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Dendritic nanocomposite for delivery of antibacterial agent

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ABSTRACT

Objective: To develop and explore the use of PEGylated poly (propylene imine) dendritic architecture for the delivery of an anti bacterial bioactive, Trimethoprim. **Methods:** For this study, PEGylated poly(propylene imine) dendritic architecture was synthesized and loaded with Trimethoprim and targeted to the resistant producing strains of both gram positive and gram negative. The antibacterial activity was carried out by agar well-diffusion method to compare zone of inhibition with standard drug and plain PPI dendrimer. **Results:** The study showed that the Trimethoprim loaded dendrimer has significant antibacterial activity than the plain PPI dendrimer, but standard drug was not shown zone of inhibition upon both microorganisms but *Klebsiella pneumoniae* (*K. pneumoniae*) the pure drug showed activity. **Conclusions:** In this study antibacterial activity of synthesized system is also relatively safer and holds potential to deliver any other antibacterial agent to the resistant producing strains.

1. Introduction

Dendrimer represents a novel type of polymeric material. It is also known as starburst[1] or cascade[2] or molecular trees[3] or arborols, or polymers. They attract the increasing attention of all because of their unique structure, high degree of control over molecular weight and the shape that has led to the synthesis of unimolecular micelles[4]. Considering the use of dendrimers for drug delivery, it is necessary that they are nontoxic and biocompatible. However, it has been demonstrated that widely used dendrimers, such as PAMAM and poly (propylene imine) (PPI) dendrimers bearing primary amino group termini, are quite cytotoxic, and also these dendrimers were cleared rapidly from the circulation when administered intravenously[5].

Poly ethylene glycol (PEG) is typically a clear, colorless odorless substance that is soluble in water,

stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm, as reviewed earlier[6].

It has been shown that covalent attachment of poly (ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time[7]. Moreover, a number of studies have demonstrated that poly (ethylene glycol) chains grafted to surface of polymer micelles and liposomes suppress their interaction with plasma proteins and cells and prolong their blood elimination half-life[8].

On the basis of these findings, it seems that dendrimers covered with poly (ethylene glycol) grafts are attractive compounds as drug carriers in *in vivo*. Such molecules are expected to encapsulate drugs in their dendrimer moiety and reveal biocompatibility due to their hydrophilic shell consisting of poly (ethylene glycol) grafts. The present study was aimed at developing and exploring the use of PEGylated newer EDA-PPI dendrimers for delivery of anti-bacterial drug, Trimethoprim. Trimethoprim was selected for

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incorporation into PEGylated ethylenediamine (EDA)–PPI dendrimers based on its antibacterial activity. PEGylation of EDA–PPI dendrimers establishes suitability of PEGylated dendrimer as a drug delivery system for Trimethoprim.

2. Materials and methods

2.1. Materials

PEG4000, Reney Nickel (Sigma, Germany), Raney Nickel (Merck, India), triethylamine, dioxan, ethylenediamine, acrylonitrile (CDH, India), N, N dicyclohexyl carbodiimide (DCC), Cellulose dialysis bag (MWCO 12–14 Kda, Himedia, India), 4 dimethyl amino pyridine (sd–fine chemicals, India), Agar plates (Himedia, Mumbai), Trimethoprim was a benevolent gift from Shasun Pharmaceuticals, Chennai, India.

2.2. Methods

2.2.1. Synthesis of 5.0 G EDA–PPI dendrimers

EDA–PPI dendrimers were synthesized by the previously reported and established procedure. The half generation EDA–dendrimer–(CN) $4n$ (where n is generation of reaction or reaction cycle) was synthesized by double Michael addition reaction between acrylonitrile (2.5 molar times per terminal NH_2 group of core amine moiety) and aqueous solution of ethylenediamine or previous full generation dendrimers. After the initial exothermic phase, the reaction mixture was heated at 80 °C for 1 h to complete the addition reaction. The excess of acrylonitrile was then removed by vacuum distillation (16 mbar, bath temperature 40 °C).

The full generation EDA–dendrimer–(NH $_2$) $4n$ was obtained by hydrogenation in methanol at 40 atm hydrogen pressures and 70 °C for 1 h with Raney Nickel (pretreated with hydroxide and water) as catalyst. The reaction mixture was cooled, filtered and the solvent was evaporated under reduced pressure. The product was then dried under vacuum. EDA–PPI dendrimers up to 5.0 G were prepared by repetition of all the above steps consecutively, with increasing quantity of acrylonitrile.

