Cyclic β-(1, 2)-glucan blended poly DL lactic co glycolic acid (PLGA 10:90) nanoparticles for drug delivery

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ABSTRACT

Our group had previously reported the encapsulation efficiency of cyclic β-(1, 2)-glucan for various drugs. The current study is aimed at evaluating the use of glucan as a drug carrier system by blending with poly lactic-co-glycolic acid (L:G = 10:90). Nanoparticles of glucan (0.5, 5, 10 and 20 wt %) blended with PLGA and gentamicin were synthesized. Encapsulation efficiency was higher for the blends (93% with 20 wt % of glucan) than the PLGA alone (79.8%). The presence of glucan enhanced both the biodegradability, and biocompatibility of PLGA. Degradation of the nanoparticles in vitro, was autocatalytic with an initial burst release of active drug and the release profile was modeled using the Korsmeyer-Peppas scheme. In vivo studies indicated that the drug released from the blends had high volume of distribution, and greater clearance from the system. Pharmacokinetics of the drug was predicted using a double exponential decay model. Blending with PLGA improved the drug release characteristics of the cyclic β-(1, 2)-glucan.

1. Introduction

Cyclic β-(1,2)-glucans (CβG) are water soluble, biocompatible, and biodegradable, extracellular polysaccharides produced by Rhizobiaceae and Agrobacteriaceae family [1, 2, 3]. They help the bacterium in osmoregulation, and during plant infection [4, 5, 6, 7]. The molecular weight of these polymers, ranges between 1 to 6 kDa, with the degree of polymerization from 14 to 28. Each ring size of the CβG exists as a mixture of conformations that have cavities of variable size and polarity [8, 9, 10, 11]. As a result, they form inclusion complexes with both polar and nonpolar compounds including poorly water-soluble molecules such as melenamic acid, vitamins, phenylbutazone, indomethacin, steroids, paclitaxel, vitamins, ergosterol, amphotericin B, flurbiprofen, luteolin and betulinic acid [8, 9]. CβG had been reported to have good encapsulation efficiency for various drugs (with 85–99 % efficiency) including dexamethasone, umbelliferone, curcumin, 6-methylcoumarin, 4-hydroxycoumarin and 4 methyl umbelliferone [12]. Since the glucans are completely water soluble, the encapsulated drugs exhibit initial burst release which depends on the specificity of drug and the binding constant. In order to control the initial burst release, and prolong the release of drug, we propose to blend CβG with a synthetic polymer (PLGA).

Synthetic poly DL lactic co glycolic acid (PLGA), copolymers of lactic acid and glycolic acid, is widely used and tested as a carrier molecule for the delivery of proteins, peptides, drugs, DNA and RNA [13]. PLGA was blended with pluronic (poly (ethylene oxide) (PEO), polypropylene oxide (PPO) or poly (ethylene oxide) (PEO) triblock co polymer to achieve sustained drug release (paclitaxel) [14, 15]. Nanoparticles of copolymer monomethoxy (polyethylene glycol)-poly (D, L lactide-co-glycolide) -poly (L-lysine) were tested for targeted drug delivery of adriamycin (anti-cancer drug) and siRNA (small interfering RNA-negative) in tumor cells [16]. Fab-conjugated nanoparticles of PLGA were developed to effectivly target cancer cells expressing CD44v6 [17]. Among different polymers tested, PLGA10:90 (molar ratio of lactic acid 10, glycolic acid 90) was reported to have better encapsulation efficiency and sustained drug release for hydrophilic drug meropenem than the higher lactic acid polymers [18].

In the present study, CβG was blended with PLGA (10:90) and polyvinyl alcohol (PVA) to improve the drug release characteristics of CβG. PVA is a hydrophilic polymer and it has been used as a surfactant to form nanoparticles of uniform size [19].

2. Materials and methods

Gentamicin (Abbott, India), PVA (Sigma Aldrich, India), Escherichia coli (E.coli) (National Collection of Industrial Microorganisms (NCIM),
Pune), L6 myoblast cells (National Centre for Cell Science (NCCS), Pune, India), Dulbecco's modified Eagle's medium (Himedia, India), Fetal bovine serum (Gibco, India). All other chemicals used were of analytical grade. In vivo experiment was carried out in C. L. Baiid Metha College of Pharmacy, Chennai, India. Animal use and the experimental protocols were approved by the institutional animal ethics committee (IAEC).

