International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 2, Special Issue
September 2015

Rationally Engineered Biodegradable Nano Particles for the delivery of Ccisplatin in solid Tumors.

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Abstract

Cisplatin is a promising anticancer agent and is effective against wide variety of cancers including ovarian, bladder,breast, cervical, stomach, prostrate, head and neck cancer etc. It is dCTP analogues that cross links purine bases on DNA, causing DNA damage, finally leading to apoptosis. But there exists substantial challenges with of delivery of cisplatin which includes limited solubility, lack of selectivity towards cancer and non-cancerous cells, multidrug resistance and numerous side effects. Therefore, nanoparticle approach is gaining attraction due to its favourable properties of sustained circulation, better patient compliance and better accumulation of drug in cancerous tissue. We have encapsulatedcisplatin in block copolymer PLA-PEG-PPG-PEG (FDA approved) based nanoparticle system which acts as target drug delivery vehicles. The latter is a biodegradable shell of 70-100 nm in size. The nanoparticle was synthesised by nanoprecipitation method and was characterised by FTIR, NMR and particle size analyser. The drug loaded nanoparticle based system used in this study will be explored in vitro and in vivo for therapeutic efficacy.

Keywords: *Nanotechnology, Cisplatin, Cancer*

Abbreviations: *NMR, nuclear magnetic resonance, FTIR, fourier transformed infrared spectroscopy, PLA, polylactic acid, PEG, polyethyleneglycol, PPG, polypropylene glycol, DCC, N,N-dicyclohexylcarbodimide and DMAP, 4-dimethylaminopyridine.*

Introduction

Cancer remains one of the world's most deadly diseases and is recognised by the condition wherein abnormal cells divide without any control and are able to invade other tissues. It can be classified as Carcinoma, Sarcoma, Leukemia, Lymphoma and Myeloma according to their site of origin [(1-3)].Treatment of cancer includes various therapies such as surgery, chemotherapy, hormonal therapy, radiotherapy, targeted therapy etc[(4)]. Chemotherapy involves the use of chemical medication to treat the disease. Patients may receive monotherapy in which given just one drug is given or combination therapy which involves administration of more than one drug.Paclitaxel, Doxorubicin, Cisplatin, Docetaxelare few of the chemotherapeutic drugs given as standardised chemotherapy regimen [(5,6)].Cisplatin is one of the most potent chemotherapeutic agent .It is basically a platinum containing anticancer drug which cross links DNA by reacting with N-7 of purine bases and forms intrastrand cross links. This further activates signal transduction pathways including p53, ATR, MAPK that triggers apoptosis[(7)].Conventional chemotherapy has certain disadvantages which includes non-selective nature of chemotherapeutic agents, poor accessibility of antineoplastic agents to the tumor site and numerous side effects [(8)].In order to overcome these challenges nanoparticle mediated drug delivery is gaining attention worldwide, which confers various advantages over the conventional chemotherapy including increased circulation time, reduced toxicity and better endocytic uptake[(9)].

In this study, we have encapsulatedcisplatin in biodegradable copolymeric PLA-PEG-PPG-PEG based nanoparticles for passive targeting of cisplatin to tumor site. Biodegradable nanoparticles prepared from this type of copolymer will have hydrophobic core of PLA and hydrophilic PEG-PPG-PEG tail which will prevent the uptake of these nanoparticles from the immune cells e.g. macrophages.The proposed biodegradable

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nanoparticle system is anticipated to have better efficacy due to increased circulation time,lower toxicity due to encapsulation of the drug in the nanodelivery system,better cellular uptake pertaining to EPR(Enhanced Permeability and Retention) effect, improved pharmacokinetic profile and better patient compliance [9,10].

Materials and Methods

Materials

Polylactic acid (PLA) was purchased from Bermingham Polymer Inc.,USA, polyethylene glycol (PEG), cisplatin (cis-platinum diammine dichloride), acetonitrile, dichloromethane, poloxamer-F127,N,N-dicyclohex-ylcarbodimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Sigma-Aldrich,USA.

Synthesis and characterization of PLA-PEG-PPG-PEG copolymer

PLA segments (Mw~70000,SigmaAldrich) were conjugated to PEG-PPG-PEG copolymer to obtain amphiphilic PLA-PEG-PPG-PEG(Mw~12,000) block copolymers. Carboxy terminated PLA gets coupled to the hydroxyl group of PEG-PPG-PEG by condensation reaction in DCM using DCC as coupling agent and DMAP as catalyst.(10) In a standard experiment, equimolar of PLA and PEG-PPG-PEG were dissolved in 50 ml of dicholoromethaneand was kept at magnetic stirrer at 0°C. Further 5 ml of 1% DCC was addedslowly followed by addition of 2ml of 0.1% DMAP for 16-18 hours. The PLA-PEG-PPG-PEG block copolymer thus formed was then precipitated with 1:1 mixture of methanol and diethylether to remove the unreacted PLA and PEG-PPG-PEG. The synthesized block copolymer was freeze dried under vacuum and kept at -20° C until used. NMR of PLA, PLA-PEG-PPG and PLA-PEG-PPG-PEG was performed in $CdCl_3$ at 300Hz (Bruker).

