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Preparation of Etoposide Loaded Poly butyl Cyano Acrylate Nanoparticles and It's Effect on Human Brain Carcinoma Cells BE(2)-C

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ABSTRACTS

The main purpose of this studywas preparation of a pharmaceutical formulation with etoposide loaded Poly Butyl Cyano Acrylate (PBCA) nanoparticles and evaluation of its effect on brain cancer. Etoposide loaded nanocarrier was synthesized using micro-emulsion polymerization method and characterized for shape morphology, particle size, zeta potential and drug-release properties. In the next step, BE (2)-C brain cell line was used to determine the rate of nanocarrieretoposide cytotoxicity. Etoposide loaded nanoparticles were characterized and size and zeta potential of nanoparticles containing drugs were measured as 225.3±21.7 nm and -26.3 ±1.5 mv, respectively. Drug loading and encapsulation values were estimated 8.7±0.15 and 97.3±2.1%, respectively. The release amount of drug from PBCA nanoparticles indicated the retention strength corresponding to the formulation. Cytotoxicity studies showed that the cell toxicity effect of drug loaded PBCA nanoparticles is almost two times higher than the free drugs. Finally, it was found that the obtained formulation has a proper stability and drug maintain its stability after storage in 37℃ for two months.
According to the obtained results, this nanoparticle has a potential to be used as a drug delivery system.

Keywords: brain cancer, etoposide, PBCA nanoparticles

INTRODUCTION

Brain tumors are a collection of neoplasms which have its own treatment, knowledge and biological properties. These tumors are a set of neoplasms which are in the skull, but some of them are not produced by brain tissue (such as meningioma, lymphoma and …), thus it can be concluded that most of the inside-skull tumors have the same clinical symptoms, diagnosis method, and initial treatment [1]. Etoposide is an inhibitor for topoisomerase II enzyme which prevents from re-closing of DNA strands and lead to deficiency in DNA synthesis. However, this drug has also some disadvantages including low solubility in water, chemical instability in aqueous solutions. Cancer cells can be resistant to etoposide due to some various mechanisms of resisting to some drugs, (drugs related to regulating topoisomerase II enzyme) or reduction of drug accumulation. Capturing of anti-cancer drugs in colloidal drug release systems can be so much useful in cancer chemotherapy. Some of the side effects of etoposide included dermal symptoms, piedra, digestion disorders, constipation and reducing the blood platelet and white blood cells (WBC)[2]. One way for reducing the chemotherapeutic symptoms is performing drug delivery to the object cell

using carrier systems (nanoparticles, Nano-capsules and liposomes) for the drug. Increase in drugs efficiency, reduction of side effects and overcoming drug resistance are consequences of using Nano-carriers for performing drug delivery [3]. According to the process used for their production, there are produced two kinds of Nano capsules and Nano sphere nanoparticles. Nano spheres have a hard and though beds on which drug is attached, while, Nano capsules have an oil core in which drug is placed and has surrounded by a membrane structure[4]. Nanoparticles can prepare by natural or synthesized macromolecules, which capture, encapsulate or adsorb the drugs on their surface [5].Poly butyl cyano acrylate nanoparticles are one of these nano carrier systems; these nanoparticles are suitable for targeted drug delivery. Some of properties of these nanoparticles include overcoming the multi drugs resistance, biodegradability, ability of change in drug's bio-distribution and facility in their purification and synthesis[6]. In mini-emulsion polymerization, there are almost stable emulsions of Nano-droplets of oil in water which are prepared by severe cutting of mixtures made of monomers, water, stabilizer and one non-soluble compound in water which is called hydrophobic compound. To date, various carriers have been used for etoposide delivery but no one could produce a specific nanoparticle formulation foretoposide which is attributed to specific properties of the drug including poor hydrophilic and lipophilic properties[7].

MATERIALS AND METHODS

Materials

Butyl cyano acrylate monomer was purchased from Evobond®TongShen Enterprise Co., Ltd. dextran 70000, etoposide and poly ethylene glycol $_{2000}$ were purchased from Sigma company and BE (2)-C cell line were prepared from Pastor Institute Cell Bank in Iran; the water used in this study was deionized water.

