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Preparation, characterization and cytotoxic studies of carboplatin-containing nanoniosome on ovarian cancer cell

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Abstract

Carboplatin is chemotherapy drug that is used against some forms of cancer such as ovarian carcinoma. The aim of this study is to improve the therapeutic efficacy of carboplatin using niosomal nanocarriers. Nanoniosomal carboplatin was synthesized using the reverse phase evaporation method and characterized for shape morphology, particle size, zeta potentialand and drug-release properties. In the next step, A2780CP ovarian cell line was used to determine the rate of nanoniosomal carboplatin cytotoxicity. In this research, the particle size and zeta potential of the niosomal nanoparticles (NPs) were measured 258.3±11.5 nm and -24.1±1.4 mV, respectively. The amount of encapsulated drug and the level of drug loading were determined 94.3±1.5% and 4.4±2.1%, respectively. The cytotoxic effect of this nanoniosome on A2780CP cell line was significantly increased when compared with free drug ($P<0.05$). Our findings suggest that carboplatin niosomal nanocarriers could serve as a new chemotherapy modality for ovarian cancer therapy.

Keywords: carboplatin, nanoniosome, ovarian cancer.

Introduction

Ovarian cancer is one of the most lethal gynecologic malignancies and the seventh most common cause of cancer among women worldwide (Ovarian cancer, 2014)(A. Kim et al, 2012)(M. Arab et al, 2010). The incidence of ovarian cancerworldwide ranges from more than 11 per 100,000 women in Central and Eastern Europe to fewer than 5 per 100,000 in parts of Africa (Ovarian cancer, 2014)(M. Arab et al, 2010). reported that overall rates of ovarian cancer in Iran and the United States were 3.9 and 16.2 per 100,000, respectively. One of the most important anticancer drugs is carboplatin, which is used to treat many types of solid cancer, such as ovarian cancer (C. Della Pepa et al, 2015). Carboplatin is a platinum-based anticancer drug that covalently binds to DNA to form DNA-platinum adducts and induces apoptosis of cancer cells (Y. Ando et al, 2014). This drug, like the other anticancer drugs, has a narrow therapeutic index, as its clinical use is hampered by several undesirable adverse effects, includingmyelo suppression and especially thrombocytopenia (A. Schmitt et al, 2010). Thus, much effort has been made to target carboplatinto cancer tissues, improving carboplatin's efficacy and safety. In recent decades, much work has been directed toward developing delivery systems to control the fate of drugs by modifying these processes, in particular the drug distribution within the organism (Babaei.M et al, 2014)(Mujoriya.R and R,Babu Bodla, 2012). Nanoliposomes loaded with anticancer agents can penetrate the interendothelial cell gap of nascent tumor capillaries much easier than normal tissues and then deposit in the tumor. These natural characteristics can increase anticancer drug concentration in the tumor, thereby decreasing its toxicity in normal tissues (M.Zarei et al, 2013)(X.Zhang et al, 2011).

Methods and materials

Carboplatin, cholesterol, and polyethylene glycol 5000 from Sigma–Aldrich Co., UK; lecithin from Acros Co, Belgium; and RPMI 1640 cell culture medium from Gibco Co., Germany, were obtained. Also, the cell line A2780CP was purchased from the cell bank of Pasteur Institute in Iran.

Nanoniosomal drug preparation

Niosomal nanoparticles were synthesized using the reverse phase evaporation method. Briefly, approximately 15 mg of carboplatin, 250 mg of lecithin, 100 mg of cholesterol, and 110 mg of polyethylene glycol 5000 (with the molar ratio of 65: 50: 8) were dissolved in 120 ml of ethanol 96% by heating at 37˚C and stirring for 1 hour at 160 rpm. After perfect dissolving, the solvent was separated using a rotary. The obtained thin film was dissolved in 26 cc phosphate buffer (pH 7.2), which was added two times. Finally, the formulations were sonicated for 5 min using a ultrasonic bath (BandelinSonorexDigitec, Germany). Light microscopy (S-4160 scanning microscope, Hitachi, Japan) was employed to determine the surface and shape morphology of the produced nanoniosomes. To determine the rate of the entrapped drug, 20 mg of the formulation was centrifuged for 1 hour at 4˚C and at 15000 rpm. Then, optical absorbance of the supernatant of each formulation was measured at 240 nm using a spectrophotometer (UV1800, Shimadzu Co). Encapsulation efficiency and the rate of drug loading were calculated by using Formulas 1 and 2, respectively.
Results were analyzed by the SPSS software version 11. Data are expressed as the Mean±SD from three separate tests that examined duplicates.

