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Dendritic architechture for the delivery of antibacterial agent against resistant producing strains

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ABSTRACT

The present study was aimed at developing and exploring the use of PEGylated poly (propylene imine) dendritic architecture for the delivery of an anti-bacterial drug, Ciprofloxacin. For this study, PEGylated poly(propylene imine) dendritic architecture was synthesized and loaded with Ciprofloxacin and targeted to the resistants produce strains of Staphylococcus aureus and Cryptococcus pneumonia. The antibacterial activity was carried out by cup and bore method to compare zone of inhibition with standard drug and plain PPI dendrimer.The study showed that the Ciprofloxacin loaded dendrimer has significant antibacterial activity than the plain PPI dendrimer, but standard drug was not shown zone of inhibition upon both microorganisms. In this study antibacterial activity of synthesized system are also relatively safer and hold potential to deliver some other drugs also.

Keywords: Dendrimers, PEGylation, Ciprofloxacin, Resistant producing strains.

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 [4-7]. 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 [8-12]. 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 [13,14]. It has been shown that covalent attachment of poly(ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time [15,16]. 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 [17-22]. 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, Ciprofloxacin. Ciprofloxacin was selected for 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 Ciprofloxacin.

MATERIALS AND METHODS

Materials

PEG2000, 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 (Hi media, Mumbai), Ciprofloxacin was a benevolent gift from Shasun Pharmaceuticals, Chennai, India.

Synthesis of 5.0G EDA-PPI Dendrimers

EDA-PPI dendrimers were synthesized by the previously reported and established procedure [8,35]. The half generation EDA-dendrimer-(CN)4*n* (where *n* is generation of reaction or reaction cycle) was synthesized by double Michael addiction reaction between acrylonitrile (2.5 molar times per terminal NH2 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 80ºC for 1 h to complete the addiction reaction. The excess of acrylonitrile was then removed by vaccum distillation (16 mbar, bath temperature 40ºC). The full generation EDA-dendrimer-(NH2)4*n* was obtained by hydrogenation in methanol at 40 atm hydrogen pressures and 70ºC for 1 h with Reney 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 5.0G were prepared by repetition of all the above steps consecutively, with increasing quantity of acrylonitrile

Synthesis of PEGylated 5.0G EDA-PPI Dendrimers

To a solution of 5G 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).

Drug Loading in Formulation

The known molar concentrations of EDA-PPI dendrimer and PEGylated 5.0G dendrimers were dissolved separately in methanol and mixed with methanolic solution of Ciprofloxacin (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} 475 nm) (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.

Antibacterial assay

About two human pathogenic bacterial strains were used. *Staphylococcus aureus* and *Cryptococcus pneumonia* were tested against the Ciprofloxacin laded dendrimer. The antimicrobial activity of Ciprofloxacin laded 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 37ºC for 24h. One hundred microlitres (about 106CFU/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 6.0mm was used to bore wells in the agar plates. Subsequently, a 50µL volume of the dendrimer, pure Ciprofloxacin 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 37ºC for 24h. The zone of inhibition was recorded to the nearest size in mm [23].

Statistical analysis

Results were analysed by mean ± SEM and it was calculated by ANOVA test. $P<0.0002$ were considered as significant

RESULTS AND DISCUSSION

The polypropyleneimine dendrimer was synthesized by using ethylenediamine as a core. The synthesized dendrimer were further PEGylated with PEG_{2000}. The PEGylated dendrimer is used as carrier system in which ciprofloxacin was loaded and drug entrapment efficiency was calculated as 60.2±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 Ciprofloxacin and thus, it reveals that the PEGylated drug loaded dendrimer shows potent activity than that of plain dendrimer on both the selected organisms. (Table no 1.). Further, it was observed that the Ciprofloxacin does not shows inhibitory zone. Hence it clarifies that the selected organisms are resistant to Ciprofloxacin. Thus, the synthesized PEGylated polypropyleneimine dendrimer is an effective carrier to target any antibacterial agent for resistant producing organism.

Table 1. Antibacterial activity of Ciprofloxacin loaded PEGylated PPI dendrimer

Microorganism	Zone of inhibition (mm)			
	PPI dendrimer (10mcg/ml)	DLP dendrimer (10mcg/ml)	Ciprofloxacin (10mcg/ml)	DMSO (10mcg/ml)
S. *aureus*	0.83±0.06	1.83±.0.03	NA	NA
C. *pneumonia*	0.83±0.06	0.53±0.08	NA	NA

NA - No activity; PPI - Polypropyleneimine dendrimer; DLP - Drug loaded dendrimer; DMSO- Dimethyl sulphoxide.

CONCLUSION

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

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