Investigation of Characteristics and Behavior of Loaded Carboplatin on the Liposomes Nanoparticles, on the Lung and Ovarian Cancer: an In-Vitro Evaluation

Majid Hasanzadegan Roudsari¹, Nasim Saeidi², Nahid Kabiri³, Afrooz Ahmadi⁴, Maral Mazloumi Tabrizi⁵, Meysam Ebrahimi Far⁶, Hasan Ebrahimi Shahmabadi⁶, Azim Akbarzadeh Khiyavi⁷, Behnaz Reghbati⁸

¹Department of Chemical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran. ²Department of Medical Genetic, International Campos, Shahid Sadoughi University of Medical Science, Yazd, Iran. ³Graduated from Veterinary Medicin, Shahid Chamran University of Ahvaz, Iran. ⁴Department of Toxicology and Pharmacology, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. ⁵Department of Toxicology, Faculty of Pharmacy, Islamic Azad University, Shahreza Branch, Shahreza, Iran. ⁶Department of Microbiology, Parasitology and Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. ⁷Department of Pilot Nanobiotechnology, Pasteur Institute of Iran, Tehran, Iran. ⁸General Biology Payam Noor University of Bahar, Iran.

Abstract

Carboplatin is a chemotherapy compound for treatment of patients with cancer, such as lung and ovarian cancer. Liposomes are biodegradable, biocompatible, safe and effective carriers for drug delivery. This study aims to prepare nanoliposomal carboplatin and evaluate its cytotoxicity against ovarian and lung cell lines. Liposomes were prepared by reverse phase evaporation method. For this purpose, a certain amount of Lecithin, carboplatin, PEG-3350 and cholesterol were mixed together in methanol solvent. The solvent phase was evaporated by rotary evaporator and the remaining gellose phase was hydrated in phosphate buffer saline. Mean size, size distribution and zeta potential of liposome was measured by Zetasizer instrument. And then, productive nanoparticles Scanning by light microscopy. Releasing pattern of drug was evaluated by dialysis method and the cytotoxicity of nanoliposome against ovarian and lung cell lines, was inspected by MTT assay. Our findings suggest that carboplatin liposomal nanocarriers could serve as a new drug formulation for breast cancer therapy.

Keywords: Carboplatin- nano liposomes nanoparticle- lung and ovarian cancer

Introduction

One of the most important anticancer drugs is carboplatin, which is used to treat many types of solid cancer [1]. Carboplatin is a platinum-based anticancer drug that covalently binds to DNA to form DNA-platinum adducts and induces apoptosis of cancer cells [2]. This drug, similar with the other anticancer drugs, has a narrow therapeutic index, as its clinical use is hampered by a lot of unfavorable adverse effects, consisting of myelosuppression and more common thrombocytopenia [3]. Thus, much endeavor has been made to target carboplatin to cancer tissues, improving carboplatin’s efficacy and safety. Recently, a lot of works have been conducted to develop a delivery system by changing the process to control the destiny of drugs, particularly drug distribution within the organism. Liposome nanoparticles loaded with anticancer agents can capillary endothelial cells, the distance between nascent tumor from normal tissue penetration much easier and then quits tumor. It can cause the increase of natural anti-cancer drug concentration in the tumor and subsequently decrease its toxicity in normal tissues [4, 5]. A variety of delivery systems to improve performance, carboplatin have been developed. For example, it’s using biodegradable polymer nanoparticles [5], sodium alginate nanoparticles [6],...
Chitosan [7], solid lipid nanoparticles [8], Liposomes [9], silica nanoparticles [10], as drug nanoparticles have been extensively employed for the enhancement of efficiency. Different carriers have been used for delivering carboplatin, but researchers have yet to be successful in producing the appropriate Nano liposome formulation from carboplatin. In this research, carboplatin Nano liposomes were optimized, and their toxic effects were assessed on against various malignancies such as ovarian and lung carcinoma.

Materials and Methods

Nano liposomal drug preparation

Liposomal nanoparticles were synthesized using the reverse phase evaporation method. Briefly, approximately 7 mg of carboplatin with 125 mg of lecithin, 46 mg of cholesterol and 50 mg of polyethylene glycol (PEG3350) were mixed in 50 ml of ethanol 96% by heating in 37°C, 60 min at 150 rpm. After perfect dissolving, the solvent was separated using a rotary (Heidolph, Germany) in 37°C, 140 min at 130 rpm. The achieved Gel layer was dissolved in Phosphate Buffer Saline (pH 7.4, 10 M), which was added two times. In that layer the final concentrations of carboplatin, lecithin, cholesterol, and PEG3350 were estimated 0.56 mg/ml, 10 mg/ml, 3.6 mg/ml and 4 mg/ml, respectively. Finally, the formulations were sonicated for 4 min using an ultrasonic bath (Bandelin Sonorex Digitec, Germany).

