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Reducing cytotoxicity while improving anti-cancer drug loading capacity of polypropylenimine dendrimers by surface acetylation

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ABSTRACT

Polypropylenimine (PPI) dendrimers have been widely used as effective delivery vehicles for drugs and nucleic acids during the past decade. However, biomedical applications of PPI dendrimers were limited because of their serious cytotoxicity and low drug loading capacity. In the present study, acetylated PPI dendrimers with different degrees of acetylation ranging from 14.2% to 94.3% were synthesized and used to encapsulate drugs, including methotrexate sodium, sodium deoxycholate and doxorubicin. Acetylated PPI dendrimers with a degree of acetylation >80% showed a significantly decreased cytotoxicity (>90% cell viability) on MCF-7 and A549 cells. The drug loading capacity of acetylated PPI dendrimers increased proportionally with the degree of acetylation on the dendrimer surface. In addition, 94.3% acetylated PPI dendrimers exhibited a pH-responsive release profile of anticancer drugs loaded within the nanoparticles. The cytotoxicities of methotrexate sodium and doxorubicin on MCF-7 and A549 cells were significantly reduced when they were complexed with acetylated PPI dendrimers with high degrees of acetylation (>80%), owing to sustained drug release from the dendrimers. The results suggest that surface acetylation can reduce the cytotoxicity and improve the anticancer drug loading capacity of cationic dendrimers, and that acetylated PPI dendrimers are promising vehicles for anticancer drugs in clinical trials.

1. Introduction

Polypropylenimine (PPI) dendrimers are the first commercially available dendrimers synthesized by a divergent strategy using diaminobutane as the central core and propylene imine as repeat units [1]. They are widely used as templates for the synthesis of dendrimer-encapsulated nanoparticles and as scaffolds for the construction of magnetic resonance imaging contrast agents, especially in drug and gene delivery [2–4]. PPI dendrimers have excellent aqueous solubility, and therefore a large number of hydrophobic cavities in their interior can effectively improve the solubility and stability of various hydrophobic drugs [5]. In addition, PPI dendrimers with a high density of active groups on their surface can be easily functionalized with therapeutic agents, targeting moieties, solubilizing ligands and imaging units for targeted cancer diagnosis and therapy [6]. However, PPI dendrimers, especially those with a cationic surface, are not ideal candidates for biomedical applications, owing to their serious toxicity [7–9]. For instance, G5 amine-terminated PPI dendrimer at a low concentration of 1 μg ml^{-1} caused 83.2% and 76.9% cell death on HepG2 and COS-7 cells, respectively [7]. Also, G5 cationic PPI dendrimer showed significant decreases in red blood cell count, hemoglobin content and mean corpuscular hemoglobin value, as well as a substantial increase in white blood cell count [7]. Exposing macrophages to G2 or G3 cationic PPI dendrimers caused dramatic changes in macrophage cell size and significant fluctuation in mitochondrial membrane potential [10]. Cationic PPI dendrimers showed rapid clearance from the blood circulation system after intravenous or intraperitoneal injection, leading to low bioavailability of the administered drugs [11]. Administration of G4 cationic PPI dendrimer caused obvious changes in the behavior of animals, such as decreased food and water consumption, and lower rate of gain in body weight [12].

Besides the non-negligible in vitro and in vivo toxicity, PPI dendrimers have extremely low drug loading capacity for a list of hydrophobic drugs [13]. PPI dendrimers have smaller molecular size and interior cavities compared with polyamidoamine (PAMAM) dendrimers [14]. Though PPI dendrimer with a more hydro-

