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Short Communication

Poly (ε-caprolactone) nanoparticles of carboplatin: Preparation, characterization and in vitro cytotoxicity evaluation in U-87 MG cell lines

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ABSTRACT

Carboplatin is a platinum based drug used in the treatment of several malignancies. Due to poor cellular uptake, generally, a larger dose of drug is administered to achieve therapeutic levels, causing harmful side-effects such as hematologic toxicity. In order to enhance the cellular uptake of carboplatin, we have developed carboplatin loaded nanoparticles using the biodegradable polymer poly (ε-caprolactone) (PCL). Nanoparticles ranging from the size of 23.77 ± 1.37 to 96.73 ± 2.79 nm with positive zeta potential and moderate entrapment efficiency (54.21 ± 0.98%) were obtained. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) confirmed the spherical morphology and smooth surface of all nanoformulations. The concentrations of PCL and the stabilizer (DMAB) are found to play a role in determining the size and the entrapment efficiency of the nanoparticles. Drug release from nanoparticles followed a biphasic pattern with an initial burst release followed by a sustained release for 10 h. Results of in vitro cellular uptake and cytotoxicity studies revealed that carboplatin in the form of PCL-nanoparticles were efficiently up taken and displayed profound cytotoxicity to U-87 MG (human glioma) cells than the free drug. Importantly, unlike the free carboplatin, carboplatin in the form of PCL nanoparticles did not present any haemolytic activity in rat erythrocytes, a major side effect of this chemotherapeutic drug. This suggests that poly (ε-caprolactone) nanoencapsulation of carboplatin might be an efficient approach to treat cancer, while reducing carboplatin induced haemolysis.

1. Introduction

Carboplatin (cis-diamine (1,1-cyclobutanedicarboxylato)-platinum (II)), a cisplatin analogue and cell-cycle non-specific drug that modifies DNA structure and inhibits DNA replication, thereby causing cell death. This alkylating agent is used in the treatment of malignant gliomas, metastatic brain cancer and also recommended for chemotherapy of ovarian, head, neck and lung cancer [1,2]. Carboplatin and its analogue, cisplatin has their own merits and demerits when used as chemotherapeutic agents. In terms of treatment efficiency, both of them show similar activity. But, if the toxicity profiles are taken into consideration, carboplatin shows hematologic toxicity [3], whereas its analogue cisplatin shows renal and neurotoxicity [4,5]. Although cisplatin based nano-formulations have been reported to increase in vitro and in vivo efficacy with lower systemic cytotoxicity [6,7], no such study has been performed with carboplatin.

Polymeric nanoparticles possess wide applications and capable of targeting tumour tissue both actively and passively. Strategies such as, localized catheter-based infusions or receptor-mediated targeting and enhanced permeation/retention effects are adapted for active and passive targeting of nanoparticles to tumours, respectively [8,9]. Poly (ε-caprolactone) (PCL) is a FDA approved biodegradable and biocompatible polyester polymer that has been used to encapsulate a variety of drugs (hydrophilic and hydrophobic) for controlled release and targeted drug delivery [10,11].

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In the present study, we have prepared carboplatin loaded PCL nanoparticles by double-emulsification solvent evaporation method and characterized the nanoparticles for their size, shape and in vitro drug release profile. Further, drug loaded nanoparticles were studied for their in vitro cellular uptake and cytotoxicity in human glioma cell line U-87 MG. Haemolysis, being one of the important side effects of carboplatin, the haemolytic activity of carboplatin loaded PCL nanoparticles and free drug were compared.

2. Experimental

2.1. Materials

Poly (ε-caprolactone) (M.W.: 14,000) and FITC (Fluorescent isothiocyanate) were purchased from Sigma Aldrich, India. Carboplatin was donated by Macchem Chemicals Ltd., Mumbai, India.

2.2. Preparation of PCL nanoparticles

Carboplatin loaded PCL nanoparticles were prepared by double-emulsification solvent evaporation using high speed homogenization method [12]. Briefly, PCL and carboplatin were dissolved respectively in ethyl acetate (EA) and de-ionized water. The aqueous carboplatin solution was slowly added to the organic phase containing PCL and emulsified by high-speed homogenization for 5 min at 15,000 rpm to form W/O emulsion. Then, the W/O emulsion was poured into aqueous solutions containing various concentrations of stabilizer (DMAB) and homogenized at 22,000 rpm for 15 min to form W/O/W double emulsion. Finally, the organic solvent was evaporated by magnetic stirring overnight to obtain carboplatin nanoparticles. The nanoparticles formulation was optimized by using different ratios of polymer, drug and stabilizer which are shown in Table 1.