2.1. Preparation of CjG and PLGA (L : G = 10 : 90) polymer synthesis

CjG was produced from Rhizobium meliloti (MTCC 3402) in mannitol glutamic acid medium [5], and the extracellular glucan was extracted by ethanol precipitation, and purified using Biogel P6 (Biorad, USA). Glucan was completely water soluble (solubility: 250 gm/L). The molar mass of CjG was 3101.46 Da (with DP size of 17–28) [20]. PLGA (10–90) (87 kDa) used in the current study was synthesized by direct melt condensation process with SnCl2 as a catalyst at 160 °C [21].

2.2. Gentamicin loaded CjG/PLGA nanoparticle synthesis

Blends of CjG and PLGA was prepared by mixing the polymers in four different weight ratios (wt %). P1 (PLGA– 100%), P2 (0.5% CjG with 99.5% PLGA), P3 (5% CjG with 95% PLGA), P4 (10% CjG with 90% PLGA) and P5 (20% CjG with 80% PLGA). Nanoparticles of CjG-PLGA blends with and without the drug was prepared by the emulsion solvent evaporation method [22]. Solutions of varying concentrations of PLGA (100, 99.5, 95, 90, 80 % w/v) in tetrahydrofuran (THF) (with 0.25% trifluoro acetic acid (TFA)) were prepared. Varying quantities of glucan (0.5, 5.0, 10 and 20 % w/v) were solubilized in water. Respective solutions were mixed together and sonicated (30 % amplitude) for 60 min. Aqueous solution of gentamicin (800 mg/ml) was added to this mixture. The mixture was then added drop wise to 0.3 % polyvinyl alcohol solution (100 ml) and homogenized for 30 min using a microtip probe sonicator (Sonics & Materials Inc., USA). Partial vacuum was applied to remove the organic phase and the remaining solution was centrifuged at 12,000 rpm for 20 min. Pellet containing gentamicin loaded nanoparticles was lyophilized and used for further analysis.

2.3. In vitro drug release from the nanoparticles

10 mg of gentamicin loaded nanoparticles was suspended in phosphate buffer saline (5 ml, pH 7.2) in a falcon tube and kept under continuous shaking (120 rpm) on a shaker. At different time intervals the tubes were centrifuged at 12,000 rpm for 20 min and the supernatant was collected. The nanoparticles were resuspended in fresh PBS and left in the shaker. Supernatant was filtered with 0.45 μm membrane filter and the absorbance was measured at 330 nm with a UV-visible spectrophotometer (Jasco V-550, Japan) [18, 23] with pure gentamicin as standard.

2.4. Characterization of the nanoparticles

Size distribution of the nanoparticles was determined by Zetatracs Particle size analyzer (Microtrac Inc., USA) at 780 nm with a backscattering angle of 180°. 1 mg/ml of drug loaded polymeric nanoparticles was suspended in distilled water to reach a final concentration of 100 μg/ml and used for the analysis. Morphology of the nanoparticles was visualized under a transmission electron microscope (Philips/FeiCM-20). Nanoparticles (P1–P5) were mixed with KBr pellets (at a ratio of 1:20) and the infra-red spectrum was recorded from 4000–400 cm⁻¹ with Perkin-Elmer PE1600 FTIR spectrometer. Thermal stability of the nanoparticles was determined by differential scanning calorimeter (Netzsch DSC 204) and thermo gravimetric analyzer (TGA Q500V20.10 Universal TA instrument) in a nitrogen atmosphere from 10 to 140 °C and 30–930 °C respectively at a heating rate of 10 °C/min. 1H NMR (Nuclear magnetic resonance spectroscopy) spectrum of the material was measured (Bruker 500 MHz spectrometer) with 10 mg of sample dissolved in D2O and a drop of TFA. Molecular weight of the polymer was determined by using gel permeation chromatography with tetrahydrofuran as the mobile phase (flow rate of 1 mL/min at 35 °C), and polystyrene (Easical, Polymer laboratories, UK) as internal standard.

