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Generation dependent cancer targeting potential of poly(propyleneimine) dendrimer

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ABSTRACT

Dendrimer-mediated delivery of bioactive is a successful and widely explored concept. This paper describes comparative data pertaining to generation dependent cancer targeting propensity of Poly(-propyleneimine) (PPI) dendrimers. This debut report reportsthe drug targeting and antciancer potential of different dendrimer generations. PPI dendrimers of different generations (3.0G, 4.0G and 5.0G) were synthesized and loaded with Melphalan. Results from loading, hemolysis, hematologic, cytotoxicty and flow cytometry assay depicted that as the generation of dendrimer increased from fourth to fifth, the only parameter i.e. toxicty is increased exponentionally. However, others parameters, i.e. loading, sustained release behavior, and targeting efficacy increased negligibly. Kaplan–Meier survival curves clearly depicted comparable therapeutic potential of PPI4M with PPI5M. *In vivo* investigations in Balb/c mice again favored 4.0G PPI dendrimer to be preferable nanocarrier for anticancer drug delivery owing to analogous anticancer potential. The outcomes of the investigation evidently projects 4.0G PPI dendrimer over 3.0G and 5.0G dendrimer in respect of its drug delivery benefit as well as superior biocompatibility. Thus, much against the common belief, 4.0G PPI dendrimers may be considered to be optimum in respect of drug delivery precluding the use of much more toxic 5.0G PPI dendrimer, which offers no benefit over 4.0G.

1. Introduction

Drug delivery systems can be defined as means to introduce therapeutic agents into the body. The rationale of drug delivery systems is to improve the pharmacological activity of drugs by enhancing pharmacokinetics and also by altering pharmacodynamic properties. The delivery systems cover a wide range of device varying in many parameters i.e. size, nature, source etc. A simple way to classify them is based on their size ranging from conventional drug delivery systems having macro-particulate range such as tablets, capsules ointments to microscopic range systems such as micro-particles, micro-emulsion, and multiple emulsions. The present era of nanotechnology provides a number of magical nanocarriers such as liposomes, nanoparticles, dendrimers and carbon nanotubes [1–3]. At present, it is easily possible to design validated nanocarriers with definite size, shape, surface charge etc, with possible applications in the field of drug delivery.

Among the available polymeric nanocarriers dendrimer is one of the most widely explored polymeric nanocarriers [4,5]. Dendrimers are three dimensional, synthetic, highly branched polymeric monodisperse systems with well-defined biocompatibility and spherical structures having diameter ranging from 1 to 10 nm [6–8]. Exclusive characteristics of dendrimers are their nano-scale spherical architecture, mono-dispersity and modifiable surface functionality along with highly defined structure. Presence of large hydrophobic cavity can be used for the entrapment of bioactives providing opportunities for controlled and sustained drug release [9–12].

Architecture of dendrimers comprises of three distinct domains namely central core, branches and many terminal functional groups. Core consists of a single atom or an atomic group having at least two identical chemical functions whereas branches, deriving from the core, comprise of repeat units having at least one branch junction, whose repetition is organized in a geometrical progression that results in a series of radially concentric layers called “generations” (Fig. 1A) [10,13]. This repetition leads to the construction of subsequent higher generations. With each subsequent generation, the number of end groups

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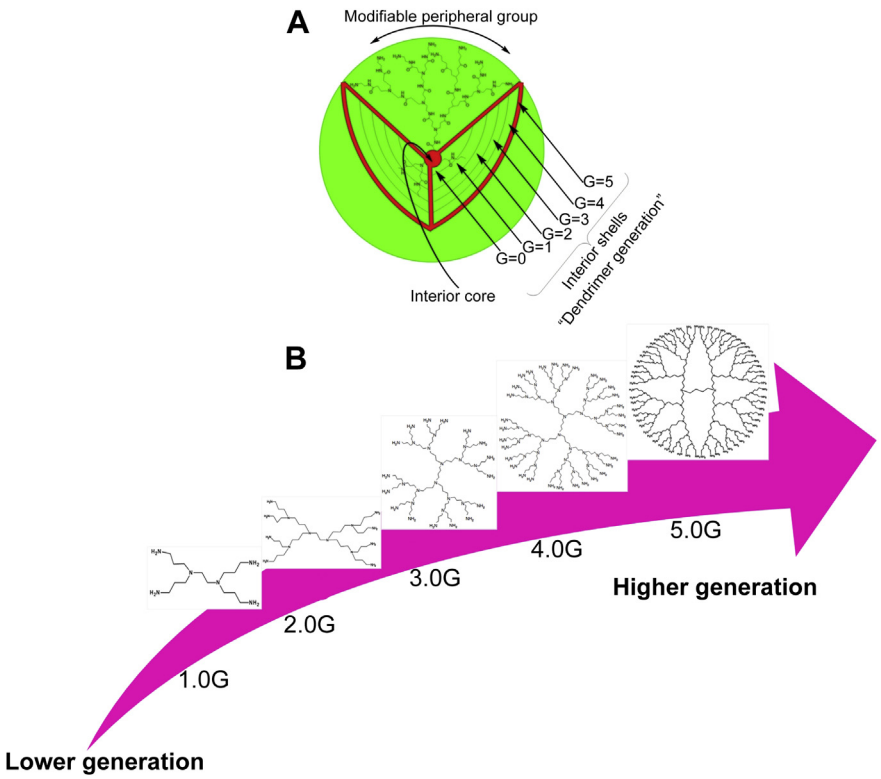