Characterization of liposomes

One milligram of the formulation was mixed in 2 ml of PBS. After the determining of its absorption in 633 nm, the zeta potential and mean diameter of the Nano liposomes were measured using a Zeta sizer (Nano ZS3600, Malvern Instruments, UK). PEGylated Nano liposomal containing carboplatin were evaluated for the morphological point of view and probable crystallization using light microscopy (Nikon, Tokyo, Japan). To determine the rate of the entrapped drug, 36.5 mg of the formulation were centrifuged (60 min at 4°C and at 21,000 rpm). Then, optical absorbance of the supernatant of each formulation was measured at 220 nm using a spectrophotometer (NanoDrop, Thermo Scientific, USA). The absorbance at 570 nm was measured by plate reader (Synergy Multi-Mode Elisa Reader, BioTek, USA). In vitro viability percent was investigated by following formula3.

\[
\text{Cell Viability percent} = \frac{\text{Abs (Sample)}}{\text{Abs (control)}}
\]

Finally, (IC50) was calculated by using statistical package Pharm-PCS program.

Statistical analysis

Results are expressed as mean ± SD. The data value from three separate tests that examined duplicates. The significance level was set at P values <0.05.

Results

Characterization of liposomes

The mean size of Nano liposomal carboplatin, potential of zeta was found to be 244.3±19.6 nm and -22.9±1.7 mV, respectively. Light microscopy confirmed preparation of nanoparticles with spherical to ellipsoid hollow forms dispersed throughout the matrix (Fig.1). According to Formulas 1 and 2, the encapsulation efficiency and drug loading contents were calculated 78.6 ± 3.7% and 2.5 ± 1.1%, respectively. Other views, 78% of used drug become associated with Nano carriers and carboplatin accounts for 5.5% of nanoparticles weight. In addition, results of size, zeta potential after lyophilized nanoparticles (Table 2) and assay MTT after 2 months were confirmed the proper stability of nanoparticles (Figure 2).
As shown in Figure 4, results showed experimental data for IC50 values on all mention cell lines. Cell viability was significantly decreased in a dose-dependent manner after exposure of cancer malignancies (lung and ovarian) cell lines to free carboplatin and its Nano liposomal formulation using the MTT assay. The experimental data for IC50 (Production time and 2 months after production) values on the ovarian and lung cell lines are summarized in Table 3.

**Discussion**

Nano carriers construction by biodegradable materials provide the possibility of drug release over a period of several days or even weeks and small size of Nano carriers make them to penetrate into target cells through
tiny capillaries, two basic features of Nano carrier drug delivery systems [11]. High encapsulation capacity confirmed reverse phase evaporation method is a preparation of PEGylated Nano liposomal containing carboplatin. PEG was used in the nanoparticles because of improve the stability of liposomal nanoparticles in the blood circulation, water soluble polymer, low immunogenicity and antigenicity and is able to extend the time of low drug-release properties[12].Nano carriers were found Unilamellar Vesicles (ULVs) that may results from sonication effects. There is a direct relationship between zeta potential of nanoparticles and suspension stability [13].Zeta potential of –22.9 mV confirmed the proper stability of particles. In a study, the roles of various drugs with Nano liposomal formulations were evaluated in different cell lines. For instance, Zhang et al. [14]investigated the cytotoxicity effect of PEGylated (PEG2000) Nano liposomal containing carboplatin against gastric cancer with the thin film hydration method. They were used diverse materials (PEG molecular weight was diverse and etc. compared to our study in which our materials were cheaper. The in vitro results of study showed that the cytotoxic effects of Nano liposomal carboplatin increase when compared with free carboplatin. In the present study, the stability of Nano liposomes was confirmed 2 months after production, by MTT assay and its Lyophilizationand then carboplatin release kinetics were studied in vitro. In the formulation showed that carboplatin release kinetics results showed an initial burst of drug release during the second2 hours and then a slowed release pattern until 10 hours and a sustained release for 27 hours. The initial burst of drug release suggests that some of the drug was localized on the surface of the Nano liposomes because of defect capsulation, and later the sustained release was due to the release of the drug from the cross-linkage site of the Nano liposomes.

The comparison between cytotoxicity effects of the Nano liposomal carboplatin with free drug shows the higher efficiency of Nano liposomal carboplatin in destroying mention all cell lines. This may be because the Nano liposomal carboplatin has a phospholipid structure like the bilayer structure of an ovarian and lung cell lines membrane and can better penetrate to these cells and there is a direct relationship between drug release and death in these cells with entering the goal cell [15]. Among these, the A2780S (P<0.01) cell line was more sensitive than the A2780CP (P<0.05) cell line to liposomal nanoparticle effects, which may be a result of the difference between genotype and/or membrane structure in the ovarian cell lines that were studied [16].

Taking collectively, our research confirms that Nano liposomal carboplatin has more cytotoxic effects than free carboplatin on ovarian and lung cancer cell lines. Thus, this formulation may be an alternative chemotherapeutic candidate for ovarian and lung cancer in the future.

References