2.5. Antimicrobial activity of released gentamicin

Activity of the released drug was determined by a method reported earlier with some modifications [24]. 10 mg of gentamicin loaded nanoparticles were suspended in phosphate buffer saline (5 ml, pH 7.2) in a falcon tube and kept under continuous shaking at 120 rpm and 37 °C. At different time intervals the tubes were centrifuged at 12,000 rpm for 20 min and the supernatant was collected and lyophilized. Cultures of E. coli NCIM 2931 prepared in Luria broth (OD of 0.005) was added to a 96 well clear bottom plate and the drug released from the nanoparticle was added to the each well and incubated at 37 °C for 24 h. The percentage inhibition of bacterial growth was calculated by the using the formula below, with pure gentamicin as positive control.

$$\% \text{ Growth inhibition} = 100 \times \left[ \frac{OD_{test}}{OD_{positive\,control}} \right]$$

2.6. Biocompatibility of nanoparticles

10 mg of gentamicin loaded nanoparticles were suspended in phosphate buffer saline (5 ml, pH 7.2) in a falcon tube and kept under continuous shaking (120 rpm) on a shaker. At different time intervals the tubes were centrifuged at 12,000 rpm for 20 min and the supernatant was collected and lyophilized. Cultures of E. coli NCIM 2931 prepared in Luria broth (OD of 0.005) was added to a 96 well clear bottom plate and the drug released from the nanoparticle was added to the each well and incubated at 37 °C for 24 h. The percentage inhibition of bacterial growth was calculated by the using the formula below, with pure gentamicin as positive control.

$$\% \text{ Growth inhibition} = 100 \times \left[ \frac{OD_{test}}{OD_{positive\,control}} \right]$$

2.7. In vivo gentamicin release

2.7.1. Experimental animals

Adult male Wistar rats weighing 220–240 g were used in the present study. The inbred animals were procured from the animal house of C.L. Baiid Metha College of Pharmacy, (Thoralipakkam, Chennai, India) and were housed six per cage under standard laboratory conditions at room temperature (22 ± 2 °C) with 12 hr light/dark cycle. The animals were provided with standard pellet chow and water ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.

2.7.2. Tail vein injection

Rats were placed in a tail flick cage with the tail part outside; tail vein was identified by applying the nominal pressure on the surface. IV infusion set was inserted into the tail vein and kept undisturbed. Syringe containing the drug solution was connected with the infusion set up and the drug solution was slowly infused in to the tail vein for about 30 s. Wistar rats were divided into four groups with 6 animals in each; group I rats were injected with 2 mg of pure gentamicin by intravenous infusion and group II to IV rats were injected with 10 mg of gentamicin encapsulated CjG/PLGA nanoparticles which contain approximately ~4 mg of the drug.

2.7.3. Detection of concentration of released gentamicin

Blood samples (500 μl) were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 24, 48 and 72 h after injection by retro-orbital plexus puncture technique [26]. The plasma was separated from the blood by centrifugation at 3500 rpm for 10 min. 100 μl of plasma was added to 200 μl of phosphate buffer saline, vortexed and centrifuged at 3500 rpm for 3 min. 20 μl of the sample was injected into HPLC (5 μm C18 reverse phase
analytical column with mobile phase consisting of 0.2 M of Na2SO, 0.02 M of sodium pentane sulfonate and 0.1 % (v/v) of acetic acid in water – methanol (97:3) at a pH of 3.4). The peaks were monitored using a UV detector at a wavelength of 300–330 nm. The amount of drug in the blood plasma was determined from the standard graph, with pure gentamicin as standard.

