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Generation dependent hemolytic profile of folate engineered poly(propyleneimine) dendrimer

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ABSTRACT

The success of any drug delivery carrier strictly depends on its toxicological profile. Apart from huge drug delivery potential of dendrimers, toxicity associated with their positively charged surfaces, constrains their biomedical applications. Engineering of these surfaces with targeting ligands not only diminishes their toxicity but also enhances the targeting potential. Conjugation of folate on the surface of poly(propyleneimine) (PPI) dendrimers is widely explored concept. The present paper investigated generation-dependent hemolytic toxicity of folate anchored PPI dendrimer (FA-PPI3, FA-PPI4 and FA-PPI5) at different concentrations (0.1–0.5% *w/v*). Further the effect of folate engineered PPI dendrimers on the surface morphology of human erythrocytes and hematological parameters was also observed. In the final outcome the extent of hemolysis was found to be concentration as well as generation dependent.

1. Introduction

Nanometric size, well-defined structure and large number of terminal groups of dendrimers confer on them tremendous advantages in intracellular and targeted delivery of drugs [1–3]. Among all classes of dendrimers, poly(propyleneimine) (PPI) dendrimers symbolize one of the most discovered classes of dendrimer, are amine-terminated hyper-branched macromolecules [4–9]. Due to the presence of large number of surface amine groups, they exhibit toxicity, which restricts their clinical applications [10–12]. For their clinical acceptance modern research has paying attention on the synthesis of dendrimers with improved biocompatibility, which will provide safeguard to dendrimer-mediated drug delivery aspects. Surface modification is one of the options to lessen the toxicity of dendrimers [13–15]. Among the different techniques of surface engineering, the folate conjugation is widely explored concept [14–18].

Red blood cell (RBCs) lysis is the simplest method to scrutinize the polymer-blood cell membrane interaction. It depends on the measurement of hemoglobin release after administration of dendritic formulations [15,19,20]. In the present study, we have synthesized folate modified PPI dendrimer of various generations (3.0G-5.0G), and compared their hemolytic toxicity using human erythrocytes (Fig. 1).

2. Materials and methods

2.1. Materials

Ethylenediamine (EDA) and Raney Nickel were purchased from Merck, India. Acrylonitrile (ACN) was purchased from Central Drug House, Mumbai (India). Methanol was purchased from Rankem, Chemical Division of Ranbaxy Labs, Mohali (India). Dimethylsulfoxide (DMSO), dichloromethane (DCM), folic acid (FA), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) hydrochloride were purchased from HiMedia Mumbai (India). All the other chemicals were purchased from HiMedia, Mumbai (India). All the chemicals used were of analytical grade.

2.2. Synthesis and characterization of PPI dendrimers

PPI dendrimers up to fifth generation were synthesized by divergent method as reported previously by our group using EDA as an initiator core and acrylonitrile as a branching moiety [5,21]. The synthesis of each generation of PPI was confirmed by FT-IR and ^{1}H NMR spectroscopic methods.

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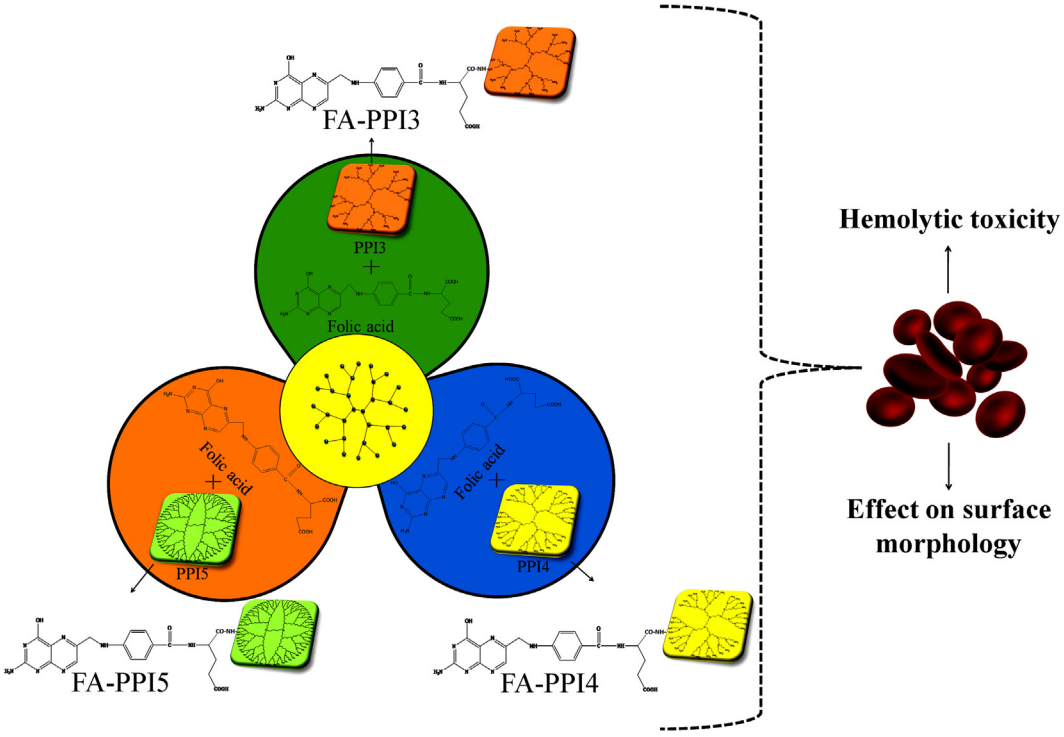

