

Investigation of Characteristics of Loaded Cisplatin on the Liposomal Nanoparticles of the Rat Glioma Cell Line C6

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Abstract

Cancer is one of the most important problems of contemporary medicine. It is main reason of mortality after cardiovascular disease. In the meantime, brain cancer is one of the most prevalent mortality causes among cancers. The use of chemotherapy drugs follow two purpose: first, prevention of irregular duplication of cells in the definite tissues of organs, Second, induction of apoptosis in tumor cells. Cisplatin (Cispt) is an important candidate in the treatment of brain cancer. The *in vitro* cytotoxicity was investigated by rat glioma cell line C6 and MTT assay. Liposomes nanocarriers (Lip-NC) prepared using reverse phase evaporation method. Prepared NC described by zetasizer. Nanoparticle's size and zeta potential obtained 331 nm and -24 mV, respectively. The amount of encapsulated drug and loaded Cispt was measured 65 and 4, respectively. The cytotoxic effect of this nanoliposome on C6 cell lines was significantly increased when compared with free drug ($P < 0.05$). On the base of obtained results, this NC can be considered as a suitable candidate for drug delivery.

Keywords: Brain Cancer; Cisplatin; Liposomes Nanocarriers

Introduction

Among all types of diseases, such as diabetes [1], blood pressure [2], cancer has always been the most important [3-5]. Brain tumors refer to all tumors found in the skull or central canal of the spinal cord. Tumors develop due to uncontrolled or abnormal division of cells and usually in the brain tissue [6]. Gliomas are known as invasive tumors due to rapid proliferation, generalized growth, and invasion to different areas of the brain as well as massive cerebral edema and high angiogenesis [6]. One of the best methods have been used to overcome this barrier including NC containing drugs [7,8]. Nanoparticles are made of materials such as PBCA, liposomal and niosomal. They can have different z-sizes and sizes shapes with unique properties on the base of the method of preparation and applied materials [9-13]. Cisplatin has widely used chemotherapeutic agent against various types of cancer. Cisplatin has affected cancer cells through binding to DNA molecule and consequently induction of necrosis and apoptosis [11]. Various liposomal formulations of Cisplatin have been produced. Lipoplatin is the most promising one. However, Lipoplatin was prepared under stringent conditions using various materials including unnatural lipids and natural. Reverse micelles between Cisplatin and DPPG under particular conditions of ionic strength, pH ethanol and other variables were formed [12]. Liposomes are nanoparticles containing watery environment surrounded by lipid bilayer membrane. Amphiphilic molecules are similar to biological membrane that used for preparation of these compounds and to improve efficiency and safety of different drugs [14].

We here encapsulated the Cispt into pegylated Lip-NC by reverse phase evaporation technique. PEG was used because of its effect on the improving of stability [15]. NC were characterized in terms of drug loading, encapsulation efficiency, size and zeta potential. Next step the cytotoxicity effects of NC were evaluated on the brain cancer cell line C6 *in vitro* environment.

Methods and Materials

Materials

Cisplatin, cholesterol, polyethylene glycol400 and lecithin were prepared from Sigma Company. C6 cell line was purchased from cell bank Iran Pasteur Institute.

Method of making of NC containing the Cispt

For preparation of control Lip-NC, at first 155 mg of lecithin, 40 mg of cholesterol and 50 mg of PEG₄₀₀ were added in the 100 ml flask. Also, for preparation of sample of Lip-NC containing Cispt, 155 mg of lecithin, 40 mg of cholesterol and 50 mg of PEG₄₀₀ were added in the 100 ml flask. 100 ml methanol was added to the test and control sample. Then, they were put on the stirrer at 140 rpm, 37°C. The samples were on the stirrer for 4h to dissolve completely. To extract the organic solvent [methanol], they were put in the rotary machine operator at 37°C with 125 rpm. After full extraction of organic solvent, 20 ml of PBS (PH:7.2) was added to control sample and 19 ml of PBS and 9 mg of Cispt dissolved 1 ml of PBS added to sample containing drug. Then, we wait until obtained gelose dissolve in phosphate buffer. After complete dissolution of samples in buffer, both formulations were put in sonic bath for 10 minutes.