Preparation of nanoparticles from PLA-PEG-PPG-PEG block copolymer

Nanoparticles made of PLA (70,000)-PEG-PPG-PEG (12,500) block copolymer are prepared using the nanoprecipitation technique[5,11,12].Briefly,PLA-PEG-PPG-PEG block copolymer was dissolved in a 5 ml organic solvent miscible to water (like Acetonitrile) and emulsified in a 20 ml aqueous phase. This dissolved PLA-PEG-PPG-PEG was slowly dropped into the emulsified aqueous phase using 5 ml syringe with a constant stirring. This primary emulsion was then added into a 20mL aqueous phase composed of Poloxamer-F127 in distilled water and stirred at room temperature for 6 to 8 hours for solvent evaporation and stabilization of nanoparticle. Particle size and distribution of PLA-PEG-PPG-PEG nanoparticles was analyzed using Particle Size Analyser(Nanosight NS 500).

Loading of anticancer drug into the nanoparticles

PLA-PEG-PPG-PEG block copolymer was dissolved in acetonitrileand emulsified inaqueous phase containing about 100 mg of emulsifying agent i.e.Pluronic F127. Cisplatinwas dissolved separately in 100 µl saline(0.9%NaCl) by gentle stirring and was further dispensed into the dissolved PLA-PEG-PPG-PEG block copolymer. The mixture of PLA-PEG-PPG-PEG addedcisplatin drug was introduced slowly into the emulsified aqueous phase with a constant stirring. Then the solution was kept for overnight stirring at room temperature to evaporate the organic solvent. The drug loaded polymeric nanoparticles were ultrafiltered using Amicon filters (Millipore, MW cut off 10,000Da) at 4000 rpm for 30 minutes in Beckman ultracentrifuge to remove the ACN and free drug outside PLA-PEG-PPG-PEG aggregates of 10kDa.The loading capacity of drug in the PLA-PEG-PPG-PEG nanoparticles was determined by deducting the amount of free drug outside the Amicon filters (filtrate) from the initial amount of drug added. The amount of free drug was determined by measuring the absorbance by UV–Vis spectrophotometry (Perkin Elmer UV–Vis Spectrophotometer Lambda 35, USA) at 314nm .The drug loading capacity of PLA-PEG-PPG-PEG was calculated as follows:

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$$\text{Loading capacity} = \frac{\text{Totalcisplatin (mg) - Free cisplatin (mg)}}{\text{Initial cisplatin}}$$

Size and zeta potential of nanoparticles was determined by using particle size analyser (Nanosight NS 500).

In vitro studies

Controlled and sustained release of cisplatin is an important parameter of the study. In vitro release behaviour of cisplatin was assessed by ultrafiltration method[10,12]. Briefly, drug loaded samples nanoparticles(4mg) were immersed in phosphate buffer saline at 37°C with mild shaking at150 rpm(orbital shaker) . At predetermined time points,upto 10 days samples were removed from the shaker and ultrafiltered through 10kDa Amicon filter (Millipore). Drug concentration was determined by UV Vis spectrophotometer at 314 nm.

Results

Preparation of copolymer

PLA-PEG-PPG-PEG nanoparticles were synthesised by condensation reaction of PLA (70kDa) and PEG-PPG-PEG(12.5kDa) using DCC/DMAP chemistry. 1HNMR of PLA showed a characteristic peak of lactic acid at –CH at 5.1 ppm and integrated signals around 1.5ppmare attributed to methyl protons of lactic acid repeated units. PEG shows a short peak between 3.7-3.4 attributed to $–(CH_2CH_2O)$.The characteristic peak of lactic acid –CH at 5.1 and a peak between 3.7-3.4 confirmed the successful synthesis of PLA-PEG-PPG-PEG (Figure 1).