3. Results and discussion

3.1. Characterization of gentamicin loaded CIG/PLGA nanoparticles

Presence of characteristic functional groups of CIG and PLGA in the blend was confirmed by FTIR spectroscopy. The peak at 3513 cm⁻¹ corresponds to the OH, 2992 and 2960 cm⁻¹ corresponds to the CN peak of gentamicin [28]. PLGA has characteristic bands corresponding to CH (2637 cm⁻¹), and carbonyl (1745 cm⁻¹). The peaks corresponding to hydroxyl (3503 cm⁻¹) and C–C–O stretch (1086 cm⁻¹) indicates the presence of CIG. These characteristic peaks were observed in all the four ratios (P2–P5) (Figs. S1–SS Supporting information). The IR spectra of all the blends confirmed the presence of both the polymers in the blend. The presence of drug in the polymer nanoparticles were confirmed from the –CN stretch of gentamicin.

| Name of the Polymer | ¹H NMR | FTIR |
|---------------------|--------|------|
| P1                  | 8.69 (s, 1H), 8.41 (s, 3H), 6.84-2.61 (m), 5.54 (d, J = 10Hz), 5.32-5.24 (m), 5.12 (s), 5.04-5.02 (m), 4.97(s), 4.94-4.84 (m), 4.60 (s), 4.62-4.85 (m) 4.49-4.39 (m), 2.91 (s), 2.37 (d), 2.34 (s), 2.21 (s), 2.02-2.00 (m), 1.82 (d, J = 5Hz), 1.74 (s), 1.72 (s), 1.652-1.58 (m), 1.572-1.28 (m), 0.90 (s) | OH (3513 cm⁻¹), CH (2992 cm⁻¹), CH (2960 cm⁻¹), CH (2637 cm⁻¹), carbonyl (1745 cm⁻¹) |
| P2                  | 8.85 (s), 8.64 (s), 6.82-6.39 (m), 5.54 (d, J = 5Hz), 5.32-5.24 (m), 5.12 (s), 5.03-5.05 (m), 4.98 (s), 4.94-4.84 (m), 4.68 (s), 4.49-4.48 (m), 4.44 (s), 4.01 (s), 3.97 (s), J = 10Hz, 3.75, 3.59 (s), 2.91 (s), 2.47 (s, J = 10Hz), 2.34 (s), 2.22 (s), 2.18 (s), 2.02 (s), 2.01 (s), 1.92 (d, J = 5Hz), 1.742-1.59 (m), 1.33-1.22 (m), 0.90 (s) | OH (3513 cm⁻¹), CH (2992 cm⁻¹), CH (2960 cm⁻¹), CH (2637 cm⁻¹), carbonyl (1745 cm⁻¹), amide (1171 cm⁻¹), CO peak (1171 cm⁻¹), CN peak (1093 cm⁻¹) |
| P3                  | 10.03 (s), 6.61 (s), 5.36-5.31 (m), 5.05-4.83 (m), 4.54-4.36 (m), 4.02 (s), 3.97 (s), 3.79 (s), 3.62 (s), 2.94 (s), 2.35 (s), 2.22 (s), 2.02 (s), 1.742-1.55 (m), 1.38-1.30 (m), 0.92 (s) | OH (3513 cm⁻¹), CH (2992 cm⁻¹), CH (2960 cm⁻¹), CH (2637 cm⁻¹), carbonyl (1745 cm⁻¹), amide (1171 cm⁻¹), CO peak (1171 cm⁻¹), CN peak (1093 cm⁻¹), glycosidic bond (1000-1100 cm⁻¹) |
| P4                  | 9.86 (s), 6.61 (s), 5.62 (s), 5.34 (s), 5.04-4.87 (m), 4.50-4.38 (m), 4.02 (s), 3.97 (s), 3.78 (s), 3.58 (s), 2.35 (s), 2.02 (s), 1.70 (s), 1.63 (s), 1.29 (s), 0.90 (s) | OH (3513 cm⁻¹), CH (2992 cm⁻¹), CH (2960 cm⁻¹), CH (2637 cm⁻¹), carbonyl (1745 cm⁻¹), amide (1624 cm⁻¹), CO peak (1171 cm⁻¹), CN peak (1093 cm⁻¹), glycosidic bond (1000-1100 cm⁻¹) |
| P5                  | 9.34 (s), 9.09 (s), 6.84-6.34 (m), 5.37-5.30 (m), 5.08-4.83 (m), 4.50 (d), 3.96 (s), 2.38 (d), 2.21 (s), 2.01 (s), 1.78 (d), 1.62 (d), 1.29 (s), 0.90 (s) | OH (3513 cm⁻¹), CH (2992 cm⁻¹), CH (2960 cm⁻¹), CH (2637 cm⁻¹), carbonyl (1745 cm⁻¹), amide (1624 cm⁻¹), CO peak (1171 cm⁻¹), CN peak (1093 cm⁻¹), glycosidic bond (1000-1100 cm⁻¹) |