Particle size and zeta potential analysis

2 milligram of the formulation was dissolved in 5 ml of phosphate-buffered saline (PBS). After the determining of its absorption in 633 nm, the zeta potential and mean diameter of the nanoniosome were measured using a Zetasizer (Nano ZS3600, Malvern Instruments, UK).

Cytotoxicity assay

A2780CP cell line was cultured in RPMI 1640 containing 10% fetal bovine serum, 100 µg/ml streptomycin, and 100 U/ml penicillin. Cytotoxicity testing was performed using MTT assay. After 48 hours of exposure, the supernatant was removed, and then 100 µl of a solution of MTT was added to the cells. After 1 hours of incubation, 100 µl of isopropanol solution was added to the culture medium. After 30 minutes, the optical absorbance of the formazan product was read at 570 nm using a plate reader (Synergy Multi-Mode Elisa Reader, Bio-Tek, USA). Finally, IC50 was calculated by using the pharm program.

Results

Particle size and zeta potential analysis

The mean size of nanoniosomal carboplatin and potential of zeta were determined as 258.3±11.5 nm (Fig 1) and -24.1±1.4 mV, respectively.

Surface morphology

As shown in Figure 2, Light microscopy images revealed that the carboplatin nanoniosomes had spherical and smooth surfaces.

Encapsulation efficiency and drug loading studies

According to Formulas 1 and 2, the encapsulation efficiency and drug loading contents were calculated 94.3±1.5% and 4.4±2.1%, espectively.

In vitro drug release studies

As shown in Figure 3, carboplatin released from the nanoniosome to PBS buffer was measured during time intervals of 1, 2, 4, 10, 23, 27, 31, and 48 hours using the standard curve of carboplatin. The results show the maximum amount of carboplatin released from nanoniosome during 48 hours at about 70.1.9%.

Cytotoxicity assay

As shown in Figure 4**,** cell viability was significantly decreased in a dose-dependent manner after exposure of ovarian cell lines to free carboplatin and its nanoniosomal formulation using the MTT assay.

Conclusion

The comparison between cytotoxicity effects of the Nano niosomal carboplatin with free drug shows the higher efficiency of Nano niosomal carboplatin in destroying mention all cell line. Thus, this formulation may be an alternative chemotherapeutic candidate for ovarian cancer in the future.

Acknowledgment

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Conflict of interest

The authors declare that there is no conflict of interest.

Equations

$$Encapsulation\ percent = \frac{PC\ - CS}{PC} \text{ x } 100 \quad (1)$$

In Formula 1, PC: primary carboplatin and CS: carboplatin in supernatant in mg/ml

$$Drug\ loading\ percent = \frac{C}{W} \text{ x } 100 \quad (2)$$

In Formula 2, C: carboplatin content in the nanoniosome and W: weight of nanoniosome in mg/ml

