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# Nonabsorbable polysaccharide-functionalized polyethylenimine for inhibiting lipid absorption

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ABSTRACT

Overweight and obesity, which contribute to various chronic diseases, are increasingly common conditions around the world. For the purpose of weight loss in patients with overweight and obesity, we developed a series of β-cyclodextrin functionalized cationic branched polyethylenimine as oral pharmaceutical agents to inhibit digestion and absorption of dietary lipids *in vivo*. Tuning the structural configuration, molecular weight, and side-chain length of the cationic polymers provided the polymer with effective inhibition of lipid absorption. Importantly, the cationic polymer significantly increased fecal elimination of bile acids, triglycerides and cholesterol by 6.3-, 4.8- and 5.0-fold higher than those of the control with high fat diet, respectively. Moreover, the polymer could reduce the plasma lipids and liver lipid level in mice. The cationic polymer exhibited low cytotoxicity and did not cause observable histological changes for normal tissue. Therefore, the cationic polymer showed effective and safe characteristics as an oral pharmaceutical agent for inhibiting lipid absorption. This work offers a new promising venue to control weight for patients with overweight and obesity.

## 1. Introduction

Overweight and obesity are rapidly increasing among adults and youth, regardless of race, ethnicity, gender or age. They are associated with cardiovascular diseases, type 2 diabetes, high blood pressure, some cancers and even an excess risk of mortality (Berrington de Gonzalez et al., 2010; Cooper et al., 2011; Finucane et al., 2011; Kotseva et al., 2016; Whitlock et al., 2009). Therefore, the prevention and treatment of overweight and obesity have become a globally relevant topic. Recently, there is growing appreciation that weight loss is associated with health benefits. Weight loss of only 5%–10% can prevent the development of type 2 diabetes, reduces risk factors of cardiovascular disease, and improves other health consequences of obesity (Knowler et al., 2002; Ryan & Bray, 2013; Wing et al., 2011). Based on the above mentioned, significant unmet need and the robust projected growth rates of antiobesity drug market are fuelling the development of weight loss drugs. Currently, marketed drugs such as lorcaserin, phentermine/topiramate, bupropion/naltrexone etc., show promising efficacy for weight loss and are used as treatment options for the management of obesity. The oral drugs are absorbed through the gastrointestinal tracts into the bloodstream and target pathways in the nervous system that influences appetite or energy use. Unfortunately, most of these oral drugs may cause side effects, such as diarrhoea, abdominal pain, cardiovascular disease risk and hypertension (Kakkar & Dahiya, 2015; Poulton & Nanan, 2014), so it is still major challenges in exploring novel drugs for weight loss.

It is well recognized that, overweight and obesity occur when energy intake exceeds energy expenditure, and are caused by an increase in the size and the number of fat cells in the body (Foreyt, Goodrick, & Gotto, 1981), thus dietary factors of obesity are particularly important topic. Accordingly, a potential effective and safe approach for the treatment of obesity is an exploration of routes to inhibit the absorption of the calorie dense ingredients of food, such as dietary lipids. The small intestine is the major site for the emulsification and micellization of dietary lipids. Moreover, emulsification of lipids along with hydrolysis and micellization is necessary for absorption through small intestinal cells into the blood circulation. Bile acids, cooperatively function with pancreatic lipase to ensure efficiency of lipid digestion and absorption, and are essential for the complete absorption of dietary lipids. Dietary lipids, including triglycerides and cholesterol, enter lipid micelles along with bile acids to form mixed micelles (Mu & Hoy, 2004; Phan & Tso, 2001; Yao et al., 2002). Therefore, it is possible to develop the new

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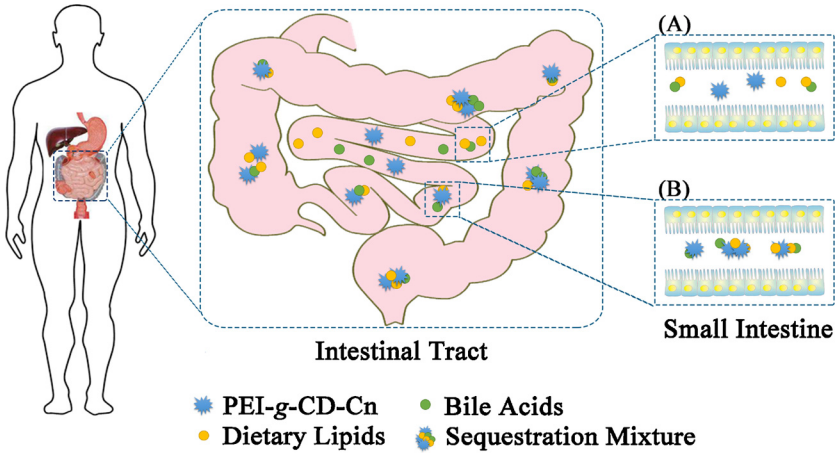


**Scheme 1.** Dietary lipids are emulsified and micellized by bile acids in the duodenum to form lipid micelles (A), and polymers bind bile acids and lipid micelles in the small intestine, and are finally excreted through feces (B).

materials to sequester the bile acids and eliminate lipid micelles, which can prevent the digestion and absorption of dietary lipids. Qian et al. prepared lipid sequestrant polymer based on poly(2-(diisopropylamino) ethyl methacrylate) and poly(2-(dibutylamino)ethyl methacrylate) that can effectively inhibit lipid absorption (Qian, Sullivan, & Berkland, 2015). The biological safety of the material as an oral agent, however, has not been investigated.