Fig. 1. Schematic representation of development of folate conjugated formulation of different generations of PPI dendrimer.

2.3. Synthesis and characterization of folate-PPI conjugates

Conjugation of folate to different generations (3.0G-5.0G) of PPI dendrimers was carried out as per the method previously reported by our group [16,18]. The schematic presentation of PPI dendrimer synthesis as well as surface engineering by folic acid is provided in Scheme 1 and Scheme 2, respectively. Confirmation of Folate-PPI (FA-PPI) was carried through FT-IR and ^{1}H NMR spectroscopic methods.

2.4. Effect on surface morphology of erythrocytes

The whole human blood from healthy donor was collected in HiAnticlot blood collection vials (Himedia, Mumbai, India) containing K_3EDTA as anticoagulant, and centrifuged (REMI, Mumbai, India) at 3000 rpm for 5 min to separate the RBCs from the blood. RBCs were then re-suspended in phosphate buffer saline (PBS) (pH 7.4) to obtain RBC suspension (5%). This was followed by treatment with developed formulations at varying concentrations (0.1–0.5 *w/v*). The effect of dendrimer formulations on morphology of RBCs was observed by optical microscopy (Leica, DMLB, Switzerland) at 400× magnification [5,18,20].

2.5. Hemolytic toxicity studies

Hemolytic toxicity was carried as per the protocol previously reported by our laboratory [5,20,21]. Hemoglobin content in the supernatant of the centrifuged RBC suspension was used to investigate the extent of hemolysis.

In short, the RBCs suspension (5%; 0.1 mL) and FA-PPI3 dendrimer formulation (0.1 *w/v* in PBS pH 7.4; 0.9 mL) were incubated at 37 ± 0.5 °C for 30 min and the mixture was centrifuged at 3000 rpm (REMI, Mumbai, India) for 10 min. The supernatant was analyzed spectrophotometrically (1601 UV–vis spectrophotometer, Shimadzu, Kyoto, Japan) at λ_{max} 540 nm (n = 3). For the determination of 0 and 100% hemolysis, RBCs suspension (0.1 mL) was added to NaCl solution (0.9%, *w/v*; 0.9 mL), and distilled water (0.9 mL), respectively. The degree of hemolysis was determined by following equation:

$$\text{Hemolysis}(\%) = [\text{Abs}_s - \text{Abs}_0]/[\text{Abs}_{100} - \text{Abs}_0] \times 100$$

where Abs_s, Abs_0 and Abs_{100} are the absorbance of the sample, solution of 0% and 100% hemolysis, respectively. Similar procedure was adapted to find out the hemolytic toxicity of all the selected concentration (0.1–0.5 *w/v*) as well as other selected dendrimer formulations (FA-PPI4 and FA-PPI5) at different time intervals.

2.6. Hematological studies

For the hematological studies the experimental protocols were duly approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of Dr. H.S. Gour Central University, Sagar (M.P.), India (Reg. No. 379/01/ab/CPCSEA). As per the approved protocol BALB/C mice were divided into four groups consisting of three mice each (fourth group was reserved as control). Various formulations (1 mg/kg bodyweight) were injected intravenously into mice every day up to 3 days. Blood samples were collected and analyzed at local pathology laboratory for RBC count, white blood cell (WBC) count and differential count of monocytes, lymphocytes and neutrophils [17,18,22].

2.7. Statistical analysis

All the statistical analysis was performed with Graph Pad Instat Software (Version 3.0, Graph Pad Software, CA, USA) using either unpaired t test or one-way ANOVA followed by Tukey–Kramer multiple comparison test. $p < 0.05$ was considered as significant difference.