The presence of both the polymer and drug was also confirmed by proton NMR spectra. The peaks were consistent with the earlier reported data for CIG and PLGA (10:90) [12, 21]. Further, the presence of both polymers in the different blends were confirmed by their ¹H NMR spectra (Figs. S6–S10 Supporting information). The chemical shift values corresponding to CIG/PLGA drug nanoparticles are listed in Table 1. The chemical shift values δ 8.69 (s, 1H) and 8.41 (s, 3H) indicate the presence of PLGA in P1, upon increasing the concentration of glucan these two PLGA peaks merged into a single peak (P2–P5). δ 5.12 (s) is the amine peak of gentamicin, and 2.91 (s) is the OH peak of gentamicin. In the blends P2 to P5, chemical shift values of CIG δ 3.79 (dd J1 = 5Hz, J2 = 3.72 (s), 3.70 (s), 3.40 (s), 3.57, 3.68, 3.60 (q), 3.44 (d), and 4.78 (m) and that of gentamicin 2.47 (t) were merged with that of the PLGA peaks (Fig. 1). The weight average molecular weight of the PLGA was 87 kDa. The TGA spectrum (Fig. 2) showed maximum weight loss at 300 °C (50 %). The melting temperature of P1 was 25.84 °C (data not included). Addition of CIG decreased the melting temperature of the blends (values for P2–P5 were 25.07, 24.06, 24.73 and 24.22 °C respectively). TGA of pure CIG indicated a maximum weight loss below 200 °C, whereas the blends (P2 to P5) showed maximum weight reduction at 300 °C.

The nanoparticles were spherical shaped (Fig. 3). These particles appeared as core-shell where the inner phase could be attributed to gentamicin loaded glucan and the outer phase to PLGA. Moreover, the phase separation was not evident, which necessitates further investigation of this blend.

Encapsulation efficiency of blends P1 – P5 (Table 2) showed that with the increasing CIG content in the blend, the percentage of drug encapsulated increased. The amount of drug per weight of nanoparticle (drug + polymer) also increased from 38 to 42 % respectively. It has been reported that PLGA (50:50) nanoparticles had poor encapsulation efficiency (11–58.2 %) for water soluble drugs [29] but it was 67.35 % for paclitaxel [30] and 82 % for meropenem [18].

In vitro weight loss of the gentamicin loaded CIG/PLGA nanoparticles and its reduction in size as a function of time (Fig. 4A and B) showed a sharp decrease followed by a relatively slow decrease in size with time. The sudden decrease in weight of the nanoparticle as well as size could be attributed to the water solubility of CIG. The degradation of nanoparticles could be modelled as an autocatalytic reaction, because the degradation of PLGA released acid which in turn catalyzes and enhances this dissolution process further. So, the weight of the particles as a function of time could be represented as

\[
W = W_0 e^{-\beta t} + \frac{W_o}{1 + k_1 t}
\]

where

\[
W = W_0 e^{-\beta t}/[\beta - Wo + Woe^{-\beta t}]
\]

\[
\beta = [k_1 + k_2 Wo]/k_2
\]

B was assumed to be the degraded product which enhanced the degradation of the blend (A further). Fig. 5 compares the experimental and model weight loss of the polymer as a function of time for P2 and P5 blends. The corresponding k1 and k2 values obtained by fitting the data was 0.017 and 0.0115 for P2; 0.018 and 0.0139 for P5 respectively. As expected, increasing CIG content in the blend (from 5 % in P2 to 20 % in P5) increased the rates of reactions since it is highly soluble in water. The model fits the degradation data reasonably well. Autocatalytic degradation [13, 31] of the nanoparticle was also observed in the drug loaded PLGA nanoparticles which was successfully modeled.
3.2. In vitro gentamicin release profile of CβG/PLGA (10:90) nanoparticles