FTIR spectra further consolidated the construct of PLA-PEG-PPG-PEG copolymerThe absorption bands at 3010 and 2955 cm-1 were C-H stretch of -CH2. A strong band at 1762.6 cm-1 was attributed to C=O stretch, and the absorption at 1184-1089.6 cm-1 was assigned to C-O stretch. The FTIR spectra of PLA and PLA-PEG-PPG-PEG had similar characteristic peaks, since they basically had same functional groups(Figure 2). The PLA-PEG-PPG-PEG block copolymer consists of multi-layered structure with the PLA hydrophobic core and the PEG-PPG-PEG hydrophilic shell interfacing with the aqueous medium.

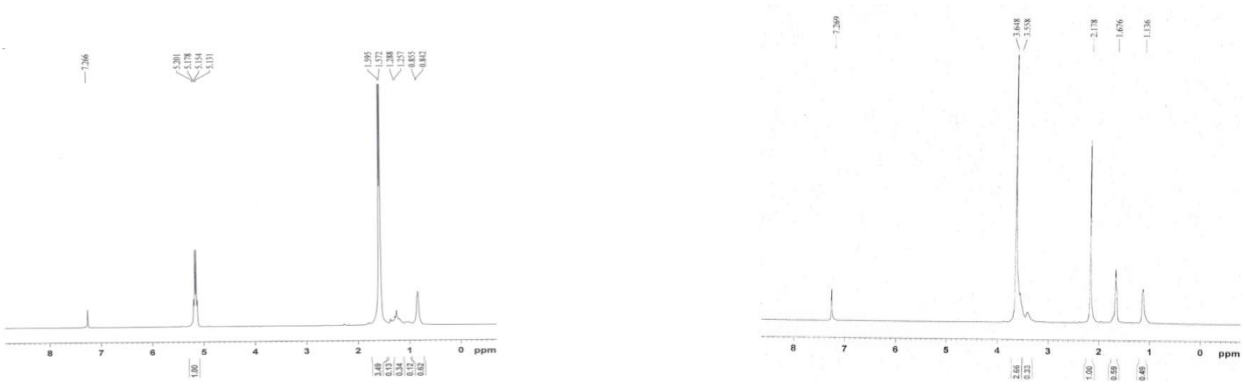

Figure 1b: NMR spectra of PEG-PPG-PEG

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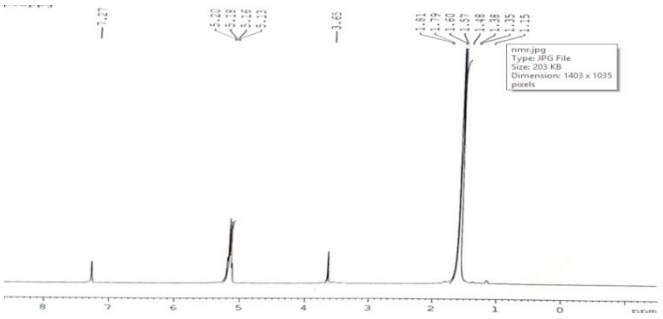

Figure 1c: NMR spectra of PLA –PEG-PPG-PEG

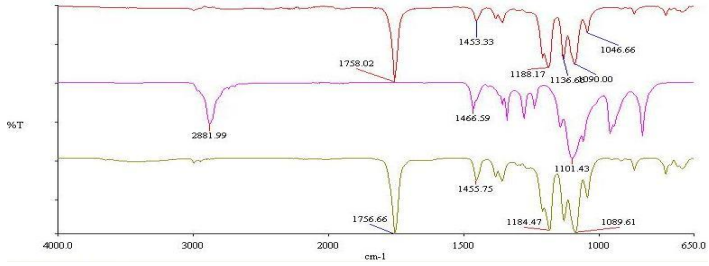

Figure 2: FTIR spectra of PLA, PEG-PPG-PEG and PLA –PEG-PPG-PEG

Preparation and characterization of cisplatin loaded nanoparticles

To ensure targeted delivery of drug along with its controlled and sustained release, we have encapsulated the drug in the biodegradable polymer based nanoparticle [13,14]. Nanoparticles were synthesised by nanoprecipitation method in which the tetrablock copolymer was dissolved in water miscible solvent like acetonitrile, and dropped slowly into the aqueous phase under stirring conditions to generate nanoparticles. The mean particle size of blank nanoparticle was 87.08 nm which was increased to 95nm on encapsulation of cisplatinwhere as the zeta potential was changed from -6.3 to -5.68 on addition of drug (Table 1). Moreover, the encapsulation efficiency was found to be 97.5% and the final loading of drug into the nanoparticle was 3.9%(w/w).

Sample	Solvent	Emulsifier	Particle Size(nm)	Zeta Potential
PLA-PEG-PPG-PEG(without drug)	Acetonitrile	Pluronic F-127	87.08±2.5	-6.3±0.98
PLA-PEG-PPG-PEG(with drug)	Acetonitrile	Pluronic F-127	95±1.8	-5.68±1.33

Table 1: Preparative condition and particle characteristics of nanoparticles.