Based on the aforementioned considerations, we designed and prepared a series of branched cationic polymers as oral pharmaceutical agents to inhibit lipid absorption in the small intestine (Scheme 1). The branched polymers have desirable features: (1) high molecular weight, allowing themselves to be administered orally but not to be absorbed in the gastrointestinal tract; (2) quaternized polyethylenimine (PEI) with positive charges, which interacts with negatively charged lipid micelles; (3) hydrophobic inner cavities of $\beta$-CD, enabling them to serve as a host to coordinate lipids, especially cholesterol.

Branched PEI is a cationic polymer containing a large number of primary, secondary, and tertiary amino groups, while linear PEI has mostly secondary amines (Wang et al., 2017; Wightman et al., 2001). To introduce the functionalized groups, primary amino groups of branched PEI are a good choice. Although the polymers based on PEI and $\beta$-cyclodextrin have been extensively studied for a variety of purposes (Guo et al., 2015; He et al., 2013; Kunath et al., 2003; Lv, Zhou, Zhao, Liao, & Yang, 2017), they have not been reported as oral pharmaceutical agents for lipid sequestration. To obtain an optimal composition of the polymer, a series of $\beta$-CD functionalized branched PEI were synthesized. To enhance the binding capability to lipids, $\beta$-CD functionalized branched PEI was further quaternized with alkyl bromide. And the biocompatibility and lipid sequestration capacity of these polymers were further evaluated. We expect that this cationic polymer, as an oral pharmaceutical agent, can bind bile acids, eliminate lipid micelles, and eventually inhibit the digestion and absorption of dietary lipids in the small intestine.

## 2. Materials and methods

### 2.1. Materials

Polyethylenimine (10 and 70 kDa) was purchased from Alfa Aesar (Ward Hill, MA, US). *p*-Toluenesulfonyl chloride, $\beta$-cyclodextrin, triglyceride and cholesterol were purchased from Shanghai Aladdin Industrial Co., China. Ethyl bromide, 1-bromobutane and 1-bromohexane were of chemical grades from J&K China Chemical Ltd (Beijing, China). Four of bile acids were purchased from Meilunbio (Dalian, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich, USA. Fed-state-simulated intestinal fluid (FeSSIF) was purchased from Biorelevant (Basel, Switzerland). Dulbecco's modified eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS), non-essential amino acid and trypsin were purchased from Gibco, USA. All solvents used were of analytical grade without further purification.

### 2.2. Synthesis and characteristics of PEI-g-CD-Cn

The synthesis of PEI-*g*-CD-Cn was performed as follows. First, OTs-$\beta$-CD was synthesized using a previously reported method (Ping et al., 2011). Briefly, $\beta$-CD (40.0 g, 35.3 mmol) was suspended in 500 mL of water, and 17 mL of NaOH (4.4 g, 110.0 mmol) solution was added dropwise to the above solution over 10 min. *p*-Toluenesulfonyl chloride (6.7 g, 35.3 mmol), dissolved in 20 mL of acetonitrile, was added dropwise over 15 min into the above solution, causing immediate formation of a white precipitate. After 2.5 h of stirring, the precipitate was removed by suction filtration and the filtrate was refrigerated overnight at 4 °C. The resulting white precipitate was filtrated and dried for 12 h, which led to 4.6 g of a pure white solid.

Secondly, PEI-*g*-CD was prepared as previously described (Ping et al., 2011). Different feed ratios of PEI (10 and 70 kDa) to OTs-$\beta$-CD were added into 10 mL of DMSO under a nitrogen atmosphere and reacted at 70 °C for 5 days. The mixture was dialyzed (3500 Da cutoff) against distilled water for 3 days and freeze-dried to obtain PEI-*g*-CD.

Finally, different molar ratios of alkyl bromides (ethyl bromide, 1-bromobutane and 1-bromohexane, respectively) to PEI-*g*-CD were added into 15 mL of distilled water, and was stirred continuously at 70 °C for 48 h. The obtained quaternized PEI-*g*-CD was dialyzed (3500 Da cutoff) against ethanol for 72 h, then further dialyzed (3500 Da cutoff) against distilled water for 5 days, and was freeze-dried to acquire quaternized PEI-*g*-CD, which was marked as PEI-*g*-CD-Cn ($n$ = 2, 4 and 6).

$^1$H NMR spectroscopy was used to characterize the synthesized PEI-*g*-CD and PEI-*g*-CD-Cn. For analysis, 5 mg of the polymer was dissolved in 0.6 mL of $D_2O$, and the $^1$H NMR spectrum of each sample was recorded with a NMR spectrometer (400 MHz, Bruker Corporation). To further confirm the synthesis of PEI-*g*-CD and PEI-*g*-CD-Cn, FTIR spectra of $\beta$-CD, PEI, PEI-*g*-CD and PEI-*g*-CD-Cn were obtained using a Fourier Transform Infrared Spectrometer (FTS-6000, Bio-Rad Co.). The molecular weight and the polydispersity of the polymers were measured using a gel permeation chromatography.

### 2.3. In vitro bile acid and lipid sequestration

The majority (approximately 80%) of the human bile acid pool is composed of four primary bile acids, including glycocholic acid