The in vitro release of gentamicin from the nanoparticles for all the blends (Fig. 6A) showed an initial burst release and the rate of initial burst release was higher with glucan blended polymers (P2, P3, P4 and P5) than PLGA alone. After the initial burst release, sustained drug release was observed with all the blends. The percentage of drug released at the end of 37 days was higher with glucan-blended nanoparticles than PLGA alone. The higher drug release in the case of glucan blended nanoparticles could be attributed to higher encapsulation, and enhanced degradation rate of glucan by hydrolysis which was also in line with physical weight loss, and decrease in size of the nanoparticles. There exists a strong positive correlation between weight loss of the nanoparticles and drug release (correlation coefficient >0.92).

Drug release from the nanoparticles could be mathematically represented assuming Korsmeyer-Peppas model [32].

\[ C_t = C_f K_t^n \]  

\( C_t \) and \( C_f \) percentage of drug released at time = t and final equilibrium condition respectively, \( n \) - exponent.

Fig. 6B compares the experimental drug release and model predictions as a function of time for P2 and P5 blends. The corresponding \( K_t \) and \( n \) values obtained by fitting the data was 0.308 and 0.245 for P2, and 0.395 and 0.22 for P5 respectively. As expected, increasing CβG content in the blend increased the drug release rate and a simple model fitted the drug release reasonably well. In vitro experiments on gentamicin and PLGA (at 50:50 weight ratio) microparticles showed 17–97 % encapsulation [33], whereas in our experiment, the particles showed 79–93 % in nano size (200 nm). Among the different synthetic polymers,
PLGA nanoparticles were well-known for their ability to associate and release molecules in a controlled manner [34, 35, 36]. In the current study, the P5 blend had high encapsulation (93 %). Several factors influence the release of the drug including its interaction with the polymer, polymer composition, size and geometry. Super paramagnetic iron oxide nanoparticles and dexamethasone acetate encapsulated by PLGA microparticles had complete release of the corticosteroid drug within a week, both in vitro and in vivo [37, 38]. In the current study the in vitro drug release was observed up to 37 days, because the polymer had a high molecular weight. PLGA nanoparticle (190–204 nm) with surface modification (human serum albumin) proved to be an ideal candidate for the delivery of anticancer drug [39].

3.3. Antimicrobial activity

The drug released from the nanoparticles inhibited the growth of *E. coli* (Fig. 7). The maximum inhibition was observed with P5 (~93.6 %) (within 1 h) and reached a plateau of 88 % inhibition and remains constant until day 37. These results indicate that P5 with 20 % glucan is a

| Name of the Polymer | Drug encapsulation (%) | Weight of drug (mg)/weight of nanoparticle (mg) |
|---------------------|------------------------|----------------------------------------------|
| P1                  | 79.80                  | 0.38                                         |
| P2                  | 82.5                   | 0.39                                         |
| P3                  | 85.7                   | 0.40                                         |
| P4                  | 86.5                   | 0.40                                         |
| P5                  | 93                     | 0.42                                         |

Table 2 Encapsulation efficiency of various CβG/PLGA blends for gentamicin.

Fig. 2. Thermogravimetric analysis of the gentamicin loaded CβG/PLGA nanoparticles.

Fig. 3. Transmission electron microscopic images of drug loaded nanoparticles, P1 (A & B - PLGA 100%), P2 (C & D - CβG/PLGA = 0.5:99.5 %), P3 (E & F - 5 % CβG with 95 % PLGA). Nanoparticles appeared as core-shell where the inner phase could be attributed to gentamicin loaded glucan and the outer phase to PLGA.
better drug carrier than other blends.

The bacterial inhibition at the end of 37th day was high with P5 (85.7 %) followed by P3 (84 %) which could be attributed to the sustained release of drug. P1 showed the least inhibition at the end of 37th day.