2.3. Characterization

The carboplatin loaded PCL nanoparticles were characterized by Zetasizer (nano ZS90, Malvern Instruments), high-resolution transmission electron microscopy (JEOL JEM 2100) and atomic force microscopy (NTMDT, NTEGRA prima, Russia) at the resonating frequency range of 47–150 KHz.

The entrapment efficiency (EE) was estimated as the following: 2 ml of formulation was centrifuged at 20,000 rpm for 30 min at 10 °C and the supernatant was collected and analyzed for free drug concentration using UV spectroscopy at 230 nm [13].

2.4. In vitro drug release studies

Briefly, 1 ml carboplatin loaded nanoparticles from each formulation was placed in a dialysis bag which was then immersed in a bottle containing phosphate-buffered saline (PBS, pH 7.4) maintained at 37 ± 1 °C under continuous magnetic stirring at 50 rpm. At various time intervals, 1 ml sample was withdrawn. After each withdrawal, 1 ml fresh buffer solution was added to maintain constant volume. Carboplatin content in the withdrawn samples was estimated by UV spectroscopy at 230 nm.

Table 1
Various compositions of polymer, drug and stabilizer used in the nanoparticle formulations.

Formulation code	Drug (mg)	Polymer (mg)	Organic solvent (ml)	Stabilizer (%)
F1	10	50	5	1
F2	10	100	5	1
F3	10	50	7.5	1
F4	10	100	7.5	1
F5	10	50	5	1.5
F6	10	100	5	1.5
F7	10	50	7.5	1.5
F8	10	100	7.5	1.5

2.5. Cell culture

Human glioblastoma–astrocytoma, epithelial-like cell line (U-87 MG) was maintained in minimum essential medium (MEM) supplemented with 1 mM sodium pyruvate, 1× non-essential amino acid, 3 μg/ml amphotericin,400 μg/ml gentamycin, 250 μg/ml streptomycin, 250 units/ml penicillin, 10% foetal bovine serum (FBS). Cultures were incubated in a carbon dioxide incubator at 37 °C with 5% CO_2.

2.5.1. In vitro cellular uptake study

FITC loaded PCL nanoparticles were prepared essentially as mentioned in Section 2.2 using 10 mg FITC instead of carboplatin and used for tracking cellular uptake. U-87 MG cells were seeded in 24-well plates at a concentration of 0.05×10^6 cells/well and incubated in a carbon dioxide incubator at 37 °C with 5% CO_2 for 24 h to allow cell attachment. Then the medium was replaced by fresh medium containing FITC nanoparticles and incubated further. After 1 and 4 h of co-incubation, the wells were washed with ice-cold PBS and the cells were visualized under fluorescent microscope [14].

2.5.2. In vitro cytotoxicity study

To compare the cytotoxicity of carboplatin nanoparticles and free drug, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed in U-87 MG cells. The cells were seeded at a density of 1000 cells/well in 96 well plates and incubated in a carbon dioxide incubator at 37 °C with 5% CO_2, overnight for cell attachment. Then, the medium was replaced with fresh medium containing either nanoparticles or free drug formulation to reach the final carboplatin concentrations of 0.125–2 μg/ml. After 48 h of incubation, 10 μl of MTT (5 mg/ml stock solution) was added to each well and incubated for another 4 h. Then, 100 μl of DMSO was added to each well to dissolve any purple formazan crystals formed and the optical density was determined at 570 nm by a microplate reader.

2.6. In vitro haemolytic assay

Carboplatin in the form of PCL nanoparticles and free drug were evaluated for their potential to induce haemolysis in rat red blood cells (RBCs) as reported before [15]. In brief, rat RBCs were separated from fresh rat blood by centrifugation at 1500 rpm for 15 min to remove plasma and other cell debris. After washing three times with normal saline, purified RBCs were resuspended in normal saline to obtain 2% (v/v) RBC suspension. Then, 10 μg of carboplatin in the form of free drug and carboplatin loaded PCL nanoparticles (10 and 20 μg) dissolved in saline were added to 2.5 ml of 2% rat RBC suspension and incubated at 37 °C. After 4 h, the samples were centrifuged at 2500 rpm for 10 min and the supernatants were observed for indication of haemolysis. Distilled water served as positive control and normal saline as negative control.

3. Results and discussion

The carboplatin nanoparticles prepared in the present study were below 100 nm in size (Table 2). Utilization of EA as solvent could be attributed to the formation of smaller size particles, because EA being a partially water miscible solvent, it has low