (MTT) solution (0.5 mg/ml) were purchased from Sigma, Ethanol and isopropanol from Merck, RPMI-1640 culture medium from Invitrogen. MCF-7 cells were provided by microbial bank of Pasteur Institute of Iran.

Preparing nanoliposomal drug

Nanoliposomal HU was prepared by dissolving phosphatidylcholine and cholesterol (portion 1:10) in 100 ml of 98% ethanol. The dissolution led to formation of a transparent and yellow suspension. Afterwards, 8 mg of hydroxyurea and 13 mg Paclitaxel were added and mixed using magnetic stirrer at room temperature and 300 rpm for 24 hours. The solvent phase was then evaporated using rotary evaporator (Heidolph, Germany) in 50°C and 100 rpm. The obtained gel was dissolved in physiologi-

cal serum and the resulting gel was sonicated for 5 minutes (Bandelin Sonorex Digitec, 60 Hz) to reduce the size of nanoliposomes and to enhance the drug loading efficiency.

To prepare nanoliposomal Paclitaxel, phosphatidylcholine and cholesterol (portion 1:15) were dissolved in 120 ml of 98% ethanol at 300 rpm at room temperature. The rest of the procedure was identical to that of nanoliposomal HU preparation.

Determining size of nanoparticles

Mean diameter of nanoliposomes was determined using Zeta sizer (Malvern Instruments Ltd).

Cytotoxic potentials

100 μ l of suspension containing 10000 MCF-7 cells was poured in sinks of 96-cell plates and incubated at 37°C and 5% CO₂ condition. After 24h the supernatant was removed and different concentrations of liposomal drugs and the control were added. The solution was incubated for 24h in the mentioned conditions. Supernatant was then removed and MTT solution (0.5 mg/ml) was added to the mixture. After 3 hours of incubation, purple color (due to the formation of formazane) was dissolved in 100 μ l of isopropanol. Absorbance was measured in wave length of 570 nm (Spectrophotometer Power Eave XS) and IC₅₀ was calculated using Pharm program.

Results

Size of nanoparticles

Mean diameter of nanoliposomes of Hydroxyurea and Paclitaxel was 402.5 nm and 421.4 nm, respectively.

Cytotoxic potentials

The results of cytotoxicity analysis of different concentrations are illustrated in Figure 1.

Discussion

In this work, nanoliposomal HU and nanoliposomal Paclitaxel were successfully synthesized. Measuring diameter of particles, validated nano-scale size of both liposomal HU and liposomal Paclitaxel standard nanoliposomes was found to have a considerably less cytotoxic effects on MCF-7 cells. In addition, it was demonstrated that nanoliposomal Paclitaxel and Hydroxyurea showed lower IC₅₀ in comparison with standard formula-

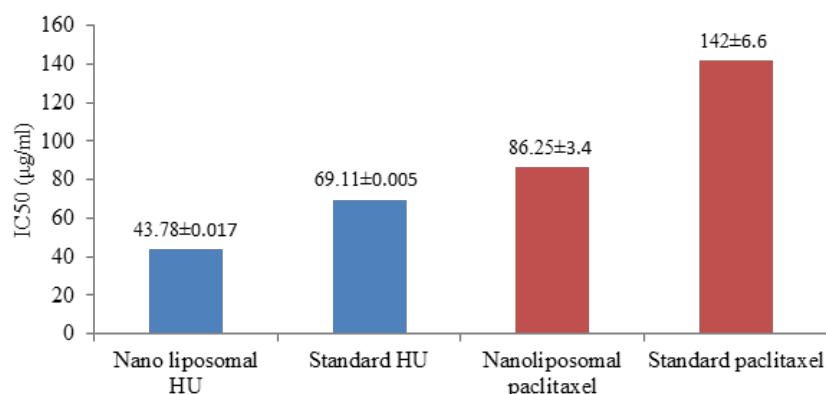


Figure 1. IC₅₀ level (µg/ml) for nanoliposomal and standard Paclitaxel and hydroxyurea

tions. Hence, nanoliposomal drugs appear to be more cytotoxic as compared with the standard formulations, thereby having higher effectiveness.

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