3.4. Biocompatibility of PLGA and different ratios of CβG

Among the five different nanoparticles tested, P5 containing 20 % glucan was highly biocompatible against L6 myoblasts which could be attributed to the presence of high glucan content [12, 40]. CβG/PLGA nanoparticles were biocompatible towards L6 myoblast cells. With P1, 75 % cells were viable when treated with a concentration of 500 μg/ml whereas the viability was above 83 % with glucan blended nanoparticles (Fig. S11 Supporting information).
3.5. In vivo drug release profile of CgβG/PLGA nanoparticles

Group I rats were injected with pure gentamicin at a concentration of 2 mg/ml, whereas group II – IV were injected with 10 mg/ml of drug loaded nanoparticles (containing 3.8, 3.9 and 4 mg of pure drug in P1, P2 and P3 nanoparticles respectively). The in vivo results of three batches were included in supporting information Fig. S12.

Area under the curve (AUC) depends on the amount of drug administered and its rate of elimination [40]. There was no significant difference between the AUC of pure drug, and drug released from CgβG-PLGA blends (Table 3), which indicated that the bioavailability of gentamicin in all cases were similar. Clearance which is a measure of amount of drug cleared from plasma in unit time [41], was high with (0.020 ml h⁻¹ kg⁻¹) CgβG-PLGA nanoparticles than the pure drug alone (0.005 ml h⁻¹ kg⁻¹). Faster clearance rate was also reported for many anticancer drug delivery systems [26]. The reason for high clearance could be attributed to three different mechanisms including disintegration of the particle by binding to the proteins, clearance by the immune cells, and removal due to filtration by the cells [42]. Clearance also depends on the physicochemical properties of the drug. Faster clearance is very much essential for drugs such as gentamicin which is toxic to the nephrons [43] as it helps to reduce the side effects. Gentamicin was detectable in the plasma up to 6 h in the case of pure drug (Fig. 8), whereas it was detectable up to 8 h with PLGA (P1) alone and glucan blended PLGA (P2 & P3).

Volume of distribution (Vd), an apparent volume of blood plasma in the body in which the drug could be completely dissolved, was higher for blended nanoparticles than the pure drug. A value of Vd < 0.07 L/kg indicated that the drug was confined to the circulatory system and it was homogeneously distributed within the blood [44]. Prolonged drug release with similar AUC, high Vd, and clearance with the CgβG-PLGA blends suggests that the blended nanoparticles could be ideal candidates for in vivo delivery of toxic drugs to achieve similar therapeutic effect of pure drug with better efficacy and safety. Similar results were also observed with paclitaxel, loaded PLGA 50:50 nanoparticles for long term treatment of breast cancer [26].

From Fig. 8 it was clear that the in vitro and in vivo gentamicin release were different. This could be due to the differences in the solubility, and degradation pattern of the polymers in PBS and blood plasma. So, the gentamicin concentration (C(t)) in the blood plasma was mathematically modeled with two exponential terms, one for the drug released from the particles into the blood stream (ka) and the other for its elimination (ke) from the body. Both the processes were assumed to be first order, resulting in the following relationship

\[
C(t) = A(e^{-kt} - e^{-kt_0})
\]  

Fig. 9 (A and B) compares the model predictions and the actual values for two different cases (P1 and P2). As seen, the model predicted the slow rise in the concentration and exponential fall, well. The values of A, ka and ke for P1 were 1300 μg/ml, 0.75 hr⁻¹, and 0.742 hr⁻¹ respectively and for P2 it was 1350 μg/ml, 0.75 hr⁻¹, and 0.742 hr⁻¹ respectively. The dotted line in Fig. 9A shows the prediction based on the burst release model (Korsmeyer-Peppas model) and could be seen this model predicts fast drug release from the nanoparticles unlike what is observed here.

| Table 3 | In vivo pharmacokinetic parameters of pure gentamicin and gentamicin released from CgβG/PLGA nanoparticles. |
|---|---|---|---|---|
| Pharmacokinetic parameters | Pure drug | PLGA (P1) | CgβG: PLGA (P2) | CgβG: PLGA (P3) |
| AUC<sub>0-∞</sub> (μg h/ml) | 203 | 196 | 201 | 200 |
| Clearance (ml h⁻¹ kg⁻¹) | 0.005 | 0.020 | 0.020 | 0.020 |
| Volume of distribution (ml/kg) | 0.1 | 0.6 | 0.6 | 0.6 |
| C<sub>max</sub> (μg/ml) | 8.4 | 5.3 | 6.2 | 7.2 |
| T<sub>max</sub> (hr) | 1.0 | 1.5 | 1.0 | 2.0 |