Figures

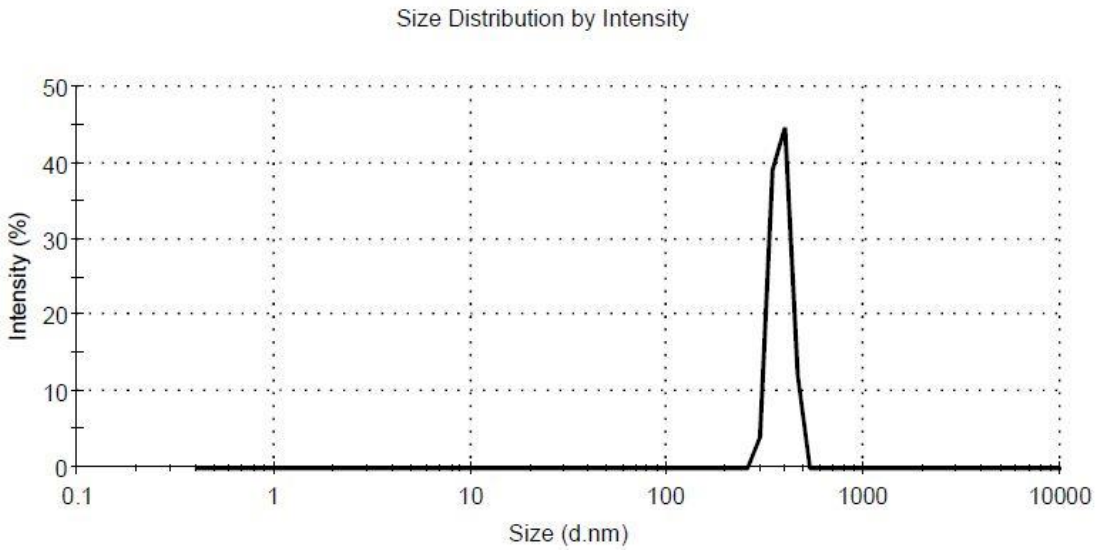

Fig 1. The mean size of nanoniosomal carboplatin

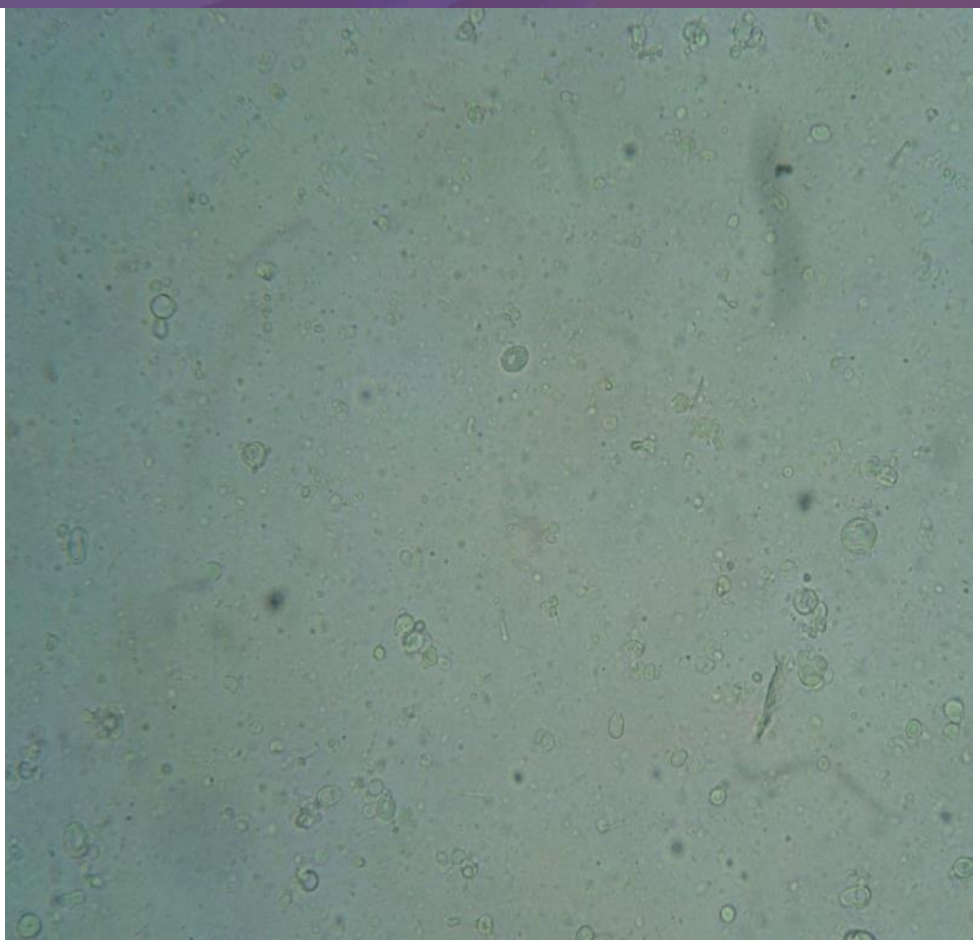

Fig 2.Light microscopy of carboplatin loaded on PEGylatedniosomal nanoparticles.