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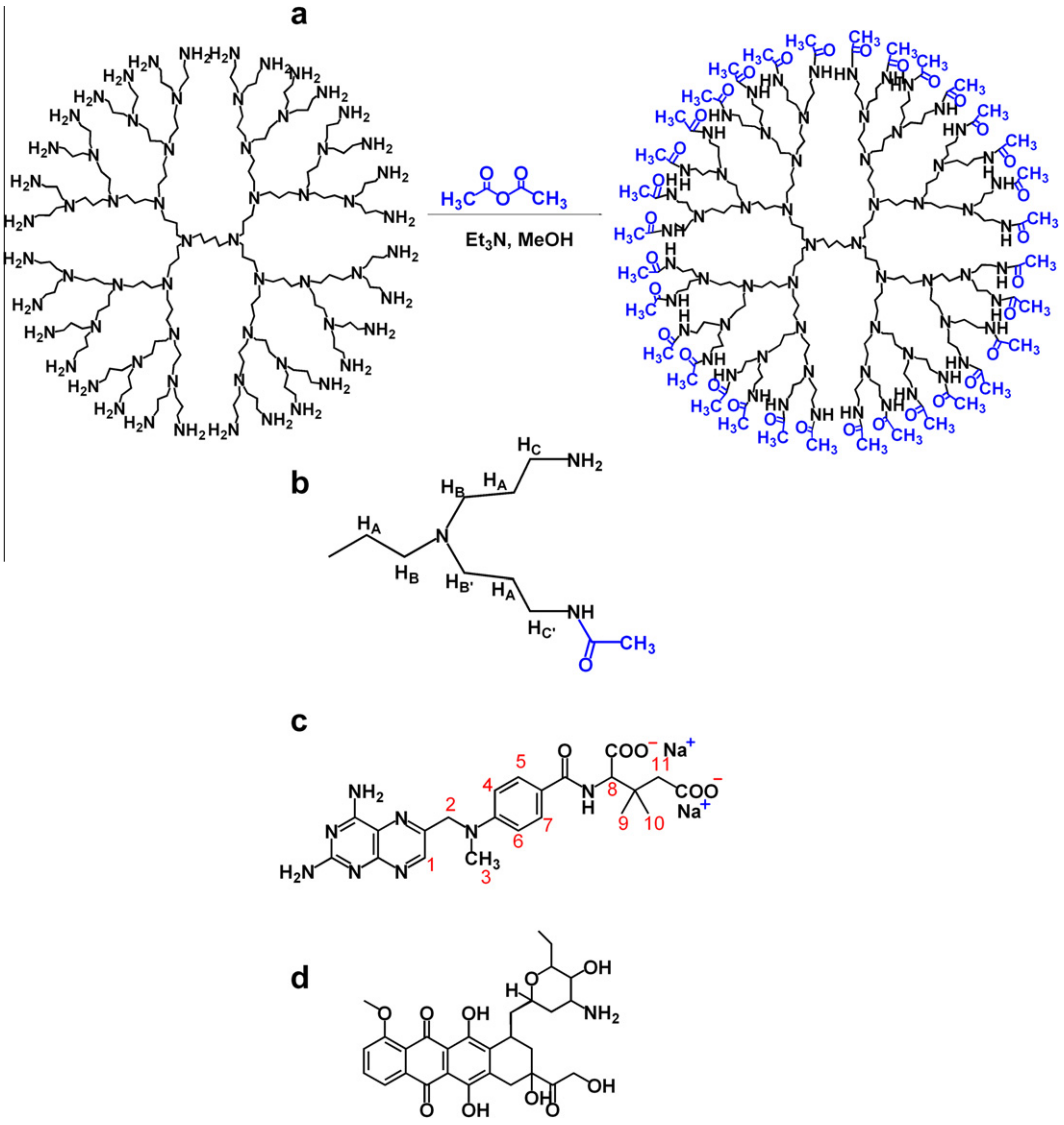

Scheme 1. (a) Synthetic route of acetylated G4 PPI dendrimer, (b) repeated unit of acetylated PPI dendrimer with proton labeling, and molecular structure of (c) methotrexate sodium and (d) doxorubicin.

phobic interior more easily encapsulate hydrophobic compounds, the yielding PPI/drug complexes are not as stable as PAMAM/drug complexes in water and may precipitate from the aqueous solution when PPI dendrimers are loaded with a large number of drug molecules [13]. These defects of PPI dendrimers prevent the development of PPI dendrimer-based drug formulations and diagnostic agents for biomedical purpose [9].

To decrease the cytotoxicity and improve the drug loading capacity of dendrimers, surface engineering of the dendrimer surface by PEGylation [15–18], acetylation [19–21], glycosylation [9] and amino acid or peptide modification [9] was proposed by several groups. Among these strategies, PEGylation and acetylation were considered to be the most efficient ones in reducing dendrimer cytotoxicity and improving their aqueous stability in physiological conditions [22]. Compared with PEGylation, acetylation is preferred for the following reasons: (1) acetylation is more facile and highly efficient, and the degree of acetylation on the dendrimer surface can be easily tailored by choosing proper stoichiometry of acetic anhydride and dendrimer [23,24]; (2) modification of PEG chains with a larger molecular size than acetyl groups on the dendrimer surface will cause significant steric hindrance and thereby affect other functional groups such as targeting moieties [25]; and (3) acetylated dendrimers can maintain the high penetration ability of cationic dendrimers across cell membranes, while PEGylated dendrimers show much reduced cellular uptake [26]. Generally, acetylation can effectively increase the aqueous solubility of dendrimer–drug conjugates, improve their biocompatibility, and optimize their in vivo pharmacokinetic behavior [9].

Though acetylated dendrimers showed several promising advantages in previous studies, the following questions are still unknown: (1) What is the least degree of acetylation on dendrimer surface that can meet the need of biomedical applications such as drug delivery, gene delivery and disease diagnosis? (2) How will the degree of acetylation influence the drug loading capacity of acetylated dendrimers? (3) Can the acetylated dendrimers with high degrees of acetylation be used directly as drug vehicles for anticancer drugs? The present study addressed these questions using methotrexate sodium, sodium deoxycholate and doxorubicin as model drugs. Sodium deoxycholate was used because it is a widely used amphiphilic guest for dendrimers. G4 PPI dendrimers with different degrees of acetylation ranging from 14.2% to 94.3% were synthesized, characterized by nuclear magnetic resonance (NMR) techniques, and used to encapsulate three model drugs. The drug loading capacity and cytotoxicity of these acetylated