4. Conclusions

The current study describes the advantages of blending the biopolymer CgβG with synthetic PLGA. A mathematical model was developed to predict the degradation of the polymer as a function of time and the drug release in vitro. Initial burst release followed by sustained release was observed in all the blends in vitro, and the drug released from the nanoparticles was active against E. coli. A double exponential model was developed to successfully predict the gentamicin concentration as a function of time in the blood. Prolonged drug release (8 h) was observed
with blend in vivo. To conclude, glucan blended polymer could be used as a carrier for delivery of toxic drugs to achieve therapeutic index similar to the pure drug with reduced toxicity.

Declarations

Author contribution statement

Geetha Venkatachalam: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Nandakumar Venkatesan: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ganesan Suresh: Contributed reagents, materials, analysis tools or data.

Mukesh Doble: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

[1] M.W. Breedveld, K.J. Miller. Cyclic beta-glucans of members of the family Sphingobacterium, Microbiol. Rev. 56 (2) (1994) 145–161.

[2] P. Piao, A. Jung, Y. Choi, M.N. Tahir, Y. Kim, S. Park, E. Cho, S. Jung. Solubility enhancement of a-naphthothiovan by synthesized hydroxypropyl cyclo(1–2)-p-glucan (cyclodextrinos). Carbohydr. Polym. 101 (2014) 733–740.

[3] A.G. Mantylogue, Epsilonpolysaccharides of Agrobacterium tumefaciens, Agrobact. Biol.: From Basic Science to Biotechnology 418 (2018) 111–141.

[4] G. Venkatachalam, S. Gumiada, M. Doble, Cyclic p-glucans from Microorganisms, Production, Properties and Applications, Springer Science & Business Media, 2012.

[5] G. Venkatachalam, D. Srinivasan, M. Doble, Cyclic p-(1,2)-glucan production by Rhizobium meliloti MTCC 3402, Process Biochem. 48 (12) (2013) 1848–1854.

[6] S.-H. Lee, E. Cho, S.-H. Jung, Periplasmic glucans isolated from Proteobacteria, BMB Rep. 42 (12) (2009) 769–775.

[7] Y. Hu, H. Kim, V.I. Shinde, D. Jeong, Y. Choi, E. Cho, S. Jung. Carboxymethyl cyclohexanoses as a flexible ph-responsive solubilizer for pindolol, Carbohydr. Polym. 175 (2017) 493–501.

[8] K. Koizumi, Y. Okada, S. Horiyama, T. Utamura, T. Higashiura, M. Ikeda, K. Koizumi, Y. Okada, S. Horiyama, T. Utamura, T. Higashiura, M. Ikeda, Carbohydr. Res. 289 (1996) 25–37.

[9] M. Kimura, K. Nemoto, K. Inoue, T. Iwata, Y. Tanaka, S. Inoue, T. Iwata, Y. Tanaka, S. Inoue, J. Biomed. Macromol. 17 (3-4) (1995) 189–198.
[41] L.Z. Benet, D. Kroetz, L. Sheiner, J. Hardman, L. Limbird, Pharmacokinetics: the Dynamics of Drug Absorption, Distribution, Metabolism, and Elimination, Goodman and Gilman’s the Pharmacological Basis of Therapeutics, 1996, pp. 3-37.

[42] S.Y. Lee, J.X. Cheng, Clearance of nanoparticles during circulation, Pharm. Sci. Encycl.: Drug Disc. Develop. Manufact. (2010) 1–32.

[43] J. Morin, G. Viotte, A. Vandewalle, F. Van Hoof, P. Tulkens, J. Fillastre, Gentamicin-induced nephrotoxicity: a cell biology approach, Kidney Int. 18 (5) (1980) 583–590.

[44] G. Evans, A Handbook of Bioanalysis and Drug Metabolism, CRC press, 2004.