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Intratumoral administration of carboplatin bearing poly (ε-caprolactone) nanoparticles amalgamated with *in situ* gel tendered augmented drug delivery, cytotoxicity, and apoptosis in melanoma tumor

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ABSTRACT

Background and objective: In a phase II clinical trial, carboplatin (CBDCA) displayed the response rate of 19% equivalent to dacarbazine in the treatment of malignant melanoma. However, besides desirable therapeutic profile, intravenous (*i.v*) administration of CBDCA delivers a subtherapeutic concentration at the target site. This entails administration of CBDCA through an alternate route by using nanovectors to achieve therapeutic efficacy in the treatment of melanoma.
Methods and results: Carboplatin loaded poly(ε-caprolactone) nanoparticles (CBDCA-PCL-NPs) were formulated and amalgamated with chitosan-β-glycerophosphate gel (CBDCA-PCL-NPs-Gel) for intratumoral (*i.t*) administration. The mean particle size and zeta-potential of CBDCA-PCL-NPs were determined to be 54.5 ± 6.3-nm and −8.1 ± 0.9-mV, in addition to spherical shape of the nanoformulation. FT-IR spectroscopy denied any issue of chemical incompatibility between drug and polymer. XRD pattern indicated the amorphous lattice of CBDCA-PCL-NPs. The drug loading capacity of CBDCA-PCL-NPs-Gel was estimated to be 152 mg/1 ml. CBDCA-PCL-NPs-Gel demonstrated prolonged drug release up to 48 h. Furthermore, CBDCA-PCL-NPs-Gel displayed the IC_{50} of 80.3-μM significantly ($P < 0.05$) lower than 162.8-μM of CBDCA-PCL-NPs and 248.5-μM of CBDCA solution in B16F1, melanoma cancer cells. CBDCA-PCL-NPs-Gel verified 80.2% of apoptosis significantly ($P < 0.01$) higher than 57.6% of CBDCA-PCL-NPs and 43.4% of CBDCA solution. Continuation to this, CBDCA-PCL-NPs-Gel significantly ($P < 0.01$) suppressed the tumor volume to 95.5 ± 8.4-mm^3 as compared to 178.9 ± 10.2-mm^3 of CBDCA solution injected *i.t.* and 210.6 ± 17.1-mm^3 displayed by CBDCA solution injected *i.v.* vis-à-vis 815.4 ± 17.1-mm^3 tumor volume of B16F1 tumor bearing C57BL6J mice.
Conclusion: The promising preclinical results of CBDCA-PCL-NPs-Gel warrant further investigations under a set of stringent parameters for the treatment of melanoma.

1. Introduction

Melanoma is a malignant tumor whose incidences are increasing continuously from the last 30 years [1]. The mortality of melanoma is faster than that of any other cancer in USA. Overall, melanoma accounts for 1–3% of all malignant tumors and incidences are increasing by 6 to 7% each year [2]. Melanoma is more frequent in men as compared to women [3]. Various treatment modalities have been recommended by USFDA for the treatment of melanoma such as carboplatin (CBDCA), dacarbazine, temozolomide, vincristine sulphate, vindesine, nitrosoureas and immunotherapy (interleukin-2, and interferon α-2b) [4]. However, each drug has its own pros and cons.

CBDCA, cis-diamine (1,1-cyclobutanedicarboxylato)-platinum (II) is a second-generation platinum compound that was developed to reduce the side effects of cisplatin, particularly neurotoxicity,

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nephrotoxicity and emesis, while maintaining comparable anticancer activity and therapeutic efficacy [5]. Furthermore, CBDCA is widely used to treat solid tumor in adults, especially ovarian and lung cancer, in addition to brain tumor, neuroblastoma, retinoblastoma, germ cell tumor, and hepatoblastoma in children [6]. Mechanistically, CBDCA modifies DNA structure and inhibits its replication, thereby causing cell death [7,8]. Both CBDCA and cisplatin being a chemotherapeutic agent, have their own merits and demerits. However, in terms of treatment efficiency, CBDCA and cisplatin demonstrated identical activity [9]. Furthermore, in a phase II clinical trial, CBDCA displayed the response rate of 19% equivalent to primary drug, dacarbazine in the treatment of malignant melanoma [10]. Besides desirable therapeutic benefits, intravenous (*i.v*) administration of CBDCA induces thrombocytopenia, mild to moderate nausea and vomiting in cancer patients [11]. This entails administration of CBDCA through a safe and biodegradable nanovector for the treatment of melanoma by following an alternate and clinically relevant route of administration.

Polymeric nanoparticles (NPs) are capable of targeting tumor tissues both actively and passively [12]. Strategies such as localized catheter-based infusions or receptor-mediated targeting as well as enhanced permeation and retention (EPR) effect can be achieved with active and passive targeting of NPs [13]. Poly (ε-caprolactone) (PCL) is a FDA approved biodegradable and biocompatible polyester polymer that has been used to encapsulate a variety of hydrophilic and hydrophobic drugs for controlled and targeted drug delivery. For instance, hydrophilic moieties cisplatin, doxorubicin, and 5-fluorouracil were enveloped with moderate to high encapsulation efficiency in stealth PCL-NPs for passive delivery to T47D and MCF-7 breast cancer cells, respectively [14]. In a similar way, GMT8 aptamer anchored stealth PCL-NPs of hydrophobic drug, docetaxel were successfully actively targeted to glioblastoma-bearing mice [15].