Preparing drug containing nanoparticles

For producing a pharmaceutical formulation, 130 µLof butyl cyano acrylate monomer were added to a mixture containing 100 mL of 0.01 N HCl, 20 mL of olive oil (Farzan Rahbar Saba Co. Iran), 80 mg of honey (Sabalan Co. Iran), 60 mg of Polysorbate 80 (Merck Co, Germany)and 30 mL of poly ethylene glycol $_{2000}$ and mixed well. In the next step, 120 mg of dextran 7000 with etoposide (Each ml contains 20 mg of etoposide) were added to the formulation and mixed again. Then, 12mL of distilled water were added to the above mixture and shaked for 12 min on stirrer (300 rpm). After placing beaker on the ice, sonication operation was performed for 10 minutes and in the next step, emulsion was placed in the stirrer and shaked slowly (130 rpm) for 2 h. The final solution was adjusted to pH 5.5 by sodium hydroxide 0.1N. Gel filtration chromatography was used for purification. To this end nanoparticles containing the drug-containing gel sephadex column with a mesh-50 identified generally is loaded and the column is passed phosphate buffer saline (PH: 7.4) to separate free drug is encapsulated drug. And for the rest of the analysis were used.

Nanoparticles description

Nanoparticles were investigated in terms of size and zeta potential using DLS method by Zetasizer instrument(Nano ZS3600, Malvern Instruments, UK) and to determine the amount of drug loaded into nanoparticles, the obtained formulation was centrifuged for 15 minutes with 49000g speed. Supernatant was separated and the solution washed for another 15 minutes to remove non- attached drugs. The etoposide content in the supernatant was estimated using ICP-MS method and the amount of loaded and encapsulated drugs were calculated by using the following formula.

$$\text{Encapsulation percent} = \frac{\text{Initially added etoposide (mg/ml)} - \text{etoposide amount in supernatant (mg/ml)}}{\text{Initially added etoposide (mg/ml)}} \times 100$$

$$\text{Loading percent} = \frac{\text{Drug amount in NP (mg/ml)}}{\text{NP weight (mg/ml)}} \times 100$$

Morphology structure

To determination of shape and size morphology of poly butyl cyano acrylate nanoparticles, the scanning electron microscopy(KYKY-EM3200, USA) was used.

Drug release Study

Drug release from PBCA was evaluated by dialysis bag method. First, 3.5 ml of etoposide loaded nanoparticles was poured and placed on dialysis tubing (cutoff 8000 Da). Then, dialysis bag was suspended in 20 ml of phosphate buffer solution (pH 7.4, 1M) and placed on a magnetic stirrer in room temperature for 31 h. In various time intervals, 3.5 mL of buffer solution was picked and replaced by a fresh buffer. Optical adsorption of collected samples in various time intervals was readapt 284 nm wavelength and the amount of drug in each fraction was determined by using a standard graph.

Cell toxicity study
Toxicity effect of nanoparticles were assessed by MTT test on BE (2)-C cell line. The used concentrations for drug were 0, 0.004, 0.008, 0.016, 0.032 and 0.064 mg/ml of Nano drug and free drug in a 48 hours incubation time.

Studying nanoparticles stability
For evaluation of the nanoparticles stability, the drug formulation was stored at 37℃ for 2 months. Changes in size, zeta potential, encapsulation percent and the amount of drug loaded in nanoparticles were studied after two months.

Statistical analysis
The gathered data from the studies was analyzed by using SPSS software, version 15 and P-Value was less than 0.05 which is statistically important.

RESULTS

Nanoparticles characterization
The present study succeeded to load etoposide on PBCA nanoparticles by using micro emulsion polymerization method. After addition of distilled water and sonication, the polymerization occurred and medium color become milky and blurred and finally completed after sonication. The obtained results from size and zeta potential were 225.3±21.7 nm and –26.3±1.5 mV, respectively; encapsulation and loading percentage value were 97.3±2.1% and 8.7±0.15%, respectively.