Characterization of NC

In order to characterize of NC, formulation of Cispt-Lip-NC were prepared using fresh PBS with PH:7.2 and the ratio 1:20. Z-Size and zeta potential of NC were examined by using zetasizer machine after measuring its absorption at 633 nm. 2 ml of obtained formulations (control and test) were centrifuged for 15 minutes with 15000 rpm at 4°C to determine encapsulation efficiency and load rate. Then, supernatant was separated. This procedure was repeated two times longer to remove not connected Cispt to the NC. Finally, encapsulation and loading of drug were calculated using formula (1) and (2).

$$\text{encapsulation percent} = \frac{\text{prime Cispt (mg / ml)} - \text{available Cispt in the supernant (mg / ml)}}{\text{prime Cispt (mg / ml)}} * 100 \quad (1)$$

$$\text{loading percent} = \frac{\text{the amount of available Cispt in the NC (mg / ml)}}{\text{weight of NC (mg / ml)}} * 100 \quad (2)$$

Obtained data was analyzed by SPSS software version 11 and all stages of toxicity was evaluated by Pharm software.

Drug release study

The experiment was performed using dialysis membrane technique. Briefly, sediment of cisplatin loaded liposome was prepared by centrifugation process as mentioned previously. The sediment was resuspended into 2 ml fresh PBS and with standard cisplatin were poured in two separate dialysis tubes (Sigma, cut off 10000 Da) and immerse into PBS and stirred (30 ml, 140 rpm). 2 ml of buffer was withdrawn on the predetermined time intervals and replaced with 2 ml fresh PBS. The amount of released drug in the PBS was calculated using spectrophotometry technique.

Investigation of cytotoxicity

Cytotoxicity of these NC was evaluated using MTT test and rat Glioma cell line C6. Used concentrations (0, 12, 24, 48, 96, 192, 384, 750, 1500 and 3000 μM) of Cispt-Lip-NC and F-Cispt were studied during 24h incubation.

Results

Characterization of NC

In this research, we succeeded to prepare Lip-NC containing Cispt using reverse phase evaporation method. The results of size and zeta potential were calculated 331 nm (Figure 1) and -24 mV, respectively. Encapsulation and loading percent were calculated 65 and 4, respectively.

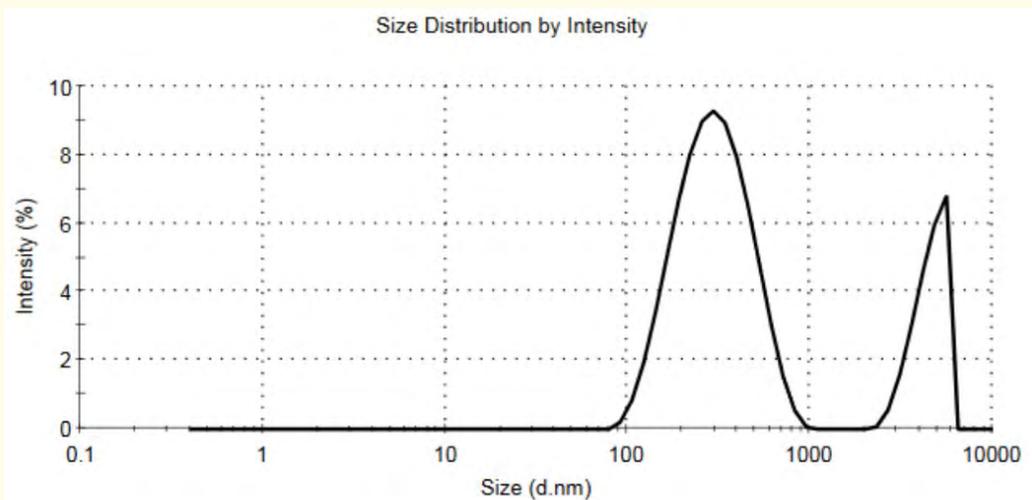


Figure 1: Size Distribution by Intensity of Cisplatin.

Drug release

The results of drug release demonstrated a sustained release pattern. Regarding the nanodrug, release curve initiated with a burst release (25% of encapsulated drug) followed by mild ascending slope with maximal release of 6% after 68h (Figure 2).