Fig. 1. Structure of dendrimer; (A) Basic structure explaining different constructed units, (B) Different generation of PPI dendrimers.

increases exponentially. Dendritic macromolecules tend to linearly expand in diameter and assume a more globular shape with increasing dendrimer generation. Addition of successive layers gradually increases molecular size and amplifies the number of surface groups present [14]. Fig. 1B represents the different generations of PPI dendrimers.

In previous research, Jain and Coworkers reported the effect of different generations of PPI dendrimers on human erythrocytes and demonstrated that the toxicological behavior of PPI dendrimer increased with the increment of the dendrimer generation [15]. The present paper further investigates the effect of dendrimer generation on other characteristic parameters of delivery i.e. drug loading, release behavior, hemolysis profile at different concentration, cell line cytotoxicity behavior and *in vivo* performance. It is envisaged that this work may help the drug delivery researcher in selecting the optimized dendrimer generation in terms of their concerning toxicity as well as drug delivery aptitudes.

2. Materials & methods

2.1. Materials

Acrylonitrile (ACN) and ethylenediamine (EDA) were purchased from CDH (India). Raney Nickel was purchased from Fluka (USA). MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] was purchased from Sigma–Aldrich (USA). Analytical grade reagents were purchased from Merck India Ltd. (Mumbai, India).

2.2. Synthesis of PPI dendrimers of different generations

EDA cored PPI dendrimers of selected generations (3.0G, 4.0G and 5.0G) were synthesized by divergent approach as reported by our groups previously [2,11,12,15,16]. The detailed synthesis is shown in Fig. 2. FT-IR spectroscopy was carried out in a Perkin Elmer FT-IR spectroscope (USA) at each generation of PPI dendrimers after adsorption of smaller amount of substance on KBr pellet. ^{1}H-NMR spectroscopy of the dendrimers was carried out at 300 MHz, after dissolving in D_2O (Bruker DRX, USA).

Further, average particle size and polydispersity index of PPI dendrimers of various generations were determined after dispersing in deionized water in a Zetasizer (DTS Ver. 4.10, Malvern Instruments, England). The particle size distributions are represented by the average size (diameter) and the variance (polydispersity) of the Gaussian distribution function in logarithmic axis mode.

2.3. Drug loading

Melphalan was loaded in PPI dendrimers (3.0G, 4.0G and 5.0G) by equilibrium dialysis method as reported previously [2,16]. Briefly, dissolved the known molar concentration of Melphalan (100 mM) in methanol and mixed with 3.0G PPI dendrimer (10 mM) and the resultant solution was incubated for 24 h under slow magnetic stirring (50 rpm) using Teflon beads at room temperature (25 ± 2 °C). Finally, the resultant mixture was concentrated by evaporation under reduced pressure and dialyzed three times using cellulose dialysis bag (MWCO 3.5 KDa Sigma, USA) against distilled water (20 ml) under sink conditions (sink condition ensures the complete dissolution of the drug it refers to the excess solubilizing capacity of the dissolution medium) for 30 min to remove unloaded bioactive (free drug). Amount of loaded drug was then estimated by HPLC method [17] to determine indirectly the amount of drug loaded within the formulations. Similar protocol was followed for drug loading inside 4.0G PPI, and 5.0G PPI. All the formulations were lyophilized using 2% lactose as cryoprotectant and used for further characterization. Different drug-loaded formulations so formed were labeled as Melphalan loaded 3.0G PPI dendrimers (PPI3M), Melphalan loaded 4.0G PPI dendrimers (PPI4M) and Melphalan loaded 5.0G PPI dendrimers (PPI5M).

2.4. In vitro drug release study

To monitor the release of Melphalan from different generation dendritic formulations (PPI3M, PPI4M and PPI5M) and plain drug, equilibrium dialysis was performed using cellulose membrane (3.5 KDa, Sigma, USA). Ten milligrams of drug loaded dendrimer formulations (PPI3M, PPI4M, and PPI5M) and plain drug was placed inside dialysis bag separately and hermetically sealed from both sides [2,12]. This bag was placed in 100 ml release medium [PBS (pH 7.4) containing 0.1% *v/v* Propylene glycol] maintained on continual stirring (400 rpm) at 37 ± 2 °C. At predetermined time points from 0.5 to 48 h, half milliliter of the release medium was withdrawn and replaced with an equal volume of fresh medium to maintained sink condition. The sample was estimated by HPLC for released Melphalan content [17]. The investigation was performed thrice and the results are presented as mean ± SD.

2.5. Hemolytic toxicity

The percent hemolytic toxicity was determined by hemoglobin content in the supernatant of the centrifuged RBC suspension spectrophotometrically (λ_{max} 540 nm; UV-1601, Shimadzu, Japan) following a reported methodology with slight