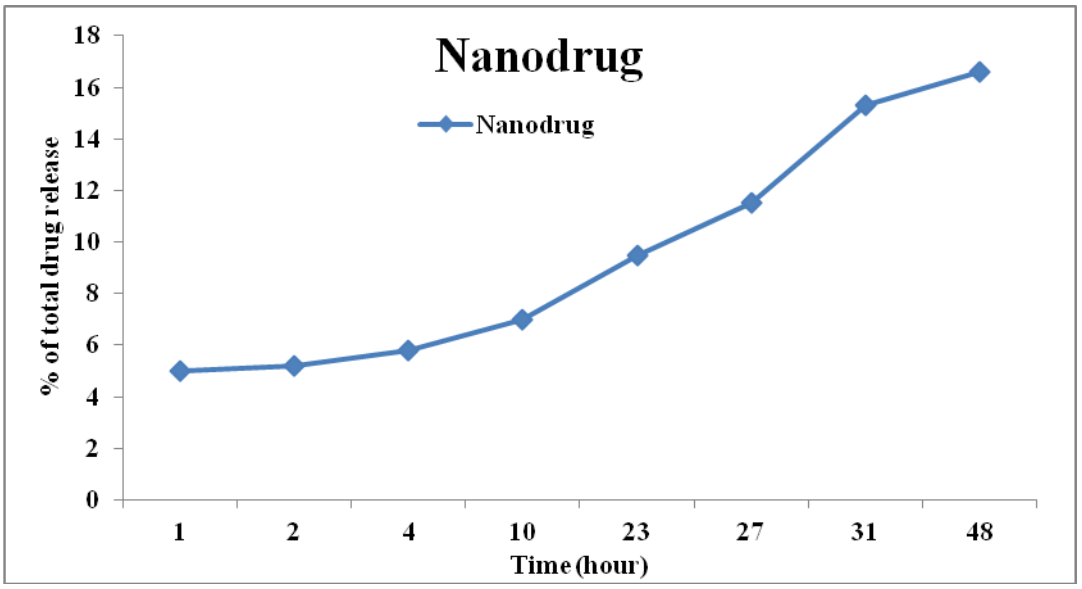

Fig3. Release of carboplatin from carboplatinNano Niosome

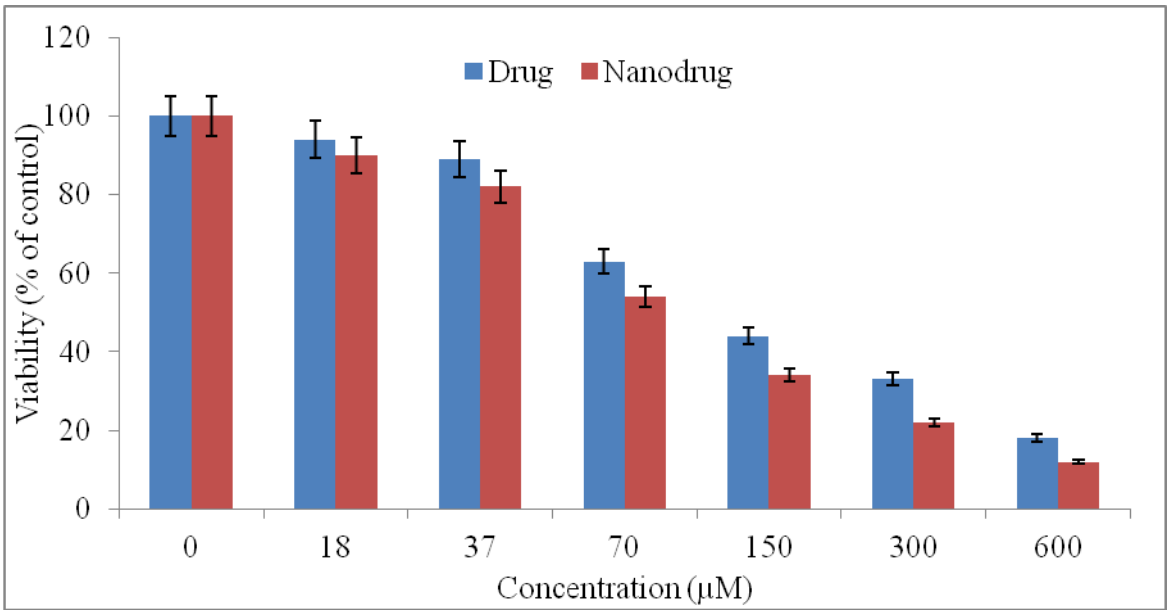

Fig. 4 the cytotoxicity effects of Carboplatin in the standard form or encapsulated into Niosome nanoparticle. Results were expressed as mean ± 5% values of three independent experiments.

References

Ovarian cancer (2014) report, Available from: http://www.aicr.org/continuous-updateproject/reports/ovarian-cancer-2014-report.pdf.

A. Kim, Y And Ueda And T. Naka And T. Enomoto. (2012). Therapeutic strategies in epithelial ovarian cancer, J Exp Clin Cancer Res. 31 (14) , pp. 1-8.

M. Arab And M. Khayamzadeh And A. Tehranian And M. Tabatabaeefar And M. Hosseini And R. Anbiaee And F. Golfam And M.E. Akbari. (2010).Incidence rate of ovarian cancer in Iran in comparison with developed countries, Indian J Cancer. 47 (3), pp. 322-327. Page 9 of 16

C. Della Pepa And G. Tonini And C. Pisano And M. Di Napoli And S.C. Cecere And R. Tambaro And G. Facchini And S. Pignata. (2015). Ovarian cancer standard of care: are there real alternatives?, Chin J Cancer. 34 (1) pp. 17-27.

Y. Ando And T. Shimokata And Y. Yasuda And Y. Hasegawa. (2014). Carboplatin dosing for adult Japanese patients, Nagoya J Med Sci. 76 (1-2) , pp. 1-9.