Apart from polymeric nanoparticles, injectable *in situ* gel has also gained a wide concern as a polymeric drug carrier over the preceding few years [16,17]. In this context, chitosan-β-glycerophosphate *in situ* gel (Gel) has delivered a high payload of chemotherapeutic drug through intratumoral (*i.t*) route and thereby elevated its clinical aspects [18]. Moreover, for localized therapy, injection of Gel leads to the formation of a depot at the site of administration that consequently releases the cytotoxic drug in a continuous and sustained manner [19]. Interestingly, the ability of Gel to deliver the drug throughout the tumour diminishes the systemic toxicity and thereby accomplished the superior therapy vis-à-vis active or passive therapy [20].

Therefore, in present investigation, carboplatin loaded poly (ε-caprolactone) nanoparticles (CBDCA-PCL-NPs) were prepared by solvent evaporation method [21] that later amalgamated with chitosan-β-glycerophosphate *in-situ* gel (CBDCA-PCL-NPs-Gel) by cold method [22]. The CBDCA-PCL-NPs were characterized for particle size, zeta-potential, shape and *in vitro* drug release. Furthermore, spectral techniques were also employed to address any issue of incompatibility between drug and excipients. The *in vitro* and *in vivo* therapeutic efficacy of CBDCA-PCL-NPs-Gel were tested using standard cell proliferation assay [23] and flow cytometry based apoptosis assay [24], respectively. The tumor regression analysis was carried out in mouse melanoma, B16F1 cells implanted in C57BL6J mice [25].

2. Material and methods

2.1. Chemicals and reagents

Carboplatin (M_W∼ 373.272 Da) was purchased from TCI Chemical Private Limited, Chennai, India. Chitosan (M_W∼1,50,000 Da, 75–85% deacetylated), MTT Assay Kit and Dulbecco's Modified Eagle's (DMEM) medium were purchased from Himedia, Mumbai, India. Poly(ε-caprolactone, M_W∼14000 Da) and pluronic F68 were purchased from Otto Chemie Private Limited, Mumbai India. Dichloromethane (DCM) was purchased from Lobba *Chemie*, Mumbai, India. Dimethyl sulfoxide (DMSO) was procured from Ozone International, India. Sodium-β-glycerophosphate was obtained from Molychem, Mumbai, India. All other chemicals used were of highest analytical grade.

2.2. Cell culture and mediums

Mouse melanoma cancer cell line, B16F1 was maintained in 95% air and 5% CO_2 at 37° C using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. All experiments were performed with asynchronous populations in exponential growth phase (24 h after plating) [26].

2.3. Preparation of carboplatin loaded poly (ε-caprolactone) nanoparticles

Carboplatin loaded *poly (ε-caprolactone)* nanoparticles (CBDCA-PCL-NPs) were prepared by solvent evaporation method [21]. In brief, 100 mg of PCL was dissolved in 16 ml of DCM. Following this, organic phase was dispersed in aqueous phase. The aqueous phase was prepared by mixing 10 mg of CBDCA and 250 mg of Pluronic F68 (PF68) into 50 ml of distilled water. Stirring was continued for 5–6 h. Lastly, the nanoparticles suspension was lyophilized (Lark Technology, New Delhi, India) to obtain the fine powder of CBDCA-PCL-NPs (Scheme 1). In addition, PCL-NPs were also customized without incorporating the therapeutic moiety.

2.4. Characterization of nanoparticles

2.4.1. Particle size and zeta-potential

The particle size and zeta-potential of nanoparticles were assessed by zeta-sizer (Malvern Instrument, Worcestershire, UK). In brief, 10 mg quantity of each nanoparticle sample was suspended in 10 ml of water for injection and serially diluted to make 100-μg/ml before analysis. An electric field of 150 mV was employed to determine the electrophoretic velocity of nanoparticles. All measurements were carried out in triplicate (n = 3).

2.4.2. Transmission electron microscopy (TEM)

The surface topography of each sample of nanoparticles was analyzed using transmission electron microscope (TEM, Hitachi, H-7500 Model) maintained at the voltage of 80 kV. In brief, an aqueous dispersion of 100-μg/ml of each sample was drop cast onto a carbon coated copper grid and grids were air dried at room temperature before loading into the microscope.

2.4.3. Fourier-transforms infrared (FT-IR) spectroscopy

FT-IR was executed to reveal any chemical incompatibility between drug and excipients. In brief, the infrared spectrum was recorded for CBDCA, PCL-NPs, physical mixture of CBDCA and PCL-NPs as well as CBDCA-PCL-NLPs by employing BRUKER (Billerica, Massachusetts, United States) infrared spectrophotometer. The specific sample was scanned thrice between 4000 and 500 cm^{-1} at a resolution of 4 cm^{-1}.

2.4.4. Powder X-ray diffraction (PXRD)

The polymorphism of drug in nanoparticles was characterized by X-Ray diffractometer (RIGAKU, Rotaflex, RV 200 (Rigaku Corporation, Japan) using Ni/filtered, CuKα-radiation, voltage of 60 kV and current of 50 mA. The range of scanning was fixed at 1°/min over 5° to 50° diffraction angle at 2θ range. The XRD pattern was