Morphology structure
As shown in the figure 1, SEM images show the smooth surface of nanoparticles.

Amount of drug release
The results of drug release study showed that the formulation hasa proper retention of drug with a release rateabout%78.23±3.2 after 31 hours. As shown in the figure 2, the amount of drug released from formulation was observed in continues and delayed pattern.

Nanoparticles toxicity in cells
At first, it was found that blank nanoparticles had no toxic effect on cells and were completely safe but the cell toxicity results indicated an increase in Nano-drug toxicity in comparison with standard drug in 48 hours incubation (figure 3). In this case the values of IC_{50} of Nano-drug and standard drug were estimated 29.6±2.6 and 56.4±5.5 mg/mL after 48 hours of incubation, respectively. Another important point was that the cell toxicity of Nano drug increased by increase in concentration in comparison with standard drugs.

Nanoparticles stability
Results of stability experiments indicated that storing for 2 months in 37℃ is a sign of maintaining stability. Changes of zeta potential, encapsulation percent and loading level in nanoparticles after two months have been brought in table 1, but no significant changes was seen in the results.

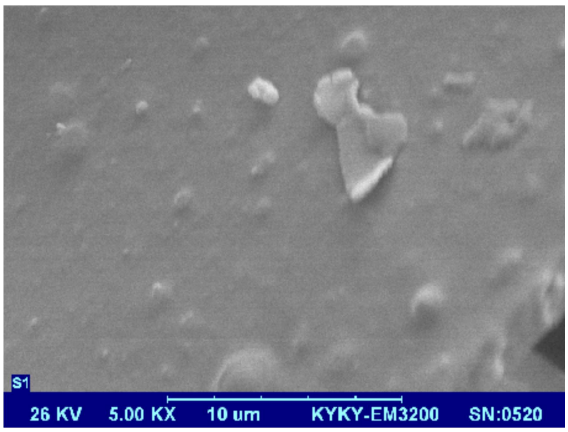

Figure 1: SEM images for PBCA nanoparticles

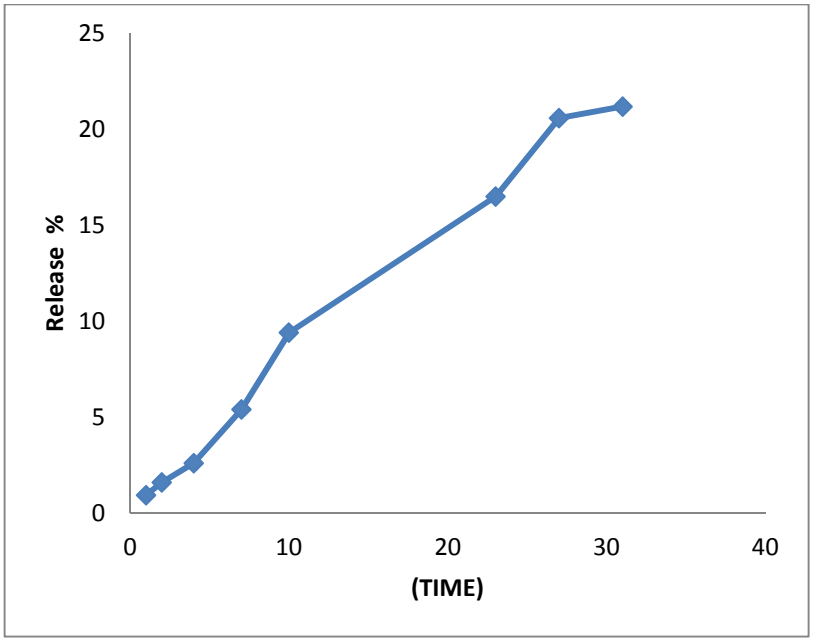

Figure 2.Etoposide release from the PBCA nanoparticles

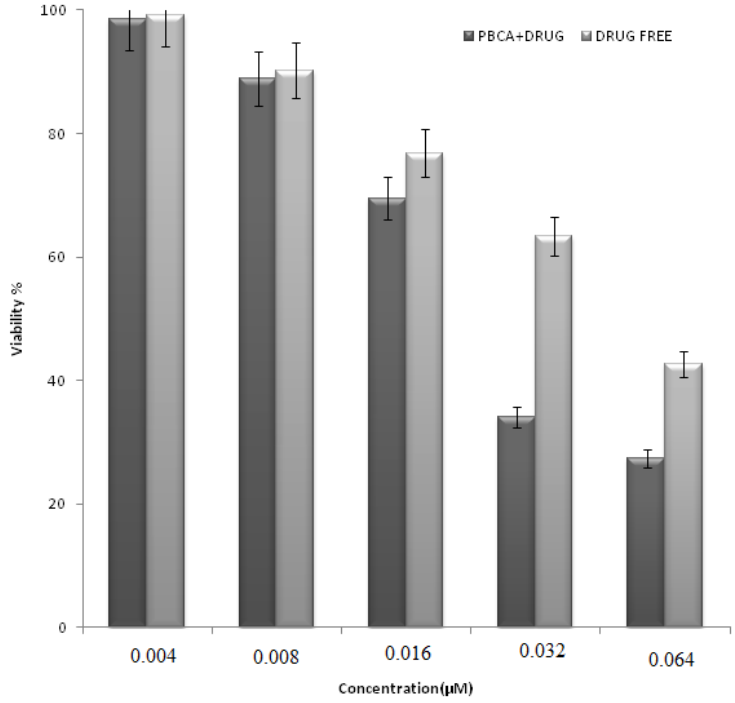

Figure 3. the cytotoxicity effects of etoposide and loaded etoposide on PBCA nanoparticles on cell line BE (2)-C after 48 hours incubation; the results were presented in surviving percentage against control group, besides, error percent was reported as 5%

Table 1: Stability results of PBCA nanoparticles during 2 months (with a 5% average error).

Time	Zeta potential(mV)	Size(nm)	Encapsulation%	**Loading**%
Drug production time	-26.3±1.5	225.3±21.7	97. 3±2.1%	8.7±0.15%
Two months after production time	-25.1±1.1	232.4±22.8	98.22±2.5%	8.8±0.19%

DISCUSSION