2.2.2. Synthesis of PEGylated 5.0G EDA–PPI dendrimers

To a solution of 5 G EDA–PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 mL), PEG 2000 (0.32 mmol) in DMSO (10 mL) and N, N dicyclohexyl carbodiimide (DCC) (0.32 mmol) in DMSO (10 mL) were added and the solution was stirred for 5 days at room temperature.

The product was precipitated by addition of water, filtered and dialyzed (MWCO 12–14 Kda, Himedia, India) against double distilled water for 24 h to remove free PEG 2000, DCC and partially PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany).

2.2.3. Drug loading in formulation

The known molar concentrations of EDA–PPI dendrimer and PEGylated 5.0 G dendrimers were dissolved separately in methanol and mixed with methanolic solution of trimethoprim (100 mol).

The mixed solutions were incubated with slow magnetic stirring (50 rpm) using teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag (MWCO 1000 Da Sigma, Germany) against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically (λ_{max} 229nm) (UV–1601, Shimadzu, Japan) to determine indirectly the amount of drug loaded within the system. The dialyzed formulations were lyophilized and used for further characterization.

2.2.4. Antibacterial assay

About six *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* human pathogenic bacterial strains were used. All six strains were tested against Trimethoprim loaded dendrimer. The antimicrobial activity of Trimethoprim loaded dendrimer was determined by agar well diffusion method against different bacteria as described. In this method, pure isolate of each bacterium was sub-cultured in nutrient broth at 37 °C for 24 h.

One hundred microlitres (about 10^6 CFU/mL, standardized by 0.5 Mac–Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller–Hinton Agar plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. Subsequently, a 50 μ L volume of the dendrimer, pure Trimethoprim and drug loaded dendrimer was introduced in triplicate wells into Muller–Hinton agar plate.

The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37 °C for 24 h. The zone of inhibition was recorded to the nearest size in mm⁹.

2.2.5. Statistical analysis

Results were analysed by mean \pm SD and it was calculated by ANOVA followed by Dunnet's test.

Table 1

Zone of inhibition of trimethoprim loaded PPI dendrimer.

Treatment	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
DMSO	NA	NA	NA	NA	NA	NA
Plain PPI dendrimer	26.00±2.65*	25.60±1.52*	25.30±2.08*	26.30±1.52*	26.33±3.21*	24.30±3.05*
Trimethoprim	NA	NA	NA	NA	NA	19.60±0.57*
DLDT(50 µg/mL)	13.30±2.08	11.30±1.52*	11.00±1.73	13.30±1.52*	12.30±1.52*	13.60±2.34
DLDT(100 µg/mL)	16.60±1.52*	20.30±0.57*	15.30±2.08*	16.60±0.57*	15.60±4.93	18.00±3.60

*: $P < 0.0001$. NA – No activity; PPI – Polypropyleneimine dendrimer; DLDT – Drug loaded dendrimer; DMSO– Dimethyl sulphoxide ($n=3$). $P < 0.0001$ were considered as significant

3. Result

The polypropyleneimine dendrimer was synthesized by using ethylenediamine as a core. The synthesized dendrimer were further PEGylated with PEG2000. The PEGylated dendrimer is used as carrier system in which Trimethoprim was loaded and drug entrapment efficiency was calculated as 60.20 ± 0.03 . The antibacterial activity of drug loaded dendrimer was performed by agar well diffusion method. The results are compared with plain dendrimer and pure Trimethoprim and thus, it reveals that the PEGylated drug loaded dendrimer shows potent activity than that of plain dendrimer on both the selected organisms (Table 1). Further, it was observed that the Trimethoprim does not show inhibitory zone against *Klebsiella pneumoniae*. Hence it clarifies that the selected organisms are resistant to Trimethoprim. Thus, the synthesized PEGylated polypropyleneimine dendrimer is an effective carrier to target any antibacterial agent for resistant producing organism.

4. Discussion

From the present study it can be concluded that the prepared Trimethoprim loaded PPI dendrimer were highly effective against resistant producing organisms. However the synthesized system was found to be suitable and safe to deliver any drug to the targeting sites.

Conflict of interest statement

We declare that we have no conflict of interest

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