Release of cisplatin from the nanoparticle in vitro

The release of drug from the nanoparticles was analysed at pH 7.4 in phosphate buffer saline for upto 10 days. The release pattern involves initial rapid release followed by the slow and steady release of the drug from the polymeric nanoparticle.The cumulative release from these nanoparticles is shown in Figure 3.

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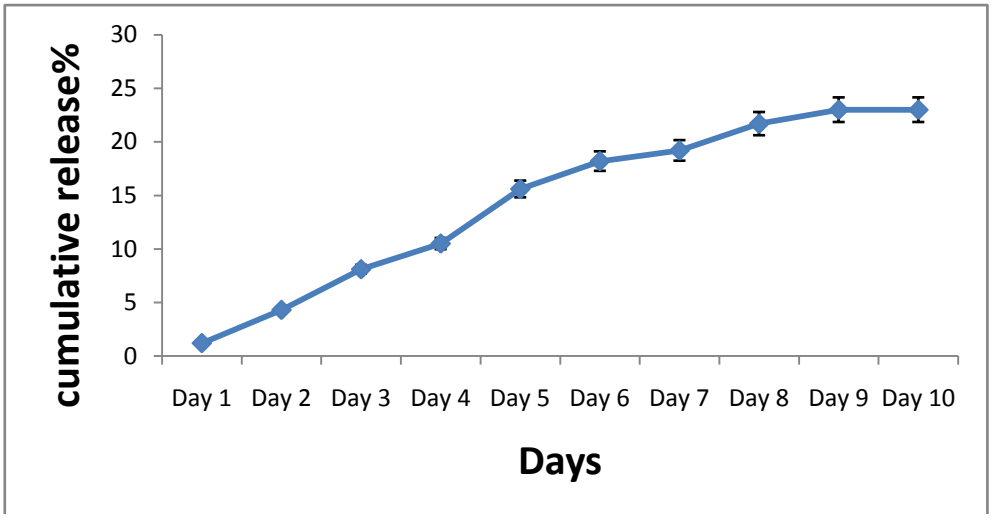

Figure 3: Cumulative release of drug from the PLA-PEG-PPG-PEG nanoparticles.

Discussion

Over the years nanotechnology in cancer therapyhas gained sincere attention due to its potential to solve numerous complications caused by conventional chemodrugs which include low aqueous solubility, toxicity, poor accessibility to tumorsite, low therapeutic index etc[15]. Over the last few years, much emphasis is made on integratingchemodrugs with the nanoplatforms for improved solubility, better bio distribution as well as prolonged and sustained circulation [9,15,16].We aim to investigate the targeted delivery of cisplatin to the tumor tissues by using PLA-PEG-PPG-PEG nanoparticles as transporting vehicle pertaining to the EPR effect. Due to the leaky vasculature of the tumorangiogenetic vessels it is anticipated that the cisplatin will accumulate in the tumor tissue exclusively[5,13,14]. It is tailored in such a way that PLA forms the hydrophobic core and PEG-PPG-PEG protrudes as ampiphilic tail. PEG is deliberately attached as it masks the nanovector from host immune response and increases the circulatory time. It also provides water solubility to the lipophilic drug[17,18]. The nanoparticles were synthesised by nanoprecipitation method and the size of nanoparticles were below 100 nm which makes it perfectly suitable to be taken up by leaky tumor vessels. Our results demonstrates that incorporation of cisplatin does not lead to significant change in size and zeta potential of nanoparticles(Table 1). Although loading of cisplatin was low around 3.9% but similar loadings of cisplatin have been obtained PLGA-mPEGnanoparticlesand polyalkylacrylocynate nanoparticles [19,20]. In order to improve loading appropriate drug to polymer ratio needs to be optimised. The release profile shows initial burst release followed by slower exponential release. It is reasonable to conclude that initial burst release could be due to the diffusion of drug that was adsorbed over the surface of nanoparticle. Similar patterns have been observed for the release of cisplatin from other polymeric nanoparticles[5,10,19]. We aim to further testify the in vitro and in vivo therapeutic efficiency of this designed nanoparticle in cancer cell lines and balb/c mice.

Conclusion

We have developed biodegradable copolymeric PLA-PEG-PPG-PEG nanoparticle for the delivery of anticancer drug cisplatin. The size of nanoparticle seems to be suitable for the EPR effect and the former exhibit sustained release of drug in the vitro system. The results appear to justify the further exploration of the developed system in the cell culture and in vivo model.

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