A. Schmitt And L. Gladieff And C.M. Laffont And A. Evrard And J.C. Boyer And A. Lansiaux And C. Bobin Dubigeon And M.C. Etienne-Grimaldi And M. Boisdron-Celle And M. Mousseau And F. Pinguet And A. Floquet And E.M. Billaud And C. Durdux And C. Le Guellec And J. Mazieres And T. Lafont And F. Ollivier And D. Concordet nd E. Chatelut. (2010). Factors for hematopoietic toxicity of carboplatin: refining the targeting of carboplatin systemic exposure, J Clin Oncol. 28 (30) ,pp. 4568-4574.

Babaei, M., et al.(2014). Efficacy comparison of nanoniosomal and pegylated nanoniosomal Cisplatin on A172 cell line. Tissue Engineering and Regenerative Medicine, **11**(5): p. 350-354.

Mujoriya, R. And R. Babu Bodla. (2012). Design and development of niosomal delivery system for Ketoprofen. available at htpp://hdl. net/123456789/97.

M. Zarei And D. Norouzian And B. Honarvar And M. Mohammadi And H. Ebrahimi Shamabadi And A. Akbarzadeh. (2013). Paclitaxel Loaded Niosome Nanoparticle Formulatian Prepared via Reverse Phase Evaporation Method: An in vitro Evalution. Pakistan Journal of Biological Scienes, 16(6): 295-298.

X. Zhang And H. Yang And K. Gu And J. Chen And M. Rui And G.L, Jiang. (2011). In vitro and in vivo study of a nanoliposomal cisplatin as a radiosensitizer, Int J Nanomedicine. 6 ,pp. 437–444.