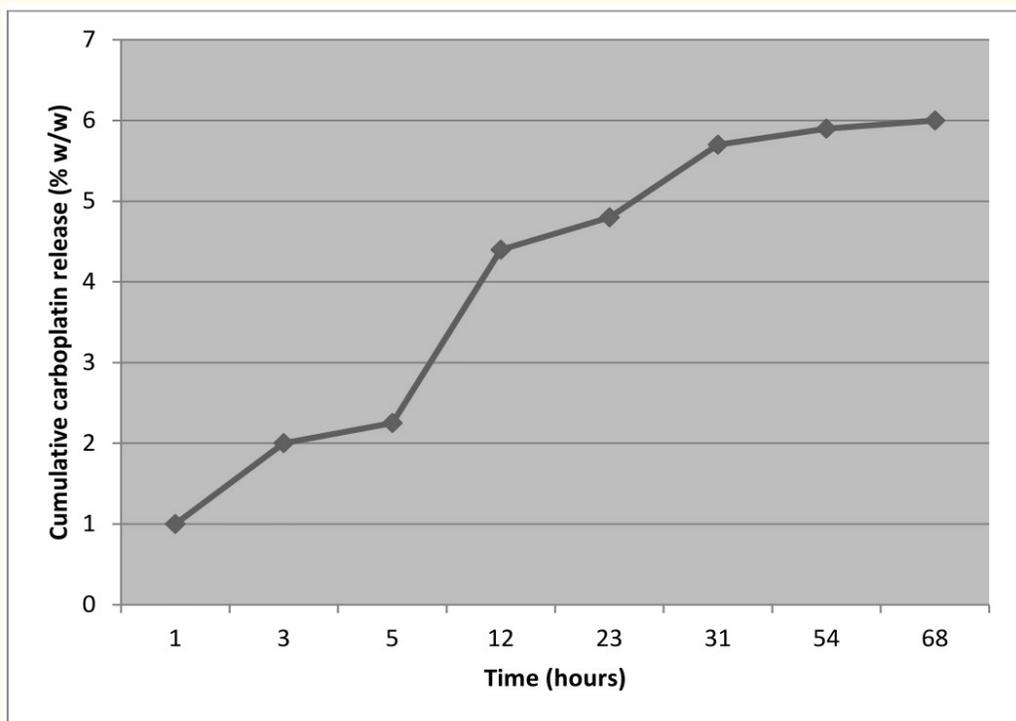


Figure 2: Release Cisplatin from Liposome.

Cytotoxicity rate and survival percentage

Results of MTT test showed that Cispt-Lip-NC with the IC_{50} of 67 μ M showed the superior cytotoxicity compared to the standard Cispt with IC_{50} of 114 μ M (Figure 3 and Table 1).