The targeted drug delivery by using nanotechnology has the ability to be used in cancer cell treatment. Nanoparticles can escape from reticuloenothelial system and cause a higher stability for drug in blood circulation. In addition, they cause the lower systematic toxicity on healthy cells and increase the efficiency of treatment. Nanoparticles would also change the new treatment methodologies like photodynamic and hyperthermia. Pharmaceutical carriers in Nano scale has the ability to overcome challenges related to resistance against drugs in treating of brain cancer[3]. Drug delivery to the target tissue is the main challenge of pharmaceutical biotechnology and Nano-carriers have the potential for doing so. PBCA is one of the polymeric Nano-carriers[2].In various studies, the effect of various drugs loaded into PBCA nanocarriers on different cell lines have been investigated. For example, A study by Hassan Ebrahimi et al, have been investigated the cytotoxic effect of cisplatin loaded PBCA on ovarian cancer on A2780CP cell line. Their study showed that 95% of the drug content were released from the nanoparticles after 48 hours [8]. Our study succeeded to proper loading of etoposide on PBCA nanoparticles by using micro emulsion polymerization. The olive oil and honey were used as a surfactant due to their anti-oxidant properties[9,10]. Toxicity tests showed that drug efficiency enhanced two times in comparison with the free drugs, which indicated controlled release of drug from formulation. Release tests showed that the formulation has proper retention strength and using poly ethylene glycol is the most possible cause of low release in the drug. Tests and experiments also showed the using of poly ethylene glycol in nanoparticles could enhance their stability in the blood circulation and make them undetectable in the blood; then there is a better chance for delivering of drug to the target tissue and this means higher efficiency of the drug[11].In general, a proper efficiency has reported for the above mentioned drug and the results also suggest their use inside the body.

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