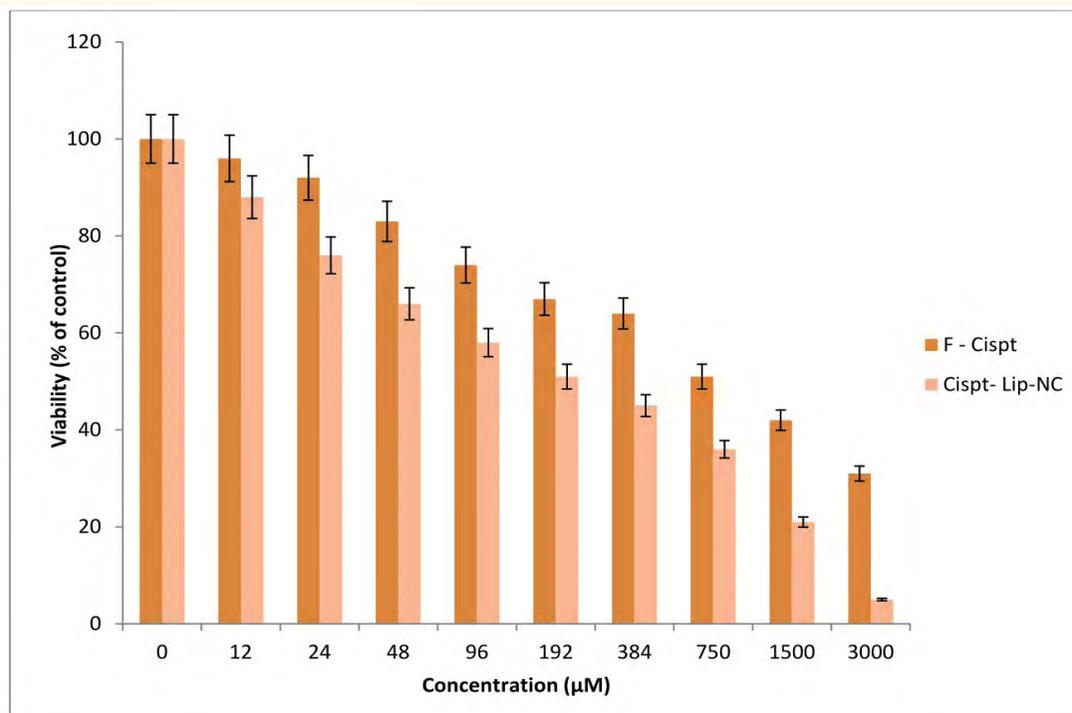


Figure 3: Cytotoxicity Effects of Free Cisplatin and Loaded Cisplatin on the Liposome NPs in the C6 Cell after 48h Incubation ($P < 0.05$).

Brain Cell Line	Cispt-Lip-NC	F-Cispt	C-NC	P value
C6	67.5 \pm 9.8	114.8 \pm 11.9	197.8 \pm 17.4	$P < 0.05$

Table 1: A comparison between IC_{50} values of F-Cispt and its Cispt-Lip-NC in the cell line, C6.

Discussion

A main problem, which introduced in cancer therapy, is the estimation of the response toward chemotherapeutics and their efficacy. In this regards evaluation the efficiency of chemotherapy is an interesting issue in cancer management and treatment outcome [16]. Indeed, the use of specific cancer cells killing agents, which provoked antitumor response, is a noticeable subject in cancer therapy [17]. Effective Chemotherapy using cytotoxic agents had imperative role in reducing the cancer rate. In addition, Nanotechnology and subsequently nanoparticles carrier applied in drug delivery system, because of numerous advantages in compared to other drug delivery systems [7,18]. In this study, cytotoxic effects of Cispt loaded Lip-NC in compared to free Cispt in glioma cell lines evaluated. Our results indicated

that cytotoxic agents such as Cispt exerted higher cytotoxicity in compared to free drug in tumor cells. Our studied Nanoparticles produced by Reverse phase evaporation technique, which was approved as proper technique for preparation of Cispt, loaded Lip-NC. The prepared drug had appropriate characteristics including size, size distribution, and Zeta potential. Poy., *et al.* (2016) prepared cisplatin loaded liposomal NPs using thin film hydration method. The prepared particles had the size of 142 nm. They were used diverse materials compared to our study. The PEG molecular weight was diverse in two studies. In both studies, lecithin was used [12]. Indeed, The MTT test as cellular viability assay technique is one of the routine methods to measuring the effects of chemotherapeutic agents so we selected this method to assessment the efficacy of Cispt loaded Lip-NC. Cispt loaded Lip-NC has lower IC_{50} than free Cispt, which displays that formulated Cispt, is more effective in destroying cancer cells. Nevertheless, further studies suggested to elevate the effectiveness of this novel drug. According to our results, liposomal formulation vigorously improved the cytotoxicity effects of drug in compared to free Cispt. In this survey, liposomal formulated Cispt increase the anticancer activity in rat glioma cell line C6.

Conclusion

Reverse phase evaporation technique was approved as proper technique for preparation of cisplatin loaded liposomal NPs. The size, zeta potential, drug loading and encapsulation efficiency and drug retention capability of NPs containing cisplatin were evaluated and was found proper. The study was followed by evaluation the efficacy of nanoliposome on the rat glioma cell line C6 which demonstrated superior cytotoxicity of nanoliposome compared to standard drug. The results of study demonstrated pegylated liposomal nanoparticles are proper carrier for cisplatin delivery to rat glioma cell line C6.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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